A follow-up study of serum cholesterol and lipoproteins in children: The effect of diet and apolipoprotein E on cholesterol metabolism, tracking, and screening

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Academic Dissertation

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ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, referred to in the text by roman numerals shown below. In addition, some previously unpublished data are presented.

I. Kallio MJT, Salmenperä LS, Siimes MA, Perheentupa J, Miettinen TA. Exclusive breast-feeding and weaning: Effect on serum cholesterol and lipoprotein concentrations in infants during the first year of life.
Pediatrics 1992;89:663-666.

II. Kallio MJT, Salmenperä L, Siimes MA, Perheentupa J, Miettinen TA. Tracking of serum cholesterol and lipoprotein levels from the first year of life.
Pediatrics 1993;91:949-954.

III. Kallio MJT, Salmenperä L, Siimes MA, Perheentupa J, Gylling H, Miettinen TA. Apoprotein E phenotype determines serum cholesterol in infants during both high-cholesterol breast feeding and low-cholesterol formula feeding.
Journal of Lipid Research 1997;38:759-764.

IV. Kallio MJT, Salmenperä L, Siimes MA, Perheentupa J, Gylling H, Miettinen TA. The apolipoprotein E phenotype has a strong influence on tracking of serum cholesterol and lipoprotein levels in children: a follow-up study from birth to the age of 11 years.
Pediatric Research 1998;43:381-385.

ABBREVIATIONS

apoA	Apolipoprotein A
apoB	Apolipoprotein B
apoE	Apolipoprotein E
CHD	Coronary heart disease
CRP	C-reactive protein
FH	Familial hypercholesterolemia
HDL	High density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
IDL	Intermediate density lipoprotein
LDL	Low density lipoprotein
SCAP	SREBP cleavage-activating protein
SD	Standard deviation
SEM	Standard error of mean
SREBP	Sterol regulatory element-binding proteins
TG	Triglyceride
VLDL	Very low density lipoprotein

INTRODUCTION

The cholesterol molecule, a complex four-ringed structure, is synthesized from a simple two-carbon substrate (acetate) through the action of at least 30 enzymes (Goldstein. Nature-90). Cholesterol serves as a precursor for adrenal and gonadal steroids and hepatic bile acids and as a structural component of animal cell membranes, in which it modulates fluidity and maintains the barrier between the cell and the environment (Brown. Science-86). Cholesterol is useful in cell membranes because it is absolutely insoluble in water. This property also causes many problems: cholesterol accumulating within the wall of an artery leads to development of an atherosclerotic plaque. The problem of cholesterol transport is solved by esterifying it with long-chain fatty acids and by further packing these esters within the hydrophobic cores of lipoproteins.

As serum cholesterol has been linked strongly to atherogenesis, many researchers have speculated that reduction of cholesterol levels might slow the progression or induce the regression of coronary atherosclerotic lesions (Brown. NEJM-90). However, atherosclerosis leading to coronary heart disease (CHD) is complex in origin. The pathogenesis of atherosclerosis involves hemodynamic, thrombotic, and carbohydrate-lipid metabolic variables, and also intrinsic characteristics of the arterial wall (Ross. Nature-93). Environmental factors such as smoking, a sedentary lifestyle, and disease processes such as diabetes also contribute to this process (Haffner. NEJM-98). In fact, the cholesterol level as such does not tell much; half of all myocardial infarctions occur in persons with normal cholesterol levels (Braunwald. NEJM-97). However, in specific subgroups observational studies have established the relationship of serum cholesterol level with CHD (Stamler. JAMA-2000), and a detailed summary of updated clinical guidelines for the detection, evaluation, and management of high blood cholesterol in adults has recently been published (NCEP Expert Panel. JAMA-2001).

Cholesterol is not the only important variable in lipoprotein metabolism; many other factors, for example apolipoproteins, can contribute significantly to CHD risk. In lipoprotein metabolism, apolipoproteinE (apoE) plays a role as a ligand for receptors of the low density lipoprotein receptor superfamily (Mahley. Science-88). The common apoE polymorphism has profound effects on susceptibility to CHD (Smith. Ann Med-2000). Because the apoE phenotype influences the serum cholesterol level and cholesterol metabolism, it also affects the tracking of serum lipids in children (Srinivasan. Atherosclerosis-96) and in adults (Srinivasan. Atherosclerosis-99).

The extent to which an individual maintains his position relative to the rest of the population is called tracking (D JAMA-90). Confirmation that children who are initially in the extremely high range of serum cholesterol become a with high serum cholesterol is the basis for cholesterol screening and possible clinical intervention in childh Screening of all children for determination of their blood cholesterol levels has been advocated by many authors, bu a complex issue. The target of the screening would be to identify children having high cholesterol values, heterozygous familial hypercholesterolemia, but the prevalence of this condition is only approximately 0.2% ar identify these children by screening would be difficult and expensive. Further, even if one were to find a child with cholesterol values, there is no consensus about the age at which drug therapy should be started in such children (S JAMA-99). Further, primary prevention programs have little effect on cardiovascular morbidity and none on mort as shown in a thorough meta-analysis (Ketola. Ann Med-2000).

For prevention of CHD, some experts recommend replacement of saturated fats with monounsaturated fats (Katan. NEJM-97), while others recommend a low-fat, high-carbohydrate diet (Connor. NEJM-97). Conflicting recommendations concerning diets and further screening compound public confusion about nutrition and CHD. In light of the many different isolated research results, this is not only a matter between scientists, but can have a huge impact on the attitudes of the general public to the impression of cholesterol and further on the daily lives of many children (the influence of milk, fat, and eggs on health). It seems that the more arteriosclerosis, thrombs, lipoproteins, and genes are investigated, the more complicated the situation becomes. There is a necessity for a wide perspective in the attempt to understand the basis for dietary and screening recommendations for children. In what follows, we will examine at a selection of these questions in greater detail.

CHOLESTEROL METABOLISM

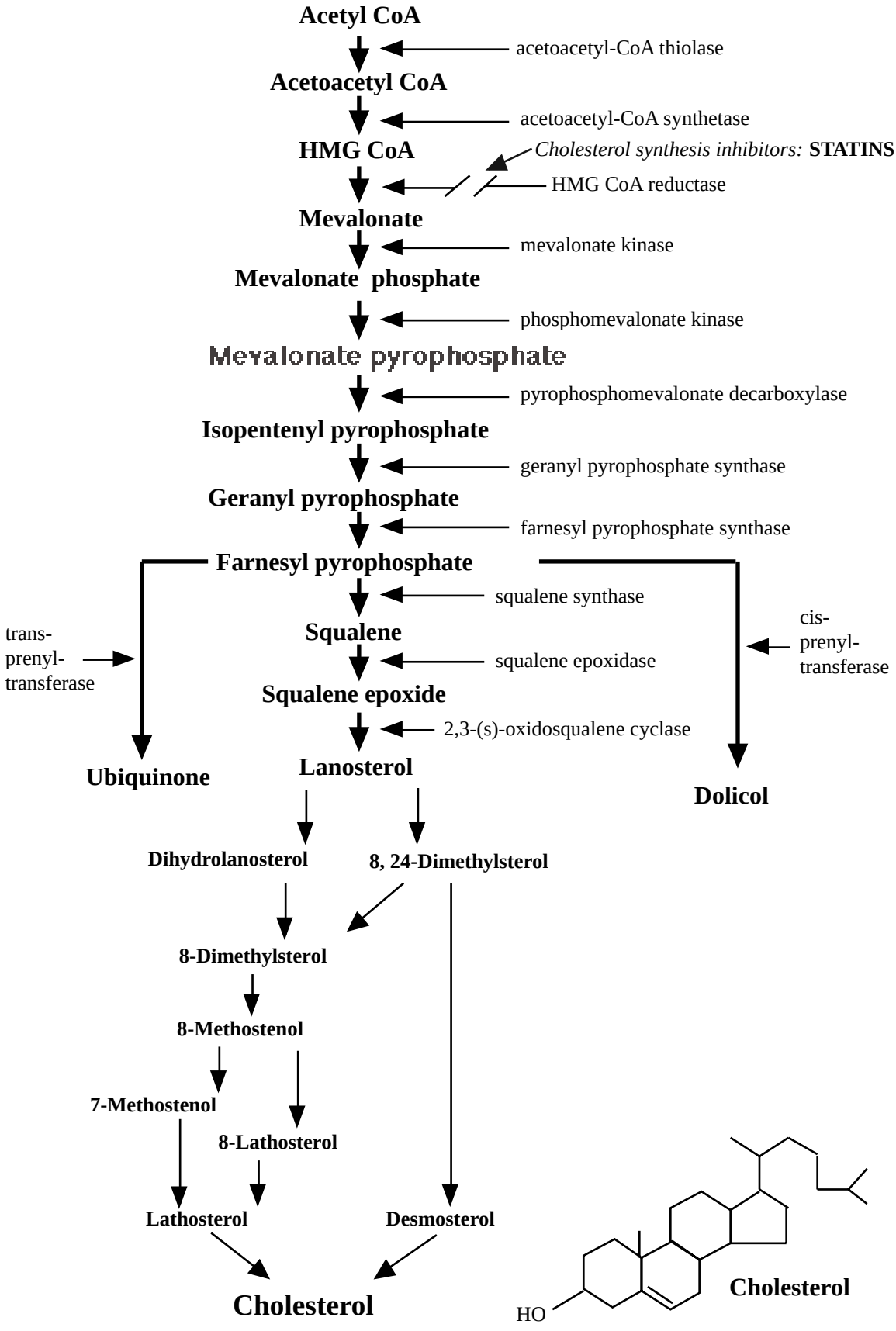
Cholesterol is mostly synthesized in the liver cells (Goldstein. Nature-90) from acetate in the endoplasmic reticulum (Fig. 1) (Brown. Science-86). Liver also converts cholesterol into bile acids, this being the major route of excretion from the body (Dietschy. NEJM-70). Cholesterol plays an important role in modulating fluidity and phase transitions in the plasma membranes of animal cells (Brown. PNAS-99). Together with sphingomyelin, cholesterol forms plasma membrane rafts or caveolae that serve as sites at which signalling molecules are concentrated (Simons. Nature-97). To perform these functions, membrane cholesterol must be maintained at a constant level. This is achieved by a feedback regulatory system that senses the level of cholesterol in cell membranes and modulates the transcription of genes encoding enzymes of cholesterol biosynthesis and uptake from plasma lipoproteins.

A feedback regulatory system that modulates the transcription of genes encoding the enzymes of cholesterol biosynthesis are a family of membrane-bound transcription factors called sterol regulatory element-binding proteins (SREBPs) (Brown. PNAS-99). To act, these must be released proteolytically from membranes (Brown. Cell-97). Transcription is enhanced when the active NH2-terminal domains of SREBPs are released from the endoplasmic reticulum membranes by two sequential cleavages. The NH2-terminal domain then travels to the nucleus, where it binds to sterol regulatory elements in the enhancers of multiple genes. In the cholesterol biosynthetic pathway, well-defined target genes include HMG-CoA synthase, HMG-CoA reductase, and squalene synthase.

Sterols block SREBP processing by the SREBP cleavage-activating protein (SCAP). This is a regulatory protein that serves as a sterol sensor, losing its activity when sterols over-accumulate in cells (Nohturfft. PNAS-99). As a result, transcription of all of the target genes declines (Horton. J Clin Invest-98). SCAP is the central regulator of lipid synthesis and uptake in mammalian cells. Molecular variants in the gene for SCAP may lead to alterations in plasma lipoprotein levels and/or derangement of intracellular lipid metabolism (Nakajima. J Hum Genet-99). These regulated proteolytic cleavage reactions are ultimately responsible for controlling the level of cholesterol in membranes, cells, and blood.

The key regulatory step in complex biosynthetic pathway of cholesterol is catalyzed by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Drugs of the statin class are structurally similar to hydroxymethylglutaryl-coenzyme A and are competitive inhibitors of HMG-CoA reductase. Statins inhibit cholesterol synthesis in the liver, but there is a compensatory response; firstly, hepatocytes synthesize increased amounts of HMG-CoA reductase, and, secondly, hepatocytes synthesize increased numbers of low density lipoprotein (LDL) receptors (Kovanen. PNAS-81a). As a result of the increase in LDL receptors, the plasma LDL level decreases. In patients with and without coronary artery disease, statins can induce regression of vascular atherosclerosis and reduce cardiovascular-related morbidity and mortality (Scandinavian 4S. Lancet-94, Shepherd. NEJM-95). However, subjects with the homozygous form of familiar hypercholesterolemia do not respond to statins, because they cannot synthesize LDL receptors (Goldstein. J Biol Chem-74).

Figure 1. **The pathway of cholesterol metabolism**



LIPOPROTEIN SYNTHESIS and METABOLISM

Cholesterol, phospholipids, and triglycerides in lipoproteins

Cholesterol and triglycerides are carried in the blood by lipoproteins because they are water-insoluble lipids. A lipoprotein is essentially an oil droplet composed of cholesteryl esters and triglycerides, solubilized by a surface monolayer of phospholipid and unesterified cholesterol and stabilized by a protein. Triglycerides and cholesteryl ester molecules are nonpolar and hydrophobic; they form the core of the lipoprotein, while amphipathic phospholipids and apoproteins form the surface (Fig. 2).

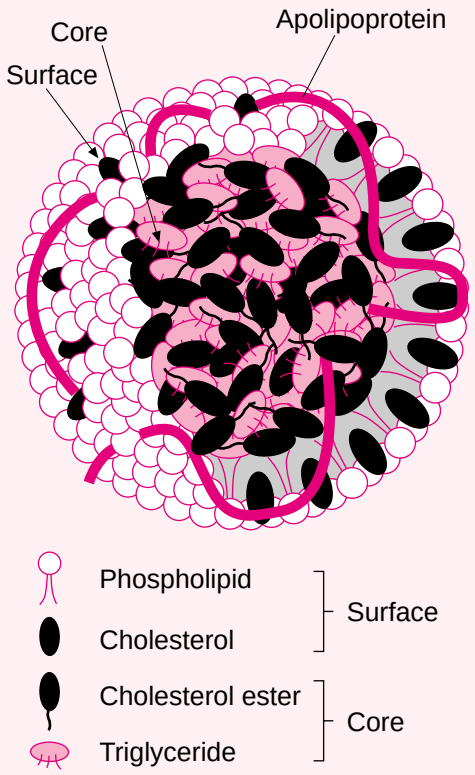


Figure 2. A lipoprotein is essentially an oil droplet composed of cholesteryl esters and triglycerides, solubilized by a surface monolayer of phospholipid and unesterified cholesterol and stabilized by a protein. (adapted from the Kovanen and Viikari, Endokrinologia, Kustannus Oy Duodecim, 2000).

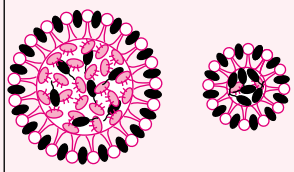
The LDL core is composed of some 1500 molecules of cholesterol esters and 200 molecules of triglyceride. The cholesterol esters are the main lipid of the lipoprotein core, the majority of the fatty acyl chains in these esters being linoleate. This core is shielded by a layer consisting of 500 molecules of phosphatidylcholine, 200 molecules of sphingomyelin, 600 molecules of unesterified cholesterol, and one molecule of apoB-100. The phosphatidylcoline and sphingomyelin and two-thirds of the unesterified cholesterol form an oriented amphipathic surface monolayer on the LDL particles, their polar heads being oriented outward and their nonpolar tails inward.

Although the lipoproteins comprise a continuum of particles differing gradually in density and in lipid and apoprotein composition, there are accumulations of relatively distinct subclasses that can be isolated by various physical methods. Several major classes of lipoproteins have been defined by their physicochemical characteristics. These are chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). LDL and HDL mainly transport cholesterol, and VLDL is the major carrier of triglycerides, whereas chylomicrons carry triglycerides of dietary origin. The physicochemical characteristics of the major lipoproteins are presented in Table I. The surface proteins of the lipoproteins are called apoproteins. Some of the apoproteins confer structural stability on the lipoproteins, while others determine the metabolic fate of the lipoprotein, and still others act as cofactors for the plasma enzymes involved in lipoprotein metabolism. Apoprotein B and E serve as ligands for the specific uptake of lipoprotein particles (Kowal. PNAS-89).

Phospholipids together with apolipoproteins help to solubilize triglyceride and cholesterol in the different lipoproteins. Phospholipids make up the majority of the surface of the lipoproteins, forming bilayers that act as interfaces with both the polar plasma components and the nonpolar lipids of the lipoprotein core. Phosphotidylcholine (lecithin) is the major phospholipid in plasma and is the source of linoleate for cholesteryl ester formation by the lecithin:cholesterol acetyltransferase reaction.

Triglycerides are the storage or carrier form of fatty acids in tissue and plasma and consist of three fatty acid molecules attached by an ester linkage to a glycerol molecule. Lipoprotein triglyceride synthesis occurs in the small intestine and the liver. Triglycerides are the major lipids in chylomicrons and VLDL, and serve as energy substrates in the liver and peripheral tissues, particularly the muscle tissue. Triglyceride molecules are nonpolar and must be carried in the core of the lipoproteins. Triglycerides can be transferred between lipoproteins in association with the carrier protein, the cholesteryl ester transfer protein.

Table I. Lipoprotein molecules. VLDL = very low density lipoprotein, LDL = low density lipoprotein, Lp(a) = lipoprotein (a), HDL = high density lipoprotein, surface proteins; apoproteins A, B, C, E and (a).
(adapted from the Kovanen and Viikari, Endokrinologia, Kustannus Oy Duodecim, 2000).

	Dietary (exogenous) lipids		Hepatic (endogenous) lipids				
							
Particle	Chylomicron	Chylomicron-remnant	VLDL	VLDL-remnant	LDL	Lp(a)	HDL
Diameter (nm)	1 000–80	100–50	70–30	30	20	20	10
Major lipid in the core	Triglyceride	Cholesterol ester	Triglyceride	Triglyceride	Cholesterol ester	Cholesterol ester	Cholesterol ester
Surface proteins	A B-48 C E	A B-48 E	B-100 C	B-100 E	B-100	B-100 a	A C E
Location of synthesis	Intestine	Circulation	Liver	Circulation	Circulation	Liver	Liver Intestine Circulation

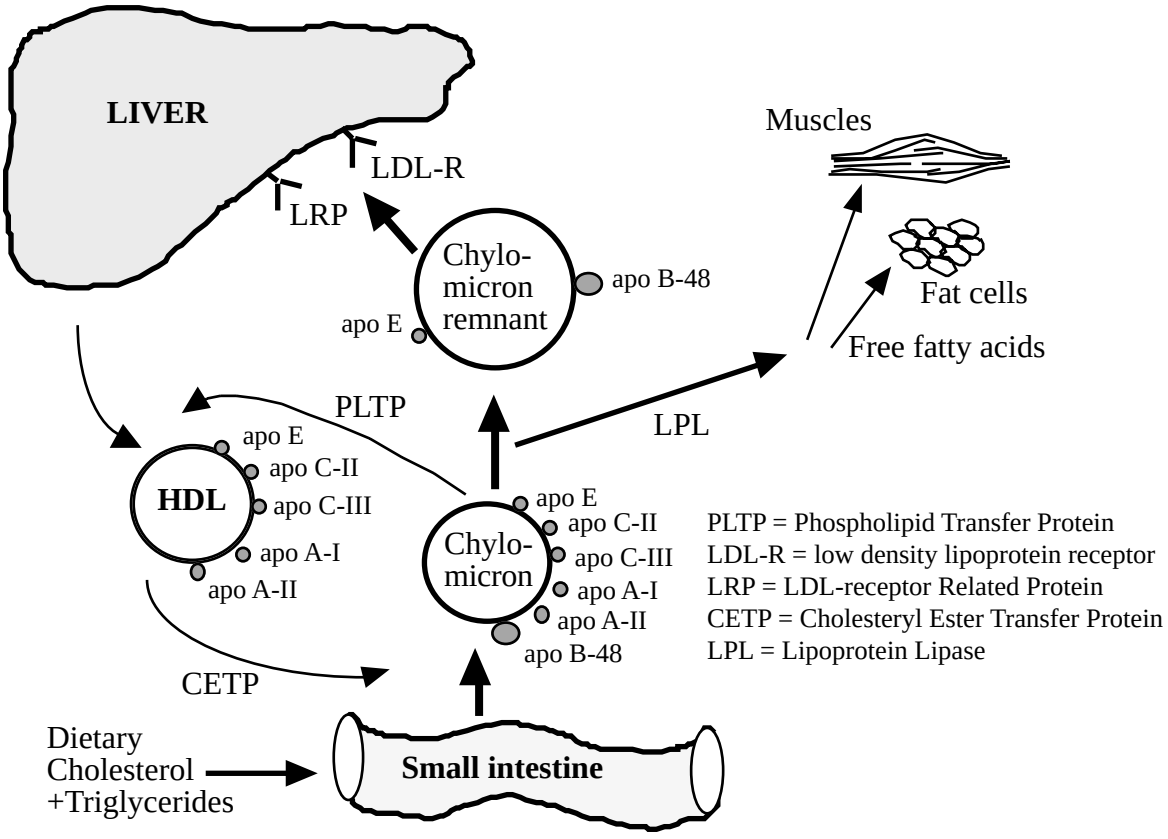
Transport of dietary lipids

Chylomicrons

Lipoprotein metabolism can be divided into exogenous and endogenous metabolism. In exogenous lipid metabolism, dietary lipids are carried to the liver whereas, in endogenous metabolism, lipids, mainly VLDL, are secreted by the liver. Chylomicrons are synthesized in the endoplasmic reticulum of gut mucosal cells and transport dietary lipids (Fig.3) (Ginsberg. Endocrin and Metab Clin N Am-98).

Dietary triglyceride, as fatty acids, and cholesterol are taken up by the enterocyte after being transported to the cells in bile salt micelles. Inside the mucosal cells occurs re-esterification to triglyceride and cholesteryl ester. After secretion via the thoracic duct into the circulation these hydrophobic lipids are incorporated into the core of nascent chylomicrons. The surface of the chylomicron is composed of phospholipid, apoB-48, apoA-I, apoA-II, and apoA-IV, which are synthesized in mucosal cells. Triglycerides constitute about 90% of weight of the chylomicron. The chylomicrons acquire apoC-II, apoC-III, and apoE by transfer from HDL, and cholesterol and phospholipids also move to chylomicrons from HDL. After gaining apoC-II, the chylomicron can bind to LPL on the surface of capillary endothelial cells, and chylomicron core triglyceride can be hydrolyzed. The hydrolysis of chylomicron triglycerides occurs in the capillary beds of adipose tissue, lung, and muscles. Chylomicrons attach to the capillary walls and undergo degradation by lipoprotein lipase which is bound to cell surface glycosaminoglycans. Large chylomicrons are rapidly metabolized resulting in the formation of remnant particles. The pathway for uptake of chylomicron remnants by the liver involves LDL receptors that recognize apoE, specific apoE receptors, and cell surface proteoglycans that can bind apoE, which is crucial in this process.

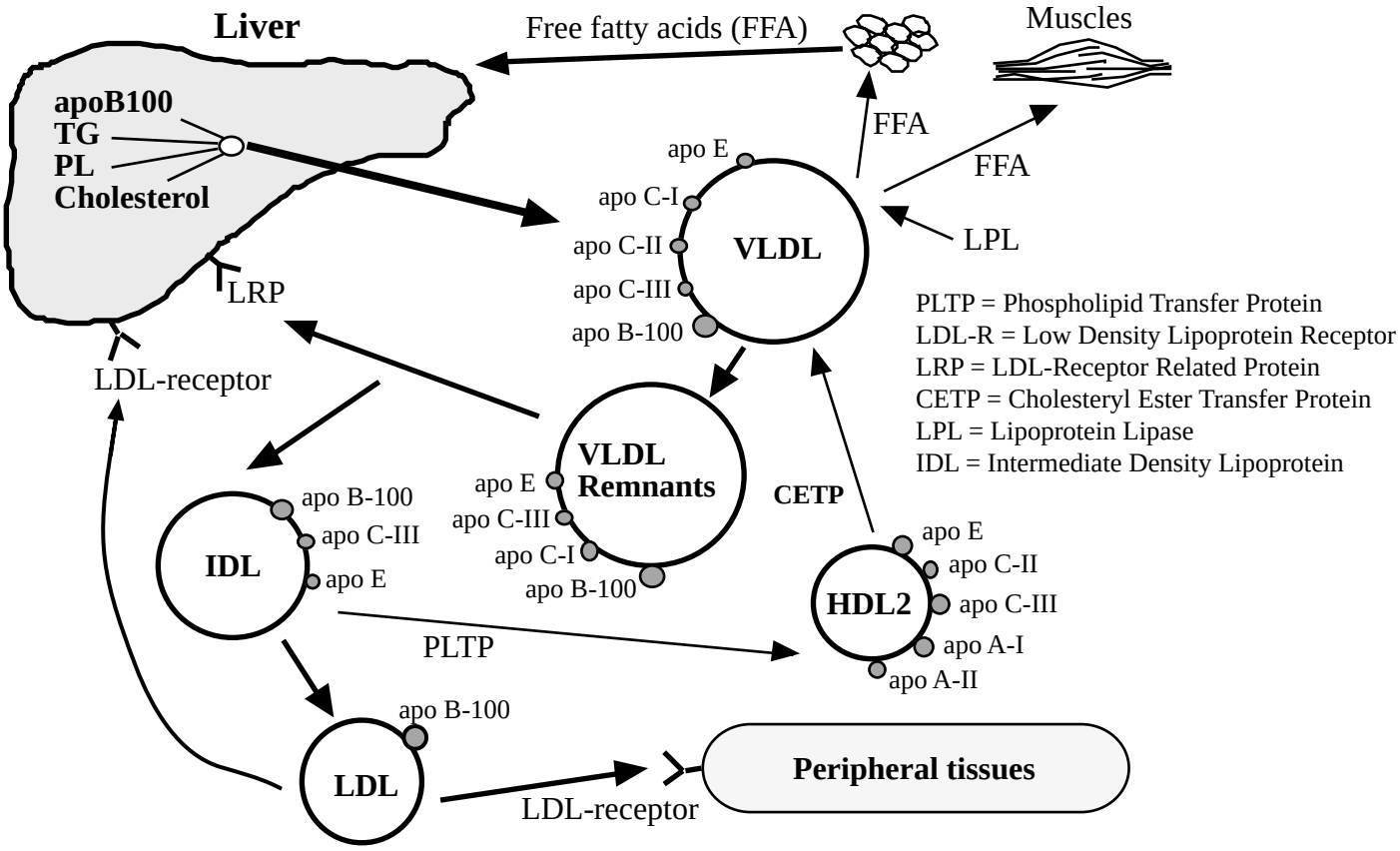
Figure 3. **Transport of chylomicron and chylomicron remnants**



Transport of endogenous, hepatic lipids via VLDL, IDL and LDL.

The liver assembles and secretes apoB-containing lipoproteins, mainly VLDL, which transports endogenous lipids (Packard. Arterioscler Thromb Vasc Biol-97). In hepatocytes, apoB-100, triglycerides and cholesterol esters, are packed together with phospholipids into nascent VLDL (Fig.4) (Ginsberg. Endocrin and Metab Clin N Am-98). The fatty acids are derived from multiple sources, namely synthesis from acetyl-CoA units produced by utilization of carbohydrate, from free fatty acids taken up into the cells from plasma albumin and from hydrolysis of lipids transported to the liver in plasma lipoproteins such as chylomicron remnants. In plasma, VLDL also contains apoC-I, apoC-II, apoC-III, and apoE, most of which are added to VLDL after their entry into plasma. The size of the VLDL seems to be determined by the availability of triglyceride for packaging into the VLDL. Large triglyceride-rich VLDL are secreted when excess triglycerides are synthesized, which is the case in obesity and in persons consuming a diet high in simple carbohydrates. After secretion from the liver VLDL attach to the capillary walls and undergo degradation by lipoprotein lipase which is bound to cell surface glycosaminoglycans activated by apoC-II. VLDLs become smaller and more dense and are converted to VLDL remnant. The rate of lipolysis depends on amounts of lipoprotein lipase, the attachment of the enzyme to the endothelium as well as on the composition of the lipoproteins and their size. During VLDL catabolism loss of triglyceride results in the transfer of surface cholesterol, phospholipids, and apoproteins to HDL. Loss of triglyceride from VLDL also generates IDL, which become LDL after interaction with the liver. VLDLs and their remnants are removed by receptors that recognize apoE.
The role of VLDL in atherogenesis has been controversial. Some subpopulations of VLDL particles may have atherogenic potential, while others do not. Small chylomicron remnant particles are implicated in the development and progression of CHD, as failure to regulate endogenous triglyceride-rich lipoproteins in postprandial dyslipidemia (Karpe. Metabolism-99).

Figure 4. **Transport of VLDL, IDL and LDL**



Low-density lipoprotein

LDL comprises lipoproteins with hydrated densities of 1.006-1.063 g/mL. This density range includes a number of lipoprotein subspecies, such as intermediate density lipoprotein (IDL), small dense LDL, and big buoyant LDL. Indeed, further heterogeneity may exist with in each of these major subspecies. IDLs are believed to be particularly atherogenic (Mack. Arterioscler Thromb Vasc Biol-96). Evidence indicates that small dense LDLs are associated with CHD, and that their presence predicts subsequent CHD events (Gardner. JAMA-96).

Once LDL is formed, essentially the only protein on the surface is apoB-100 (Fig. 2). About 70% of the plasma LDL is taken up into the liver by LDL receptor-dependent pathways, but the highest rate of uptake on a weight basis occurs in the adrenal gland (Kovanen. Endocrinology-79). Studies with cultured cells led to the discovery of a cell surface receptor for LDL and to elucidation of the mechanism by which this receptor mediates feedback control of cholesterol synthesis (Goldstein. PNAS-73). The LDL receptor is a transmembrane protein present on the cell surfaces in nearly all tissues of the body (Brown. Science-86). The LDL receptor makes a round trip into and out of the cell every 10 minutes for a total of several hundred trips during its 20-hour life-span (Brown. Nature-97). Once LDL receptors become saturated, the removal of LDL is proportional to the number of receptors; whenever the number of receptors is reduced, plasma LDL levels must rise. Consumption of a high-fat diet decreases the number of LDL receptors (Kovanen. PNAS-81b). This mechanism operates through feedback suppression, i.e. when excess dietary cholesterol accumulates in the liver, the liver responds by decreasing the production of LDL receptors.

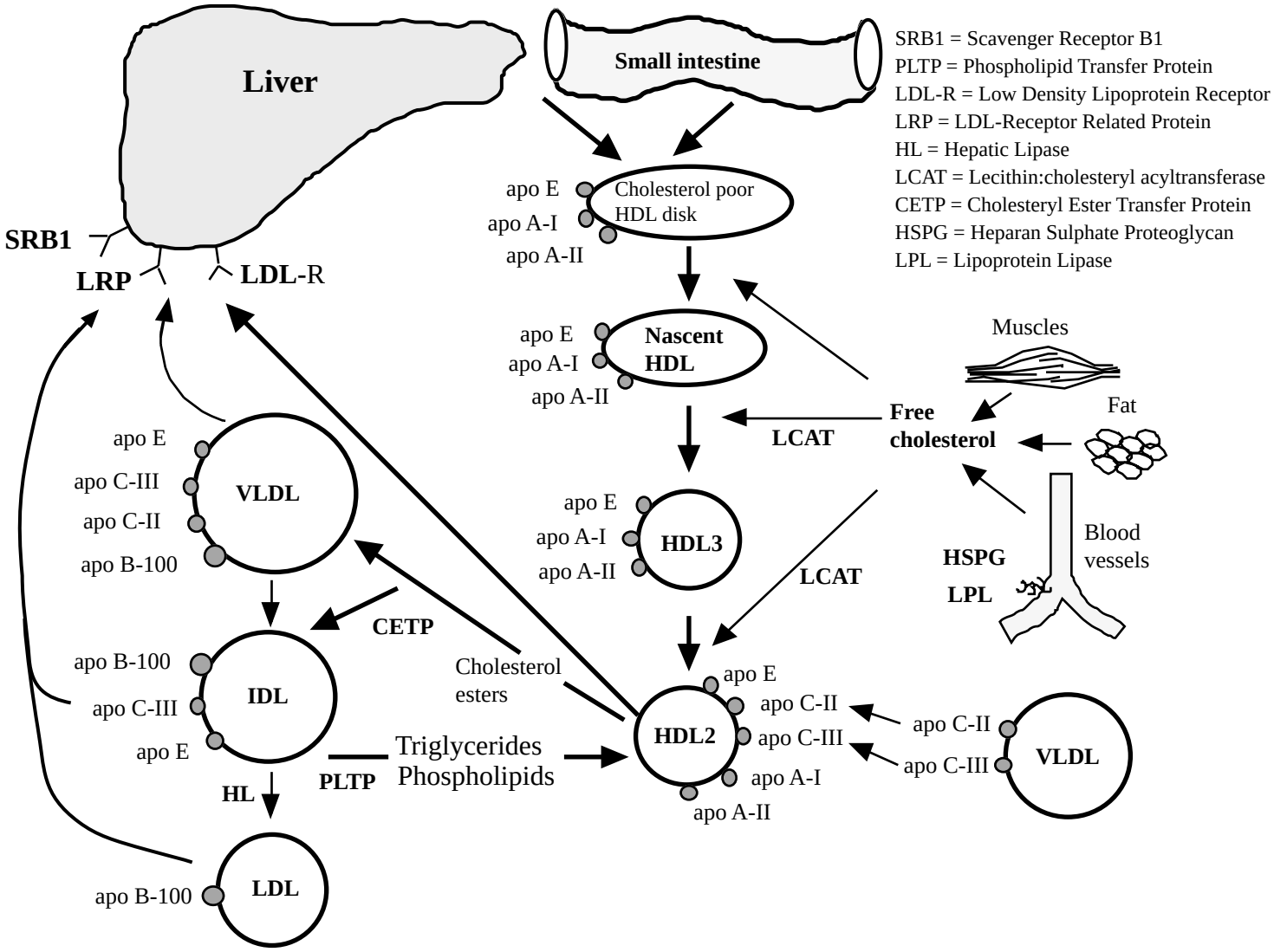
High-density lipoprotein

HDL plays an important role in shuttling apolipoproteins to and from the triglyceride-rich lipoproteins. The delivery of cholesterol to the liver by HDL proceeds differently from that of LDL (Acton. Science-96), which is catalyzed by the LDL receptor (Brown. Science-86). Nascent HDL are generated during the intravascular degradation of VLDL and chylomicrons, and are also directly secreted by intestine. Thereafter plasma phospholipid transfer protein facilitates the transfer of phospholipids and some cholesterol into the HDL pool. HDL are formed by the obvious coalescence of individual phospholipid-apoprotein disks containing apoA-I, apoA-II, apoA-IV, and possibly apoE, together with lecithin-cholesterol acyltransferase and cholesteryl ester transfer protein.

This initial step in the reverse cholesterol transport process is followed by lecithin-cholesterol acyltransferase-generated esterification of more free cholesterol in HDL3, which allows the free cholesterol on the surface to move into the core of the particle as cholesteryl ester (Fig. 5) (Ginsberg. Endocrin and Metab Clin N Am-98). As HDL3 accumulate cholesteryl ester, they enlarge and can now accommodate apoE, apoC-II and apoC-III as well as more phospholipid on their surfaces. As more cholesteryl ester accumulates, HDL3 is transformed into HDL2, the major HDL class. The fate of HDL2 is complicated, the initial transfer of cholesteryl ester from HDL to triglyceride-rich lipoproteins chylomicrons and VLDL by cholesteryl ester transfer protein is important. The cholesteryl ester transfer protein-transferred cholesteryl esters can then be taken up by the liver as chylomicron remnants or VLDL remnants, or IDL are removed by the LDL (or other) receptor-mediated pathway. HDL uptake can be mediated by the scavenger-receptor-B1 in the cells (Kozarsky. Nature-97).

An important finding in HDL metabolism is that a biochemical defect in cellular cholesterol efflux can be caused by mutations in the ATP-binding-cassette gene, which codes for the cholesterol-efflux regulatory protein (Rust. Nat Genet-99, Marcil. Lancet-99). This cholesterol efflux regulatory protein encoded by ATP-binding-cassette gene is a key gatekeeper influencing intracellular cholesterol transport. It has an essential role in the formation of HDL. The identification of ATP-binding-cassette gene has implications for the understanding of cellular HDL metabolism and also reverse cholesterol transport and its association with premature cardiovascular disease. High levels of HDL-cholesterol are associated with a reduced risk for atherosclerotic cardiovascular disease (Gordon. NEJM-89, Pekkanen. NEJM-90). The mechanism by which HDL exerts its beneficial effect on CHD is still debated, but the theory that has gained most acceptance is that the effectors of reverse cholesterol transport are HDL apoproteins and phospholipids (Stein. Atherosclerosis-99). The HDL-cholesterol level is sensitive to many factors such as exercise, which elevates HDL levels. Even the consumption of boiled rather than filtered coffee has been shown to decrease HDL-cholesterol (Aro. Atherosclerosis-90).

Figure 5. **Transport of HDL**



Role of age in determining lipoprotein levels

Many studies have examined the lipid, lipoprotein, and apoprotein levels in umbilical cord blood (Kwiterovich. Lancet-73, Viikari. Acta Paed Scand-85). Mean total cholesterol is slightly higher in females than in males, which is related to the higher levels of HDL-cholesterol in female infants. Striking changes in lipid and lipoprotein levels occur over the first months of life and throughout the remainder of the first year (Lane. Pediatr Res-86). Generally, apoB and LDL-cholesterol and triglycerides increase twice as much as apoA-I and HDL-cholesterol. The changes are due to the shift from intrauterine parenteral nutrition via the placenta to extrauterine enteral nutrition with breast milk, which has a high fat content.

By 6 months of age, there has been an increase of about a 100% in total cholesterol and of about 130% in triglycerides above cord blood levels in infants fed a polyunsaturated, fatty acid-rich, cholesterol-free formula (Farris. Am J Clin Nutr-82). The body weight almost triples and body fat increases remarkably during the first year of life. This massive accumulation of adipose tissue triglycerides prepares the infant for energy deficits that may occur during weaning. During adolescence, the mean HDL-cholesterol levels decrease by approximately 20% in males. LDL-cholesterol levels increase still further in adulthood as people become older; a part of this rise is due to the decreased activity of the LDL receptors (Miller. Lancet-84).

APOPROTEINS

There are ten major apoproteins (apoA-I, -II, -IV, apoB-48, apoB-100, apoC-I, -II, -III, apoE and apo(a)), and additionally six minor apolipoproteins, termed apoD, apoF, apoG, apoH, apoI, apoJ. Found on the surfaces of lipoproteins, apoproteins provide structural stability and have critical roles in regulating lipoprotein metabolism. Some apoproteins act as cofactors for plasma lipid-modifying enzymes.

ApoB-100 and apoB-48

The main function of both isoforms of apoB relate to their ability to bind lipids: in the absence of apoB-48 or apoB-100, no chylomicron or VLDL particles are formed and lipid absorption and transport are severely hampered. The ability of apoB to bind lipids resides in the multiple hydrophobic domains present throughout the length of the protein. ApoB-48 is a component of chylomicrons, constituting 48% of the amino terminal end of apoB-100. ApoB-48 is synthesized in significant amounts only in the intestine, and is necessary for the packing and secretion of chylomicrons from the small intestine (Higuchi. PNAS-88).

ApoB-100 is a large, hydrophobic protein of 4536 amino acids, synthesized in significant amounts in the liver. It is the major apoprotein of VLDL, IDL, and LDL, constituting approximately 30%, 60%, and 95% of the protein in the respective lipoproteins. The availability of the major lipoprotein lipids (triglycerides, cholesteryl esters, and phospholipids) determines whether apoB is degraded or secreted. The major factor in the post-translational regulation of apoB secretion is triglyceride availability (Dixon. J Lipid Res-93).

ApoB-100 seems to form a ring around the LDL particle (van Antwerpen. J Lipid Res-97). About 90% of the N-terminal apoB-100 forms a ribbon that wraps around the LDL particle, and the remaining 10% forms a bow that crosses the ribbon. It is the arginine residue in the ribbon that interacts with the C-terminal bow of apoB-100 and allows binding of the LDL receptor to residues of apoB-100 (Boren. J Clin Invest-98). Thus apoB acts as the ligand for receptor-mediated uptake of LDL. The apoB molecule contains several extremely hydrophobic areas that probably serve as strong lipid-binding domains. Polymorphism of apoB-100 partly accounts for the genetic variation in LDL-cholesterol levels (Aalto-Setälä. Hum Genet-89).

Apolipoprotein E

Apolipoprotein E (apoE) is synthesized mainly in the liver, but as much as 40% is formed in extrahepatic tissues (Reue. J Biol Chem-84). ApoE plays a major role in lipoprotein metabolism as a ligand for receptors of the LDL receptor superfamily (Mahley. Curr Opin Lipidol-99). The apoE phenotype is involved in the homeostasis, absorption, and synthesis of cholesterol, in its elimination as bile acids, in the removal of chylomicron remnants and in the hepatic clearance of dietary fat (Ehnholm. J Lipid Res-86, Kesäniemi. J Clin Invest-87, Miettinen. Arterioscler Thromb-92). Variation of apoE has been estimated to account for 8-10% of the total variation in serum cholesterol concentrations in various populations (Ye. Am J Clin Nutr-2000). ApoE interacts with several receptors in the liver, including the LDL receptor, the LDL receptor-related protein, and the VLDL receptor (Hoeg. Science-85). ApoE is also needed for the conversion of VLDL to LDL (Mahley. Science-88). apoE phenotypes regulate non-fasting serum triglyceride values in healthy infants, apoE3/4 and apoE4/4 predispose infants to higher values than apoE3/3 phenotype (Tammi. Atherosclerosis-2000). The common apoE polymorphism has effect on CHD, Alzheimer's disease, and human longevity, as well as on the common subclinical changes in cognitive function that accompany aging (Smith. Ann Med-2000). In addition, apoE polymorphism influences the tracking of serum lipids in children (Srinivasan. Atherosclerosis-99).

ApoE allele frequencies

The apoE gene exhibits genetic polymorphism with three alleles: e2, e3, e4. These alleles encode the protein isoforms E2, E3, and E4, respectively, but for convenience both the protein isoforms and the alleles will be referred to as E2, E3, and E4 (Utermann. Nature-77). Structurally, the three isoforms differ in cysteine-arginine interchanges at two positions in the protein, i.e. in the residues 112 and 158. Thus, apoE3 contains cysteine at 112 and arginine at 158, apoE2 contains cysteine at both positions, and apoE4 contains arginine at both positions (Weisgraber. J Biol Chem-81). Each subject inherits one of the three alleles from each parent, thus exhibiting either a homozygous (i.e. E2/2, E3/3, or E4/4) or a heterozygous (i.e. E4/2, E4/3, or E3/2) apoE phenotype.

The proportions of each of the six apoE phenotypes in a Western European sample were (E2/2=1%, E2/3=11%, E2/4=3%, E3/3=63%, E3/4=20%, E4/4=2%) (Menzel. Arteriosclerosis-83). Similar allele frequencies have been reported for the white population from the USA (Howard. Am J Epidemiol-98). Within Europe there is a clear gradient, with higher E4 allele frequencies in the north and lower E4 allele frequencies in the south (Tiret. Arterioscler Thromb-94). One of the highest E4 allele frequencies has been reported for the Finnish population (Ehnholm. J Lipid Res-86, Lehtimäki. J Lipid Res-90). This may be one factor responsible for the high serum cholesterol levels in the Finnish population. In fact, variation at the apoE gene locus may account for as much as 8% of the phenotypic variance in cholesterol levels in the general population (Sing. Am J Hum Genet-85).

ApoE and receptor binding

The change from a positively charged arginine to a neutral cysteine causes a dramatic decrease in the receptor-binding capacity of the E2 allele, to the level of only 1% of that found in the E3 and E4 alleles, as a result of an alteration in the structure of the receptor-binding domain (Weisgraber. J Biol Chem-82). ApoE4 binds normally to the LDL receptor but is associated with high levels of LDL-cholesterol (Davignon. Arteriosclerosis-88). Reduced delivery of cholesterol to the liver through apoE2-containing lipoproteins could up-regulate LDL receptors, thereby enhancing LDL clearance. On the other hand, although apoE3 and apoE4 bind to their receptors almost equally well, apoE4 appears to be metabolized more rapidly than apoE3. This could down-regulate LDL receptors, resulting in higher serum cholesterol levels (Davignon. Arteriosclerosis-88). In Finland the serum lipid levels have been shown to occur in the following descending order of apoE phenotypes: E4/4 > E4/3 > E3/3 > E3/2 > E4/2 > E2/2 (Ehnholm. J Lipid Res-86). Gender and diet also influence the effect of the apoE alleles on plasma lipoproteins (Lehtimäki. J Lipid Res-95).

ApoE and efficiency of cholesterol absorption

The intestinal efficiency of cholesterol absorption is related to the apoE phenotype, so that subjects having the apoE2 isoform absorb less cholesterol than subjects with the apoE4 isoform, while subjects with the apoE3/3 phenotype fall in between (Kesäniemi. J Clin Invest-87). Differences in intestinal cholesterol absorption could thus also contribute to the variability of serum total and LDL cholesterol levels between the apoE phenotypes. Subjects with the E4 allele are usually, although not consistently, responders to dietary modifications of cholesterol and fat, while those with the E2 allele are not (Miettinen. Lancet-88). Further, E4 is associated with increased and E2 with decreased prevalence of gallstones (Niemi. Gut-99), with gallstone recurrence after therapy, and with a higher cholesterol content of gallstones (Juvonen. Gastroenterology-93). These findings may be related to the role of apoE in the delivery of cholesterol to the liver and its possible role in cholesterol absorption and bile secretion (Miettinen. Arterioscler Thromb-92).

ApoE and arteriosclerosis and CHD

ApoE is important in the regulation of lipid metabolism in the arterial wall. It seems that apoE reduces atheroma formation via its role in cholesterol efflux (Lin. J Lipid Res-98). ApoE is secreted by macrophages in the arterial wall, and is located on the surface of macrophages and in the extracellular matrix surrounding them (O'Brien. Am J Pathol-94). Macrophage-derived apoE, which amounted to only 5% of normal plasma levels, restored the cholesterol efflux capacity of apoE-deficient plasma (Zhu. PNAS-98). ApoE 2/2 macrophages have a lower apoE secretion rate than E 3/3 or E 4/4 phenotypes (Cullen. J Clin Invest-98). However, apoE4 is associated with the most severe lesions (Hixson. Arterioscler Thromb-91), and among angiographically verified coronary patients the prevalence of apoE4 is increased (Kuusi. Arteriosclerosis-89). In a comparison of nine populations, there was an average increase of 0.11 mmol/L in total cholesterol and an increase of 0.024% in CHD mortality for each percent of increase in E4 allele frequency (Stengard. Hum Genet-98). Further, a meta-analysis found that the odds ratios for Alzheimer's disease, compared with E3/3 subjects, were 14.9 for subjects with the E4/4 genotypes, respectively (Farrer. JAMA-97).

As an increased level of LDL is an independent risk factor for CHD and apoE4 is associated with increased LDL, it is possible that the E4 allele is itself a risk factor for CHD. In a meta-analysis, the E4 allele was associated with a mildly increased risk for CHD compared with E3 (odds ratio = 1.3) (Wilson. Arterioscler Thromb Vasc Biol-96). About this association, however, there have been differing conclusions, even when the subjects came from the same region. A 3.5-year prospective study of 1067 elderly Finns failed to find an association between the E4 allele and CHD (Kuusisto. Arterioscler Thromb Vasc Biol-95), while a 5-year study of 666 elderly Finnish men found a twofold higher frequency of the E4 allele among those who died from CHD (Stengard. Circulation-95).

ATHEROSCLEROSIS

Justification for countless nutritional studies is the argument that nutrition has a strong influence on atherosclerosis and further on the risk of CHD. Therefore, there are numerous recommendations concerning diet, such as the often repeated statement that one should avoid eating cholesterol (no eggs, etc.). Because of the many dietary recommendations (partly conflicting, mostly based on unpowered study designs concerning CHD risk) it is important to make a thorough examination of atherosclerosis before recommending different diets.

Atherosclerosis is a slowly developing and relatively benign disease. Sometimes it changes suddenly, resulting in severe life-threatening acute myocardial ischemia (Burke. NEJM-97). In general, the angiographic evidence of the severity of the stenosis does not correlate with the physiologic and clinical effects. Coronary blood flow in the basal state and the coronary flow reserve correlate poorly with the severity of the stenosis (White. NEJM-84, Uren. NEJM-94). Studies based on angiography (DeWood. NEJM-80), autopsy (Davies. NEJM-84), and angioscopy (Sherman. NEJM-86) have shown that the key event that causes the change in symptoms is the formation of a coronary thrombus superimposed on a atherosclerotic plaque. Thrombus formation at the site of plaque rupture is thus the final step in the process leading to an acute ischemic syndrome (DeWood. NEJM-80).

Soft plaques, which cannot be seen angiographically, are prone to rupture and result in infarction (Castelli. Am J Cardiol-98). These plaques are large confluences of fat pushed out from the lumen of the artery, which have not yet become organized with large amounts of fiber and cells, so that they can hardly be seen angiographically. Examination of atherosclerotic plaques from 85 patients who had died as a result of coronary thrombosis revealed evidence of plaque rupture in 83% of them (Richardson. Lancet-89). Many vulnerable plaques are invisible angiographically because of their small size and compensatory vascular remodeling.

Atherosclerosis is at least partly an inflammatory disease and is not simply the result of accumulation of lipids (Ross. Nature-93). The cellular interactions in atherogenesis are fundamentally no different from those in chronic inflammatory-fibroproliferative diseases such as cirrhosis or rheumatoid arthritis (Ross. NEJM-99). Atherosclerosis causes damage not only to the vessels of the heart; but also to other vessels like the carotid arteries (O'Leary. NEJM-99).

Inflammation is of first importance and therefore C-reactive protein (CRP), a sensitive marker of the acute-phase response to infectious agents and tissue damage, is elevated in serum. CRP accumulates in the atherosclerotic arterial wall, suggesting a local inflammatory event. Of the 12 markers of atherosclerosis (including total cholesterol, LDL- and HDL-cholesterol) measured in previously healthy patients, CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events (Ridker. NEJM-2000). Addition of CRP to standard lipid screening would generate an improved method for identifying persons at high risk for future cardiovascular events (Lindahl. NEJM-2000, Ridker. NEJM-2001). Because inflammation has a central role in both the initiation and the progression of atherosclerosis, anti-inflammatory agents, such as aspirin, are of value in the prevention of cardiovascular disease (Alexander. NEJM-94), reducing the incidence of acute myocardial infarction in healthy men by 44% (Physicians' Health Study. NEJM-89).

Atherosclerosis is, however, a very complicated process and the most important new cholesterol lowering medicines are statins, which are inhibitors of HMG-CoA reductase (Alberts. Am J Cardiol-88). They induce regression of vascular atherosclerosis as well as reduction of cardiovascular-related morbidity and mortality in patients with and without coronary artery disease (Scandinavian 4S. Lancet-94, Shepherd. NEJM-95). But statins do much more than just lower the serum cholesterol level, like influence critical pathways that regulate plaque stability and thrombosis, and improve endothelial function (Anderson. NEJM-95), and these properties extend beyond LDL cholesterol lowering (Rosenson. JAMA-98).

Atherosclerosis and age

Evidence of focal lipid accumulation in arterial intima was found in human fetal aortas by using immunocytochemistry, which showed focal oxidized lipoprotein accumulations with macrophage foam cells (Napoli. J Clin Invest-97). These changes were present more often when the mothers had high plasma cholesterol and may thus reflect risk factors of the mothers. These earliest lesions, the fatty streaks, are purely inflammatory lesions, consisting of monocyte-derived macrophages, T-lymphocytes, and especially accumulations of small groups of foam cells, i.e. macrophages filled with cholesteryl esters (Ross. Nature-93). The incidence of these lesions was greatest in the first 6 months after birth and declined thereafter (Stary. Am J Clin Nutr-2000).

Thus the prevalence of fatty streaks in early childhood has hardly any relationship with the prevalence of atheromatous plaques in later adulthood (Stary. Am J Clin Nutr-2000). In fact, girls have more aortic fatty streaks and higher serum cholesterol values in childhood than boys, but fewer plaques in adulthood and less coronary heart disease. The location and morphology of fatty streaks change with age, suggesting that these lesions routinely resolve without sequelae (Sloop. Atherosclerosis-98, Sloop. Atherosclerosis-99).

During the second decade of life, more than one half of all children have coronary lesions characterized by accumulations of macrophage foam cells, lipid-containing smooth muscle cells, and thinly scattered extracellular lipid, and about 10% have larger accumulations of extracellular lipid resembling atherosclerotic plaques (Stary. Arteriosclerosis-89). In an analysis of coronary arteries from children at the time of accidental death, there was a continuous age-related increase in LDL-cholesterol-derived esterified cholesterol in the arterial wall (Ylä-Herttuala. Arteriosclerosis-86). In fact, in young adults, serum total cholesterol and LDL-cholesterol levels show a positive association with the extent of aortic fatty streaks (Newman. NEJM-86), and of 21-25-year-old adults in the Bogalusa Heart Study, 50% had at least one fibrous plaque lesion in their coronary arteries (Berenson. NEJM-98). Despite of this, the factors that increase the extent of lesions in arteries at puberty have not been identified, because no increase in blood lipids is associated with puberty.

Because the lipid cores of atheromas may be an underlying cause of lesion rupture, hematomas, and thrombosis, and because their development begins soon after puberty, it would be prudent to attempt to lower the influx of excessive atherogenic lipoproteins into the arterial wall by that age (eg. diet, physical activity and weight control) (Stary. Am J Clin Nutr-2000).

Familial hypercholesterolemia

If, as a result of genetic defects, LDL receptor function is diminished, cholesterol builds up in plasma and atherosclerosis ensues. Familial hypercholesterolemia (FH) heterozygotes have a single copy of a mutant LDL-receptor gene resulting in an increased number of LDL particles in the plasma. These subjects are at higher risk for CHD; among people under age 60 who suffer from myocardial infarction, about 5% have the heterozygous form of familial hypercholesterolemia, a 25-fold enrichment over the incidence in the general population (Nikkilä. Lancet-73).

Over 200 mutations of the LDL receptor gene have been reported. Their molecular diagnosis is difficult unless a single mutation occurs with high frequency because of the founder effect. This is the case in Finland in North Karelia, where two mutations account for about 90% of the receptor defects causing FH (Koivisto. J Lipid Res-93, Koivisto. Am J Hum Genet-95). The frequency of heterozygotes is about 1 in 500, and that of homozygotes 1 in a million. In fact, patients with two mutant FH alleles are referred to as homozygotes, although most have 2 different mutations at the LDL receptor locus.

In Finland, the prevalence of FH in cases of sudden death was analyzed in a series of 149 such patients who had suffered early (≤ 50 years) unexpected cardiac death from CHD. Three individuals (2%) had molecularly defined heterozygous FH, and, of the 67 subjects who had demonstrable acute myocardial infarction, heterozygous FH was present in two (3%). Considering that these two FH mutations amount to two-thirds of the FH cases in Finland, the overall prevalence of FH underlying early cardiac deaths caused by acute myocardial infarction may be estimated to be in the range of 3-5% (Vuorio. Forensic Sci Int-99).

Familial hypercholesterolemia in children

FH is expressed at birth and early in life as significant elevations of total cholesterol and LDL-cholesterol (Kwiterovich. Am J Cardiol-93). Two-thirds of 29 youths (aged 11-23 years) with FH already had coronary calcification, as determined by electron beam tomography (Gidding. Circulation-98). Typically, the plasma cholesterol concentration is above 9 mmol/L, and LDL-cholesterol is above 6.5 mmol/L. HDL-cholesterol tends to be lower than normal, and the triglyceride concentration is normal unless a second genetic defect is present. A plasma cholesterol level or LDL-cholesterol level above the 95th percentile may be the only marker of disease in childhood and adolescence, in addition to the familial context.

In a recent study, many of the parents of 10-17-year-old heterozygous FH children had symptoms of CHD (Stein. JAMA-99). Such symptoms were present in 59% of fathers with FH and in 19% of mothers with FH. The mean age at onset of CHD in the affected parents was 37 years. Eight fathers with FH (20%) had died of CHD, the mean age at death being 39 years. None of the mothers with FH had died of CHD. The early onset of clinically manifest CHD in these patients, sometimes in their 30ies, highlights the need to treat patients with heterozygous FH, especially boys, early and aggressively. To delay effective lipid-lowering therapy in these high-risk groups until early adulthood easily results in their being lost to the medical system until they present with CHD or sudden death.

The reason for screening cholesterol levels in children is that in patients with heterozygous FH medical therapy should already be initiated in childhood, given the tendency of these individuals to experience CHD events as early as the third decade of life (Rifkind. JAMA-99). Diet is not very effective in these patients because type II diets decrease their total cholesterol only with some 10-15% (Connor. Arteriosclerosis-89). Recently it was shown that treatment with lovastatin for 1 year was effective in lowering LDL-cholesterol without apparent effect on sexual maturation in boys aged 10-17 years with heterozygous FH (Stein. JAMA-99). LDL-cholesterol levels of these patients receiving lovastatin decreased significantly by 17%, 24% and 27% with doses of 10, 20 or 40 mg/day, respectively. In another statin study with pravastatin, 72 children (66% females) ranging in age from 8 to 16 years, LDL-cholesterol reductions ranged from 23% to 32% (Knipscheer. Pediatr Res-96), and when lovastatin was given to 69 adolescent boys receiving 10-40 mg/day, LDL-cholesterol reductions were 21-36%, without any safety concerns being reported (Lambert. Pediatrics-96).

Risk factors for CHD in children in addition to cholesterol

When considering large scale screening programs for high cholesterol values in children, one have to take into account also risk factors other than lipid values. Overweight children and adolescent are at increased risk of early death and morbidity (Must. NEJM-92). A study involving 2990 subjects followed for 57 years revealed that a childhood body mass index above the 75th percentile was associated with a twofold increase in the risk of ischemic heart disease compared with a body mass index between the 25th and the 49th percentiles (Gunnell. Am J Clin Nutr-98). The relative risk of CHD morbidity and mortality increases with an increasing body mass index at the age of 18 years, each kilogram of weight gain from this age on being associated with a 3.1% higher relative risk of CHD in women (Manson. NEJM-95). Unfortunately the prevalence of obesity (body-mass index > or = 30 kg/m2) has increased substantially from 25.3% (in 1995) to 32.8% (in 2000) among adult CHD patients in Europe (EUROASPIRE. Lancet-2001). The risk of CHD with an increasing body mass index is highest in young adults and seems to attenuate with aging (Stevens. NEJM-98).

Cigarette smoking is an very important risk factor for CHD (Howard. JAMA-98). Early atherosclerotic lesions in youths (aged 15-34, n = about 3000 persons) have been reported in association with cigarette smoking and high cholesterol levels (PDAY. JAMA-90). In both sexes, current smokers have higher rates of cardiovascular mortality than those who have never smoked, with a relative risk of 2.0 (LaCroix. NEJM-91). Campaigns against cigarette smoking should be very active in adolescents, because smoking is a habit that usually begins in that age group.

Risk factors tend to cluster in individual subjects and a greater number of risk factors predicts more extensive lesions. This relation appears to be curvilinear, indicating a synergistic effect of multiple risk factors. The relation of the risk factors to the increasing extent of raised lesions in arteries at approximately the age of 25 years suggests that risk factor modification should be initiated by about the age of 15-20 years. This conclusion is supported in part by reports that the serum cholesterol level at the age of 22 years predicts the risk of CHD in middle age (Klag. NEJM-93).

Children learn from their parents and tend to emulate the lifestyle practices they see in their homes and schools. Since many lifestyle changes are difficult to achieve in adulthood and even harder to maintain over the long term, it seems reasonable to attempt to alter these risk factors early in life.

DIET

Human milk

Human milk is a complex biological fluid composed of thousands of constituents in several compartments: an aqueous phase with true solutions (87%), colloidal dispersion of casein molecules (0.3%), emulsion of fat globules (3-5%), fat-globule membranes, and live cells (Picciano. Pediatric Clinics-2001). The disaccharide, lactose, which is second only to water as a major constituent of human milk, is present at an average concentration of 68 g/L (Jensen. Lipids-99). Milk lactose content increases steeply in early lactation, and it is one of the most stable constituents of human milk.

Dietary fat during the first 6 months of life is controlled in infants fed human milk and infant formulas, which both provide about 50% of energy as fat. A review of studies from Europe and North America, however, indicated wide variation in the fat content of diets of children 6-36 months of age; mean dietary fat ranged from 27% to 42% of energy (Michaelsen. Eur J Clin Nutr-95). Despite this variation, no association between fat intake and infant growth has been found, in fact, in STRIP baby trial moderately restricted fat intake (25-30% of energy) was not associated with compromised infant growth (Niinikoski. Pediatrics-97). Fat is also the most variable constituent of human milk, and the mechanisms for all observed changes are not well understood. Circulating lipids in mothers, which are a reflection of the diet and adipose stores, are the main substrates for milk fat. When the maternal diet is low in fat content and rich in carbohydrates, mammary de novo synthesis is increased, and milk rich in medium-chain (C6-C10) and intermediate-chain (C12-C14) fatty acids are secreted (Jensen. Lipids-99).

Milk-fat globules also protect infants from infection by two mechanisms: the fat globule membrane glycoconjugates act as specific bacterial and viral ligands, whereas the digestive product of the core triglycerides, free fatty acids have a detergentlike lytic action on enveloped viruses, bacteria, and protozoa (Hamosh. Semin Perinatol-99). Although products of lipolysis are qualitatively similar among formula-fed and breast-fed infants, quantitatively, breast-fed infants benefit from higher rates of gastric lipolysis and duodenal lipolysis associated with the structure of milk-fat globules and the presence of milk bile salt-dependent lipase, respectively (Hamosh. Pediatric Clinics-2001).

The high long-chain polyunsaturated fatty acids secreted in the milk of women who deliver prematurely may reflect the enhanced need for these essential fatty acids by premature infants. The assimilation of fatty acids by young infants is crucial not only for energy to support growth but also for the synthesis and development of retinal and neural tissues. Human milk is rich source of the essential fatty acids, linoleic acid (C18:2n-6, 8-17%) and alpha-linolenic acid (C18:2n-3, 0.5-1.0%), and the long-chain derivatives, arachidonic acid (C20:4n6, 0.5-0.7%) which is the major n-6 fatty acid of neural tissue, and docosahexanoic acid (C22:6n-3, 0.2-0.5%) which is the major n-3 fatty acid of neural tissue and comprises as much as 40% of the total fatty acids of retinal photoreceptor membranes (Martinez. J Pediatr-92).

Docosahexanoic acid, a long-chain polyunsaturated fatty acid present in large quantities in the brain and retina, is present in human milk but not in commercial formula. A large meta-analysis indicated that, after adjustment for appropriate key cofactors, breast-feeding was associated with significantly higher scores for cognitive development than was formula feeding (Anderson. Am J Clin Nutr-99). The meta-analysis also found that duration of breast-feeding correlated with developmental and cognitive outcome, the mean difference increased as the duration of breast-feeding increased (Heird. Pediatric Clinics-2001). Although children who are breast-fed have better neurodevelopmental outcomes, whether this is a biological or nutritional effects, an environmental effect, a genetic effect, or some combination of these factors is unclear (Reynolds. Pediatric Clinics-2001).

Dietary cholesterol

Prospective cohort studies on the relationship between dietary cholesterol and the risk of CHD have been inconsistent, an association being found in some (Shekelle. NEJM-81), but not in most of the studies (Kushi. NEJM-85, Hu. NEJM-97). In metabolic studies conducted in humans, dietary cholesterol raises the levels of total and LDL-cholesterol in the blood (Hegsted. Am J Clin Nutr-65, Keys. Am J Clin Nutr-66, Caggiula. Am J Clin Nutr-97), but the effects are relatively small compared with those of saturated and trans fatty acids (Clarke. BMJ-97, Howell. Am J Clin Nutr-97). Studies based on monitoring plasma cholesterol levels have shown that individuals vary widely in their responses to dietary cholesterol (Hegsted. Am J Clin Nutr-86).

Additionally, the plasma LDL-cholesterol response to dietary cholesterol, as well as to dietary fatty acids, is influenced by a large number of genetic factors such as apoE genotypes (Miettinen. Lancet-88). However, diet is not the only factor influencing serum lipids, as dieting failed to lower LDL-cholesterol concentrations in individuals who took little exercise (Stefanick. NEJM-98). In metabolic ward studies, mean reductions of LDL-cholesterol with a stringent diet have been approximately 15%, but there is wide individual variation in the response (Schaefer. Am J Clin Nutr-97). In the outpatient setting, only 5% reductions in LDL cholesterol have been reported after dieting (Hunninghake. NEJM-93). The responsiveness to dietary therapy is strongly related to compliance.

The Dietary Intervention Study in Children (DISC) assessed the efficacy and safety of lowering dietary intake of total fat, saturated fat, and cholesterol to decrease LDL-cholesterol levels in children (DISC. JAMA-95). Prepubertal boys and girls were randomized into an intervention group and a usual care group. The diet provided less than 150 mg of cholesterol per day. At 3 years, the levels of LDL-cholesterol was 0.09 mmol/L lower in the intervention group than in the control group, and in the DISC study the factor associated with the greatest difference in LDL-cholesterol was sexual maturation (Kwiterovich. Circulation-97). According to the results of the DISC study, the current public health recommendations for moderately lower fat intakes in children during puberty may be followed safely (Obarzanek. Pediatrics-97), but the cholesterol lowering of LDL-cholesterol achieved by intervention was only less than 0.1 mmol/L.

Raising cholesterol intake from 200 to 400 mg/day (=100%) has been estimated to increase the serum cholesterol by an average of about 0.013 mmol/L (=2%) (Keys. Metabolism-65). To avoid elevations in blood cholesterol and to reduce the risk of CHD, the public has been advised to consume no more than 300 mg of cholesterol per day and to limit the consumption of eggs, which contain about 200 mg of cholesterol per egg. However, eggs contain many other nutrients besides cholesterol, including unsaturated fats, essential amino acids, and B vitamins. The problem with egg consumption is that it is positively associated with smoking, lower physical activity, and a generally unhealthy eating pattern (i.e. bacon and eggs). This speaks of the importance of considering the overall eating pattern when examining the effects of cholesterol consumption. In a study of about 117,000 people it was found that the consumption of up to 1 egg per day did not have an impact on the risk of CHD among the participants (Hu. JAMA-99).

An interesting question is whether the addition of cholesterol to formula at a concentration mimicking human milk would influence endogenous cholesterol synthesis or circulating cholesterol levels (Bayley. Ped Res-98). The addition of cholesterol to regular formula elevated total and LDL-cholesterol but did not suppress the high fractional synthetic rate observed when the low-cholesterol formula was fed. These findings suggest that factors in human milk other than cholesterol may be responsible for the regulation of cholesterol synthesis. The form of cholesterol may be important, human milk cholesterol is incorporated into a membrane matrix encompassing the fat globule, rather than being present as the crystalline pure cholesterol form. This envelope contains phospholipids, long-chain polyunsaturated fatty acids, glycolipids, and multiple other specialized membrane glycoproteins (Jensen. Lipids-99).

It must be emphasized that the human body synthesizes all the cholesterol it needs from acetyl CoA. In general, the larger the amount of dietary cholesterol absorbed, the smaller the rate of biosynthesis of cholesterol (Grundy. Arteriosclerosis-88). In most people this homeostatic control is nearly perfect, but in others the reduction in the biosynthesis of cholesterol with increased dietary input is imperfect and LDL-cholesterol levels increase. In a

study to examine the effects of dietary fat and cholesterol on cholesterol homeostasis in man about 70% of the subjects compensated for the increased cholesterol intake by decreasing cholesterol fractional absorption and/or endogenous cholesterol synthesis (McNamara. J Clin Invest-87). When an increase in plasma cholesterol levels was observed there was a failure to suppress endogenous cholesterol synthesis. Plasma cholesterol levels were more sensitive to dietary fat quality than to cholesterol quantity. These results demonstrated that the responses to dietary cholesterol and fat are highly individualized and that most individuals have effective feedback control mechanisms.

Dietary plant sterols

Sterols are an essential component of cell membranes, and both animal and plants produce them. Cholesterol is exclusively an animal sterol, while there are more than 40 plant sterols; the most abundant are sitosterol, stigmasterol and campesterol. Plant sterols are present in the Western diet in amounts almost equal to dietary cholesterol intake, that is 160-360 mg per day (Miettinen. Am J Epidemiol-90). Stanols are saturated sterols, so they have no double bonds in the sterol ring. A novel development has been to convert plant sterols in to the corresponding stanols and esterify them to fat-soluble forms (Miettinen. Curr Opin Lipidol-99). Stanol esters reduce the efficiency of cholesterol absorption by up to 65%, increase elimination of cholesterol in feces, and stimulate cholesterol synthesis. Plant stanol esters can easily be consumed in soluble form in different fat-containing food constituents during normal food intake and have a potent cholesterol-lowering effect (Miettinen. NEJM-95). Lowering cholesterol with stanols amounts to approximately 10% for total and 15% for LDL cholesterol.

In children with heterozygous familial hypercholesterolemia, sitostanol has been found to be effective in reducing LDL-cholesterol by up to 33% and has been suggested as the treatment of choice (Becker. J Pediatr-93, Gylling. J Lipid Res-95). Plant stanol ester margarine lowers even serum total (5.4%) and low-density lipoprotein cholesterol (7.5%) concentrations of healthy children (Tammi. J Pediatr-2000), but had no effect on endogenous cholesterol synthesis (Tammi. J Nutr-2001). If sitostanol is free of adverse effects in the long run, margarine containing sitostanol, may be beneficial for young members of high-risk populations.

Saturated fatty acids

Dietary fats are classified on the basis of the number and type of chemical bonds that they possess. Saturated fatty acids contain no double bonds and vary in chain length from 6 to 18 carbon atoms. The most prevalent saturated fatty acid in the diet is palmitic acid (16:0), followed in order of abundance by stearic (18:0), myristic (14:0), and lauric (12:0) acids.

Some individuals are especially sensitive to the cholesterol-raising action of saturated fatty acids, which leads to hypercholesterolemia (Grundy. Am J Clin Nutr-88). However, not all saturated fatty acids affect total cholesterol concentrations in the same manner. Stearic acid has little effect on plasma cholesterol concentrations, whereas myristic and palmitic acids have been reported to be the most potent fatty acids with regard to cholesterol-raising potential (Bonanome. NEJM-88). It is postulated that the relatively neutral effect of stearic acid is the result of its rapid conversion to oleic acid (18:1), a monounsaturated fatty acid in the body (Grundy. J Lipid Res-90).

A high intake of saturated fatty acids increases serum cholesterol, partly by suppressing LDL-receptor activity (Spady. PNAS-85). However, when diets containing 15% or 30% of fat were compared in a 1-year study of free-living subjects, no difference in LDL-cholesterol lowering was observed (Brown. J Am Diet Assoc-84). Similarly, in a 2-month, cross-over design feeding study, no difference in LDL-cholesterol was observed between diets containing 20% and 30% fat (Grundy. JAMA-86). In a large five year study in Finland the mothers and fathers of the intervention children used less butter, more margarine and more skim milk (less total fat, less saturated fat and more polyunsaturated fat) than parents of the control children, the serum cholesterol concentration of the intervention mothers was slightly lower than that of the control mothers (4.86 and 5.09 mmol/L, respectively), while the values of the intervention and control fathers showed no differences (Lagstrom. Eur J Clin Nutr-99). Japanese children, on the other hand, consume less fat than children in USA, but despite of this lower consumption, it is striking that cholesterol concentrations among children in Japan have surpassed those of USA children (Couch. Am J Clin Nutr-2000).

If saturated fats were replaced by monounsaturated or polyunsaturated fats, the fasting serum total and LDL-cholesterol levels could possibly be decreased without significantly decreasing the total fat intake. However, replacing fat with fat does not take into account the several disadvantages of a high-fat diet. Postprandial lipemia is atherogenic, and obesity is a problem. Further, the advantages of a lower-fat diet in the prevention of cancer and the control of hypertension are lost when saturated fat is replaced by other fats rather than by vegetable foods (Appel. NEJM-97).

In the Seven Countries Study, total fat intake ranged from 40% of calories in Finland to less than 20% of calories in Japan, with saturated fat intake ranging from 20% to less than 10% of calories. The study showed a correlation between saturated fat intake and CHD mortality (r=0.84) (Kromhout. Prev Med-95). In this classic study, however, CHD mortality was six times as high in Finland as in Japan. Reliable information on causes of death is essential for all epidemiologic studies concerning CHD mortality. The Global Burden of Disease Study made corrections for miscoding of CHD to estimate regional cause-of-death patterns (Murray. Lancet-97). Coding of cardiovascular disease in Finland did not change at all; whereas the figures for Japan changed considerably. Before correction, the ratio of the ischemic heart disease mortality rates in Finland to Japan was 6 to 1, but after correction the ratio was 2 to 1.

Monounsaturated fatty acids

The major monounsaturated fatty acid in the diet is oleic acid, which contains one double bond at the number 9 carbon (18:1n9cis). Monounsaturated fatty acids have a hypocholesterolemic effect when substituted for dietary saturated fatty acids (Dreon. JAMA-90). Oleic acid reduces LDL-cholesterol levels but not HDL-cholesterol levels (Grundy. NEJM-86). This is possibly one of the reasons why age-adjusted rates of CHD and total mortality are low in Mediterranean countries where olive oil, mainly oleic acid, is consumed in large amounts and total fat intake is high. It has been suggested that the majority of saturated fats should be replaced by oils high in monounsaturated fats such as rapeseed oil and olive oil.

Some experts recommend replacement of saturated fats with monounsaturated fats, but there is controversy in regard to the recommendation of dietary fat restriction for the prevention of CHD (Katan. NEJM-97). The controversy involves whether a diet in which the total fat content is held constant but which is relatively enriched in monounsaturated fatty acids offers better protection against CHD than a low-fat diet. This argument has stemmed primarily from the observation that low-fat diets often reduce both HDL as well as LDL cholesterol concentrations (Knopp. JAMA-97). However, a study of coronary artery atherosclerosis in monkeys found that monounsaturated fat failed to provide protection against the development of atherosclerosis even when that the monounsaturated fat diet was associated with the most favorable plasma lipoprotein profile, specifically, a low LDL to HDL cholesterol ratio (Rudel. NEJM-98).

Polyunsaturated fatty acids

Dietary polyunsaturated fatty acids are subclassified as n-6 and n-3, indicating the location of the carbon involved in the first double bond. The major n-6 fatty acid in the diet is linoleic acid (18:2n-6), and is not synthesized by the body and is therefore termed as essential fatty acid. Food sources rich in linoleic acid include vegetables and vegetable oils. Linoleic acid clearly has a hypocholesterolemic effect, reducing both LDL and HDL cholesterol concentrations, but part of the lowering of total cholesterol levels appears to be due to reduced HDL-cholesterol levels (Mensink. NEJM-89). Also in infants fatty acid intake plays a predominant role in determining total and LDL-cholesterol concentrations. Infants receiving diets with high concentration of polyunsaturated fats had highest LDL-receptor activity of the lymphocytes, while human milk group had the lowest LDL-receptor activity (Uauy. Am J Clin Nutr-2000).

The proportions of linoleate (18:2n-6) and alpha-linolenate (18:3n-3) have been found to be similar in preterm and term milk and show an increasing trend from transitional (8.7-9.9% and 0.9-1.1% of total fatty acids, respectively) to mature milk (9.9-11.8% and 1.2-1.5%, respectively) (Luukkainen. J Ped Gast Nutr-94). The proportions of the major long-chain polyunsaturated fatty acids, arachidonate (20:4n-6), and docosahexaenoate (22:6n-3), were highest at 1 week and decreased thereafter in both types of milk. Thus in long-term lactation, preterm human milk provides a significantly higher relative supply of long-chain polyunsaturated fatty acids than term human milk. This higher content may be of special benefit to the development of a preterm infant, since they are vital for brain development (Lanting. Lancet-94).

A strong inverse association has been found between the intake of polyunsaturated fat and the CHD risk (Shekelle. NEJM-81). However, the epidemiologic evidence supports the findings that high intakes of linoleic acid may not be safe, as they may promote carcinogenesis. In animal studies, dietary linoleic acid has suppressed the immune system (Weyman. Lancet-75), and high intakes of linoleic acid have lowered HDL-cholesterol levels (Vega. J Lipid Res-82), and increased the risk of cholesterol gallstones (Sturdevant. NEJM-73).

Dietary carbohydrate

High-carbohydrate diets that lower serum total cholesterol levels are believed to reduce the risk of CHD. Some experts recommend a low-fat, high-carbohydrate diet because of its beneficial effect on lipids (Connor. NEJM-97), but it is also advantageous for blood-pressure reduction (Appel. NEJM-97). Carbohydrates and monounsaturated fatty acids, such as oleic acid, reduce LDL-cholesterol levels similarly but, in contrast to high oleic acid diets, high carbohydrate diets lower HDL-cholesterol and raise triglyceride levels (Grundy. NEJM-86). This effect is induced by both sugars and complex carbohydrates such as starch (Mensink. Lancet-87). Further, replacement of fat by carbohydrates has not been shown to reduce the risk of CHD (Sacks. J Cardiovasc Risk-94), because this change lowers the levels of HDL and LDL cholesterol (Mensink. Arterioscler Thromb-92). Most experts agree that a greater amount of plant-derived foods should be consumed to provide an optimal intake of fiber, antioxidants, and other protective factors. Americans have already cut their fat intake of total energy consumption, but the fat has been replaced largely by sugar, not by complex carbohydrates and fiber.

Restricting fat in the diets of children

The proponents of fat-restricted diets for children argue that low-fat diets given in childhood will prevent the development of atherosclerosis in adulthood; however, there is no evidence at the moment that low-fat diets in childhood would prevent atherosclerosis in adulthood. The second argument for low-fat diets for children is that children are conditioned to continue low-fat diets in adulthood. This prediction is not self-evident, as restricting access to palatable foods enhanced the interest of 3- to 5-year-old children in those foods and increased their desire to obtain and consume them (Fisher. Am J Clin Nutr-99). It was concluded that stringent parental controls can potentiate preference for high-fat energy-dense foods, limit children's acceptance of a variety of foods, and disrupt children's regulation of energy intake. Efforts to modify diet in children by intervention in school lunch programs and by family counseling have achieved only moderate results (Niinikoski. Circulation-96, Simell. Am J Clin Nutr-2000).

Health behavior interventions were evaluated, focusing on the school environment and home programs for the primary prevention of cardiovascular disease in 5106 initially third-grade children over three years of follow-up (Luepker. JAMA-96). In intervention school lunches, the percentage of energy intake from fat fell significantly (from 39% to 32%), and vigorous daily activity also increased significantly (59 minutes vs 47 minutes). However, cholesterol levels did not differ significantly between the intervention and control groups.

TRACKING OF LIPIDS

Tracking of cholesterol

The extent to which an individual maintains his position relative to the rest of the population is called tracking. Significant tracking of serum cholesterol levels occurs not only in adults (Davis JAMA-90), but also in children and adolescents (Porkka. Prev Med-91). Confirmation that children who were initially in the extreme high range of serum cholesterol become adults with high serum cholesterol is the basis for cholesterol screening and possible clinical intervention in childhood. Selective cholesterol screening on the basis of family history alone has been found to be neither sensitive nor specific for predicting elevated cholesterol levels in children (Dennison. J Pediatr-89). The purpose of this screening is the prevention of arteriosclerosis, which begins in childhood and depends on serum lipids. In children and young adults, serum total and LDL-cholesterol levels showed a strong positive association with the extent of aortic fatty streaks and a negative association with the HDL:LDL cholesterol ratio (Newman. NEJM-86). Because of the importance of screening, there have been many studies concerning tracking of lipids, even from Finland there are several publications based on a large multicenter study; the Cardiovascular Risk in Young Finns Study (Moilanen. Atherosclerosis-87, Viikari. Prog Clin Biol Res-88, Porkka. Prev Med-91, Moilanen. Am J Epidemiol-92, Porkka. Atherosclerosis-94, Raitakari. J Clin Epidemiol-94, Porkka. J Clin Epidemiol -95).

Tracking analyzed with correlation coefficients

Tracking of serum cholesterol can be examined by noting the correlation coefficients between serum cholesterol levels at different time points or the percentage of children persisting in high or low serum cholesterol percentiles. Correlations for total cholesterol decrease with increasing length of follow-up; the rate of this decrease is similar in children and in adults. The correlation between total cholesterol values at two testings approximately 6 weeks apart was 0.76 in children and 0.83 in adults (Kwiterovich. Circulation-86). Thus, children may have more short-term variation than do adults, but long-term tracking is similar. Tracking of serum cholesterol does not begin at birth; there is no correlation between cord blood levels and the cholesterol levels later in childhood (Kwiterovich. Lancet-73). After birth, tracking starts to develop and there is a significant correlation (r=0.42) between the serum cholesterol values at 6 months and 1 year of age (Webber. J Chron Dis-80), and between values measured at 6 months and 7 years of age (Freedman. Pediatrics-87). The correlation coefficient improves still further (r=0.46) for values measured at 1 year and 7 years of age (Freedman. Pediatrics-87), and for values measured at the age of 7-8 years compared with adult cholesterol levels (r=0.56-0.64) (Lauer. Pediatrics-88). In a study in Finland, 6-year tracking of serum cholesterol measured from the correlation coefficient was high (r=0.63) for children initially 3-18 years of age (Porkka. Prev Med-91).

Tracking analyzed with percentiles

Tracking of serum cholesterol can also be examined as the percentage of children persisting in the high serum cholesterol percentiles. Several investigators have found that about 40% of the children initially at or above the 90th percentile for total cholesterol are still at or above the 90th percentile at a repeated examination (Laskarzewski. Pediatrics-79, Frerichs. J Chron Dis-79, Webber. J Chronic Dis-83). In a more detailed analysis of children initially found to have serum cholesterol values above the 90th percentile, 43% were still above the 90th percentile, 62% were above the 75th percentile, and 81% above the 50th percentile as young adults 12-16 years later (Lauer. Pediatrics-88). On the other hand, 42% of the children with initial total cholesterol of at least the 95th percentile did not persist in this extreme range at the second visit (Morrison. Metabolism-82); however, the mean total cholesterol in these nonpersisters at retest was approximately at the 90th percentile. At a second visit individuals initially with total cholesterol in at least the 95th percentile, 58% of children persisted above this level (Morrison. Metabolism-82), compared with 68% of the adults (Jacobs. Am J Epidemiol-82).

When the cholesterol values of the children were divided into quintiles, 43% of those children who had cholesterol levels in the highest quintile at the age of 1 year still had levels in the highest quintile at the age of 7 years (Freedman. Pediatrics-87), and in the older children the percentages of children persisting in the high serum cholesterol quintile were 54%, 45%, and 55% at 4, 6, and 9 years after the initial cholesterol determination, respectively.

Tracking and lipoproteins

Tracking of different lipoproteins has been investigated with the correlation of LDL-cholesterol being 0.56 and that of HDL-cholesterol 0.31 between 1 and 7 years of age (Freedman. Pediatrics-87). In older children, the correlation between the initial and the 8-year follow-up levels of LDL-cholesterol was 0.61 and of HDL-cholesterol 0.33 (Freedman. Prev Med-85). In Finland, the 6-year correlation of HDL-cholesterol for children was 0.58 (Porkka. Prev Med-91). The correlations between childhood LDL-cholesterol levels and adult levels were between 0.47 and 0.65 at different ages, but HDL-cholesterol levels did not correlate (Lauer. Pediatrics-88). In children with familial hypercholesterolemia the correlations between follow-up measurements were 0.73 for LDL-cholesterol and 0.55 for HDL-cholesterol (Mellies. Metabolism-85).

When tracking of different lipoproteins is examined as the percentage of children persisting in the high percentiles at successive measurements, 48% of children with high LDL-cholesterol persisted in the highest quintile for several years (Freedman. Prev Med-85). The proportion of children persisting in the highest quintile for LDL-cholesterol for over 8 years increased to 64% when two lipoprotein determinations were required to be high at baseline. The correlation between the average of two HDL-cholesterol determinations at baseline with a follow-up determination several years later ranged from 0.36 to 0.57.

The phenomenon of regression toward the mean

The fact that the serum cholesterol level at the next possible measuring point was lower than the 90th percentile for some of these children is partly due to the phenomenon of regression toward the mean, i.e. the shift toward less extreme values for cholesterol at a second sampling of children initially at the extremes of the distribution (Morrison. Pediatrics-79). Intraindividual variation in serum cholesterol is substantial; some subjects show extreme variability from one blood sample to the next (Hegsted. PNAS-87), but, even so, they may belong to a risk group for CHD in future. The relationship between serum cholesterol and CHD is not a threshold, with increased risk confined to the highest quintiles, but rather is continuously graded (Stamler. JAMA-86). Each 1% rise in cholesterol has been claimed to be associated with an approximate 2% increase in CHD risk. This is an underestimate, however, because of failure to take into account intraindividual variations in cholesterol level, and in fact each 1% rise in blood cholesterol is associated with an approximately 3% increase in risk (Davis. JAMA-90). This means that the cohort of children with initial total cholesterol exceeding the 90th percentile is an elevated risk group.

The importance of initial cholesterol values for subsequent arteriosclerosis and CHD was investigated in a long follow-up study of physicians. The total cholesterol level in young adulthood significantly predicted the relative risk of CHD upon follow-up (Klag. NEJM-93). A total of 1337 medical students at Johns Hopkins Medical School were enrolled. The mean age at the beginning was 22 years and at check-up 60 years. The average of all cholesterol measurements obtained during medical school was used for each participant. During the follow-up, there were 125 cardiovascular disease events, 97 of which were due to CHD.

The serum cholesterol level at baseline was strongly associated with the incidence of events related to CHD. Participants with serum cholesterol levels in the highest quartile at baseline had a markedly higher risk of death during follow-up than those with cholesterol levels in the lowest quartile. The adjusted relative risk for the highest quartile as compared with the lowest quartile of serum cholesterol was 3.6 for total cardiovascular disease, 5.3 for CHD, and 6.0 for myocardial infarction. Those who had a baseline total cholesterol level above 5.4 mmol/L, had 35 years later, a cumulative incidence of CHD of almost 25% compared with approximately 9% in the group with a baseline total cholesterol level below 4.5 mmol/L.

This study demonstrates a strong, graded relation between the serum cholesterol level measured in men early in adult life and the subsequent incidence of CHD in midlife. Almost all the events in this analysis were premature in the sense that over 95% occurred before the age of 65. The high follow-up rate of the cohort and the accuracy of the physicians' self-reports are major advantages.

SCREENING OF SERUM LIPIDS

Cholesterol screening when a parent has a high cholesterol level

The lipid and lipoprotein levels in children are related to the levels in their family members, and there is a strong familial aggregation of total cholesterol, LDL-cholesterol, and HDL-cholesterol in children and parents (Freedman. NEJM-86). Simple linear regression has been widely used to compare serum cholesterol values of children with those of their parents. The mother-pediatric offspring correlation was 0.41 for LDL-cholesterol and 0.30 for HDL-cholesterol, while for fathers it was 0.38 for LDL-cholesterol and 0.20 for HDL-cholesterol (Morrison. Metabolism-82). Mother-pediatric offspring correlations are slightly higher than those for father-pediatric offspring. This suggests a closer sharing of the environment by mothers and their pediatric offspring than by fathers and their pediatric offspring.

As the cut-off point for parental plasma cholesterol is increased, the proportion of children who would be screened decreases, and at the same time the sensitivity with which screening identifies children with high cholesterol values decreases. When the cut-off point for parental cholesterol was ≥6.5 mmol/L, then 15% of children were screened and the sensitivity for identifying children with elevated LDL-cholesterol was 28% (NCEP. Pediatrics-92). The sensitivity no longer decreased after the parental cut-off point was raised above 6.5 mmol/L, because values were often clearly above this limit. This subset is likely to represent the children with inherited high blood cholesterol.

Cholesterol screening with positive family history of CHD

In epidemiological studies, a family history of premature CHD emerges as an independent risk factor even when other risk factors are considered (Colditz. Am J Cardiol-91). When children are screened because they have a positive family history of premature CHD in a parent or grandparent, about one third are found to have elevated plasma lipid or lipoprotein levels (Chase. JAMA-74). Despite this relatively high percentage, many parents do not have CHD on account of their relatively young age, and thus a positive family history alone is inadequate for detecting many of the children with elevated levels of LDL-cholesterol (Dennison. Pediatrics-90). When a parent has angiographically documented coronary artery disease, abnormal lipid profiles have been found in 50% of the offspring (Lee. Pediatrics-86). Offspring of CHD patients have elevated LDL-cholesterol or VLDL-cholesterol (Glueck. Am J Dis Child-74), and additionally low HDL-cholesterol (Lee. Pediatrics-86). The mean HDL/LDL-cholesterol ratio was also lower in the offspring of patients with myocardial infarction (Pometta. Atherosclerosis-80).

In the Newcastle Family History Study, the risk ratios for an acute coronary event, adjusted for proband age and sex, ranged from 2.7 for the simplest definition (two or more first-degree relatives with CHD at any age) to 5.4 for the most stringent definition (two or more first-degree relatives with CHD before the age of 55 years) (Silberberg. Am J Epidemiol-98). The results of the Bogalusa Heart study also showed that young adult offspring with a parental history of heart attack had significant elevations of total cholesterol, LDL-cholesterol, VLDL-cholesterol, and glucose after the age of 17 years, regardless of weight (Bao. Circulation-95). A familial propensity for CHD may result not only from genetic factors but also from nutritional practices, and lifestyle characteristics, or their combinations.

Families with early CHD have also been found to have abnormalities in the levels of major apoproteins (Durrington. Lancet-88). Children of fathers with CHD had lower apoA-I and higher apoB levels than controls at any age, but did not have significant differences in lipoprotein levels (Freedman. NEJM-86). The offspring of men with angiographically documented CHD had higher total cholesterol, LDL-cholesterol, and apoB, and lower HDL-cholesterol and apoA-I than controls (Van Stiphout. Atherosclerosis-86). These studies suggest that apoB and apoA-I levels in children may be even better predictors of later CHD risk than lipoprotein levels.

When premature atherosclerotic events are found in a family, the children should be examined for lipoprotein cholesterol disorders (Am Acad Ped. Pediatrics-89, Salo. Duodecim-94). However, the positive family history is frequently inaccurate and has a low predictive value (Dennison. J Pediatr-89). It must be emphasized that the numerous risk tables and computer programs based on the Framingham data do NOT take into consideration a family history of CHD.

Screening of family members of dyslipoproteinemic children

The prevalence of CHD in adult relatives of children with high cholesterol levels is significantly higher than in relatives of children with normal cholesterol levels (Schrott. Circulation-82, Moll. Circulation-83). Elevated total and LDL cholesterol levels have also been found in parents and grandparents of children with elevated cholesterol levels (Schrott. Circulation-79, Morrison. JAMA-83). CHD-combined morbidity and mortality were 1.8 times higher in grandfathers of children with high total cholesterol than in those of those children with average total cholesterol (Moll. Circulation-83). The rate of CHD in fathers of children with LDL-cholesterol or VLDL-cholesterol levels above the 75th percentile in two consecutive determinations was 2.2 times higher than in fathers of children with LDL-cholesterol or VLDL-cholesterol levels above the 75th percentile on only the initial determination (Freedman. Prev Med-85).

Recommendations for cholesterol screening of young adults

In young adults it has been suggested that total cholesterol levels should be measured at least once every 5 years in all adults aged ≥20 years, including young adult men aged 20-35 years and premenopausal women (aged 20-45 years), and that HDL-cholesterol levels be recorded at the same time (Cleeman. Circulation-97).

In contrast, the American College of Physicians has suggested that adults (young adult men aged 20-35 years or premenopausal women aged 20-45 years) should not be screened at all, a view based on concern regarding the effects and expense of life-long therapy in this population (Am College. Ann Intern Med-96). Further, those under 50 years old who are free of vascular disease are unlikely to run a risk of coronary death of 1.5% or more per year at any realistic cholesterol concentration. This feature underlines the caution against the use of lipid-lowering agents in younger subjects and sets out the reason explicitly. Since no randomized prospective trials have assessed long-term lipid-lowering therapy in this age group, no evidence-based recommendation can be made. However, the recommendation to begin screening at age 20 years would allow more modest intervention, such as diet and weight loss, to be used in some individuals and also would allow earlier identification of familial hypercholesterolemia (Ansell. JAMA-99). Despite of these advantages of screening, only less than 20% of patients having a premature CHD, had their family members screened (Swanson. Am J Prev Med-2001). This study demonstrated that physicians do not appear to follow national recommendations for the screening of family members of their high-risk patients (NCEP Expert Panel. JAMA-2001).

Therefore, if one wants to accurately identify all the patients at high cardiovascular risk, universal blood lipid screening above the age of about 45 years is the price to pay (Jackson. BMJ-2000). Selective screening will be relevant only in younger people (Salo. Duodecim-94). One should initially focus on identifying the high risk patients, whom we all agree should be treated. The issue is not whether everyone above a given threshold is identified but whether their identification is worth the additional effort. Savings from selective screening will be attained only in younger people. Selective screening may miss some people with extremely high lipid concentrations resulting from familial hyperlipidemia. The value of detecting these uncommon individuals needs to be weighed against the additional cost, resources, and harm from labeling (when “well” people become “patients”) as a result of general screening (Wallis. BMJ-2000).

When the Human Genome Project is completed, it should become possible to genotype people at risk-factor loci and thereby divide patients into subgroups for whom we can tailor specific preventive and therapeutic approaches, such as vascular gene transfer, which potentially offers new treatments for cardiovascular diseases (Ylä-Herttuala. Lancet-2000). However, most cases of hypercholesterolemia and CHD result from the interaction between multiple genes and environmental influences and are difficult, if not impossible, to predict.

AIMS OF THE STUDY

The aims of the present study were:

I. To follow exclusively breast-fed (high cholesterol) and formula fed (low cholesterol) infants during their first year of life and to analyze the effects of these diets on serum total cholesterol and lipoprotein concentrations.

II. To determine how early the tracking of blood lipid levels becomes established, and how the different dietary regimens during the first year of life influence tracking.

III. To establish the role of the apoprotein apoE phenotype in determining serum cholesterol in children fed exclusively high cholesterol human milk, and in children fed low cholesterol fat formula.

IV. To investigate how the different apoE phenotypes influence the tendency to maintain the relative cholesterol level (tracking) over the first 11 years of life.

V. To analyze correlations between lipids of child and parents, and to visualize the relation between the serum lipid values of the child and the parents with a three-dimensional regression model.

SUBJECTS and METHODS

SUBJECTS

Children

This investigation is part of a nutritional follow-up study of 200 mothers and their infants (Salmenperä. Pediatr Res-85). The recruitment criteria were a healthy, non smoking mother with an uncomplicated pregnancy and delivery and a full-term singleton infant with weight appropriate for gestational age, a 1-min Apgar score of ≥8, and no evidence of disease by age 3 days. The mothers were encouraged to breast-feed exclusively as long as possible.

The exclusively breast-fed group consisted of infants who had been fed exclusively with breast milk, without supplementary formula or solid foods. This group included 159 infants at 2 months, 109 at 6 months, 33 at 9 months and 7 at 12 months. The rest of the infants were weaned to a formula and gradually given solid foods. In a longitudinal follow-up study of these healthy children, concentrations of total serum cholesterol and triglyceride were determined at birth (n=193), and at the ages of 2 (n=192), 4 (n=192), 6 (n=190), 9 (n=188), and 12 months (n=196), and 5 (n=162) and 11 years (n=153). Concentrations of total HDL, HDL2-, and HDL3-, VLDL-, and LDL-cholesterol were determined at 2, 6, 9, and 12 months (n=36), and at 5 (n=162) and 11 years (n=153). The apoE phenotype was determined in 151 children. At the age of 5 years, a blood sample was then taken not only from the children but also from their both parents (139 complete sets of samples).

Mothers

We analyzed the mothers serum total cholesterol concentrations at delivery (n=195), and at 2 (n=165), 6 (n=119), 9 (n=74) and 12 months (n=32) of lactation. The number of mothers who were successful in exclusive lactation was 165 at 2, 116 at 6, 36 at 9, and 7 at 12 months. The effect of ending this exclusive lactation was examined by measuring serum total cholesterol 2 months after the end of lactation; which took place between 11 and 13 months. In a subgroup of 34 mothers, serum VLDL, LDL, HDL2 and HDL3, LDL apo B, and triglyceride concentrations were determined at 2, 6, 9, and 12 months of lactation. These mothers were recruited from among the last 50 mothers participating in the study. Of the original 200 mothers, 156 were re-examined 5 years later and 140 were re-examined 11 years later, and serum cholesterol, triglyceride, and lipoprotein values were determined.

METHODS

Cholesterol and lipoproteins

Serum total cholesterol (Huang. Anal Chem-61) and triglyceride levels (Kessler. Autom in Anal Chem-66) were determined with an AutoAnalyzer. Lipoprotein cholesterol was measured with an enzymatic method (von Röschla. Z Klin Chem Klin Biochem-74). VLDL was separated by ultracentrifugation at a density less than 1.006. Cholesterol was quantified from the infranatant, and HDL-cholesterol was determined after heparin-manganese precipitation of apo B-containing particles (LRCP. DHEW-74). The difference between the total cholesterol and HDL-cholesterol values in the infranatant gave the LDL-cholesterol level. After precipitation of the apo B-containing particles, HDL2-cholesterol was precipitated by addition of dextran sulfate (Gidez. J Lipid Res-82), and HDL3-cholesterol was determined from the resulting supernatant. HDL2-cholesterol was calculated as the difference between total HDL-cholesterol (heparin-Mn^{2+} supernatant) and HDL3-cholesterol (dextran sulfate supernatant). The LDL apo B concentration was determined from the infranatant after VLDL separation by immunodiffusion with commercial kits (M-Partigen; Behringwerke AG, Marburg, Germany).

ApoE phenotyping in serum samples was performed by isoelectric focusing (Havekes. J Lipid Res-87). The apoE 4/4 (n=4) and 4/3 (n=40) phenotypes were combined as the apoE4 group (n=44), and apoE 4/2 (n=2), 3/2 (n=11) and 2/2 (n=0) were combined as the apoE2 group (n=13), while those homozygous for E3 allele were denoted as the apoE3 group (n=94).

Milk samples

The volume of milk consumed by the exclusively breast-fed infant was assessed by weighing the infant before and after each feed during a 72-h period at 6 (n=17) and 9 (n=4) months of age. Each mother collected milk into a plastic container by manual pressure, 5 mL of fore- and 5 ml of hindmilk at every feed over a 24-h period. The pooled sample was kept refrigerated until the clinic visit the next morning. The sample was analyzed the same day for cholesterol, cholesterol precursors and triglyceride concentrations. Analysis of cholesterol in these samples allowed calculation of the cholesterol intake and the relation of serum cholesterol levels to dietary cholesterol.

The total daily cholesterol output in milk was estimated by determining milk cholesterol concentrations and volumes in 17 mothers at 6 months of lactation. The volume of milk consumed by the exclusively breast-fed infant was assessed by weighing the infant before and after each feed during a 24-h period.

Infant formula

The infant formula used was Tutteli® (Valio Ltd, Helsinki, Finland); which contains 3.5g fat / 100 mL (67% milk fat, 33% soya oil). Of its fatty acids, 56% were saturated, 27.7% monounsaturated, and 16.3% polyunsaturated. The amounts of the most important fatty acids expressed as percents of the total fatty acid contents were: myristic (14:0) = 7.8%, palmitic acid (16:0) = 22.5%, stearic (18:0) = 11.0%, oleic (18/1) = 25.3% and linoleic (18/2) = 12.6%

Growth

Finnish standards (Saarinen. Acta Ped Scand-79) were used for determining relative length and weight in standard deviation scores. The growth velocities between measurements were calculated by dividing the increments (mm or g) by the actual time interval (days). The weight for length2 index was calculated (kg/mm2) (Cole. Am J Clin Nutr-81).

Statistics

For statistical analysis we used simple linear regression, Pearson's correlation coefficient, and Spearman's non-parametric correlation. Analysis of variance was used for repeated measures. The significance of the differences between population means was evaluated with the pairwise or the non-pairwise t-test. Logarithmic transformation was used for total triglyceride (TG), VLDL-TG and VLDL-cholesterol.

The three-dimensional model building was done using LOWESS, a locally weighted regression smooth (Cleveland. J Am Stat Assoc-79). LOWESS fits a regression within a local window around each point, the weights decreasing with distance from the point being smoothed. Distance-weighted least squares, a variant that will smooth in two directions, is available in commercial software packages and was used (Wilkinson. SYGRAPH-88). In this way a smoothed plot can be produced of the simultaneous dependence of one variable on two other variables.

Ethics

The study was approved by the Ethical Committee of the Childrens' Hospital, and is in accordance with the Helsinki Declaration.

RESULTS

Effects of apoprotein E phenotype, breast- and formula-feeding on serum lipids (I, III).

The serum total cholesterol concentrations increased gradually throughout infancy. However, the rise after birth was higher in the exclusively breast-fed than in the weaned infants (Fig. 6). In fact, for the breast-fed infants, the mean values more than doubled from 1.5 mmol/L at birth to 3.8 mmol/l at 2 months of age. The mean values for the infants weaned to milk formula increased from 1.5 mmol/L at birth to 4.4 mmol/L at age 12 months. The difference between the mean concentration of cholesterol in exclusively breast-fed and weaned infants was greatest at the age of 2 (0.9 mmol/L, $p<0.001$), 4 (0.6 mmol/L, $p<0.01$) and 6 months (0.5 mmol/L, $p<0.01$).

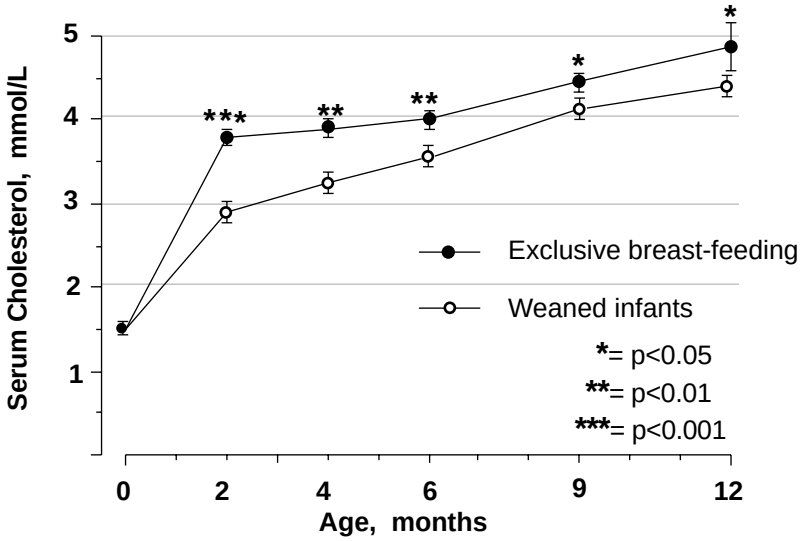


Figure 6. Serum cholesterol concentration (mean±SEM) in exclusively breast-fed and weaned (formula+solid-fed) infants during the first year of life.

Weaning from exclusive breast feeding at 6 and 9 months was associated with a significant decrease in the cholesterol values as compared with the levels in the infants continuing on exclusive breast feeding. In the infants who had received human milk exclusively for 6 months and were weaned completely during the next 3 months, the mean value of total cholesterol at 9 months was lower, 4.0±0.8 mmol/L, than in the infants who continued on exclusive breast-feeding, 4.5±0.7 mmol/L, ($p<0.05$) (Fig. 7). A similar phenomenon was seen between 9 and 12 months, when total cholesterol levels were 4.3±0.5 for weaned infants and 4.9±0.8 mmol/L for exclusively breast-fed infants at the age of 12 months ($p<0.05$).

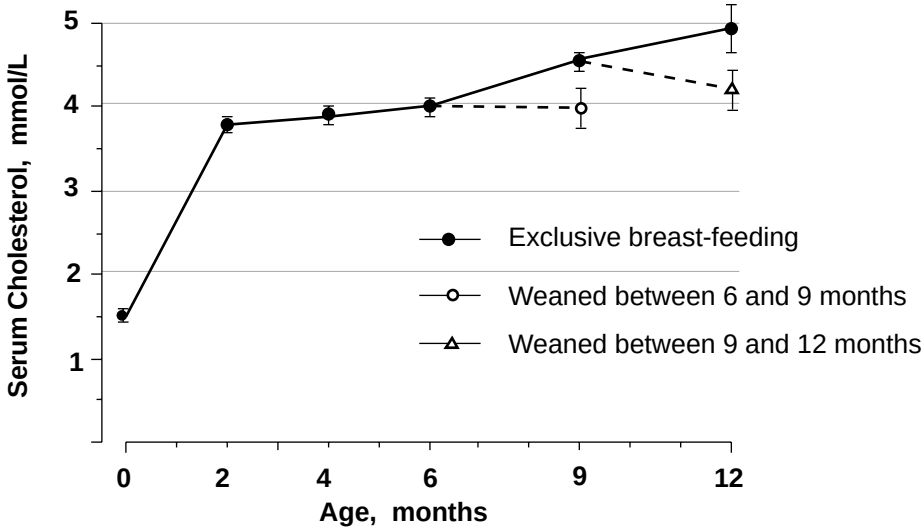


Figure 7. The mean serum cholesterol concentration (±SEM) of exclusively breast-fed infants (solid line) compared with that of infants completely weaned between 6 and 9, or 9 and 12 months (dashed lines).

The LDL-cholesterol concentration explained most of the difference found in the total cholesterol between the two groups of infants. The weaned infants had lower LDL-cholesterol concentrations than the breast-fed infants (Table II). The mean difference was 0.9 mmol/l at age 2 months ($p<0.001$), 0.7 mmol/L at age 6 months ($p<0.05$) and 0.2 mmol/L at age 9 months ($p>0.05$). The apo B concentration was also lower in the formula-fed than in the exclusively breast-fed infants at 2, 6, and 9 months of age, and the apo B/LDL-cholesterol ratio was stable and similar in the two groups throughout the year.

The mean concentration of HDL2-cholesterol tended to be lower in the formula-fed than in the breast-fed infants (Table II). Despite this, the HDL-/ LDL-cholesterol ratio was higher in the formula-fed than in breast-fed infants at 2 and 6 months of age. HDL3- and VLDL-cholesterol concentrations were independent of the diet at any age in infancy. The mean triglyceride concentrations were equal; however, the individual variation was large.

The mean (±SD) daily cholesterol intake of the exclusively breast-fed infants was 131 ± 38 mg at 6 months and 165 ± 34 mg at 9 months of age. The high daily cholesterol intake, with only a slight variation, 15-20 mg/kg, was not correlated with serum total cholesterol in the exclusively breast-fed infants.

We were also interested in whether there was a correlation between the total serum cholesterol level and the growth of the infants, since the growth pattern of exclusively breast-fed infants is unique at the same time when changes in total serum cholesterol are greatest. However, no association was observed between total serum cholesterol and the most sensitive parameters of growth, such as growth velocity, relative length, or the weight/length2-index.

Table II. The mean (±SD) serum concentrations of VLDL-, LDL-, HDL2- and HDL3-cholesterol, apoprotein B and triglyceride of exclusively breast-fed (B), formula-fed (F), breast+solid-fed (B+S), breast+solid+formula-fed (B+S+F), and formula+solid-fed (F+S) infants at 2, 6, 9 and 12 months of age. The values are expressed as mmol/L, except for apoprotein B as mg/dL.

Age	**2 months**		**6 months**		**9 months**			**12 months**	
Type of feeding:	**B**	**F**	**B**	**F+S**	**B**	**B+S**	**F+S**	**B+S+F**	**F+S**
VLDL-chol	0.20±0.13	0.17±0.14	0.23±0.18	0.25±0.15	0.23±0.19	0.18±0.13	0.23±0.11	0.11±0.02	0.17±0.13
LDL-chol	2.5±0.54	1.6±0.31	2.9±0.80	2.2±0.47	2.7±0.95	2.8±0.73	2.5±0.4	3.0±0.64	2.8±0.68
HDL2-chol	0.74±0.37	0.55±0.22	0.50±0.18	0.44±0.09	0.55±0.15	0.47±0.13	0.42±0.19	0.50±0.17	0.42±0.20
HDL3-chol	0.47±0.13	0.50±0.10	0.48±0.09	0.51±0.05	0.50±0.07	0.50±0.10	0.52±0.06	0.53±0.13	0.50±0.13
Apo-B	80±15	56±12	106±23	76±19	87±23	82±23	77±17	94±17	90±21
Triglyceride	2.2±1.2	2.4±2.1	2.2±0.99	2.0±0.80	1.5±0.39	1.2±1.0	1.2±0.65	1.1±0.31	1.4±0.57

Effects of the apoprotein E phenotype

At birth, the mean (±SEM) total cholesterol was 1.4±0.1 mmol/L in the E2 group, 1.5±0.05 mmol/L in the E3 group, and 1.6±0.01 in the E4 group. It rose faster and higher in the exclusively breast-fed infants of the E4 group than in the others. Thus, in the exclusively breast-fed infants, the total cholesterol concentrations were higher in the E4 group than in the E2 group at all time points after birth (Fig. 8). The values increased linearly between the ages of 2 and 12 months. The values of the E3 group were always between the values of the E2 and E4 groups.

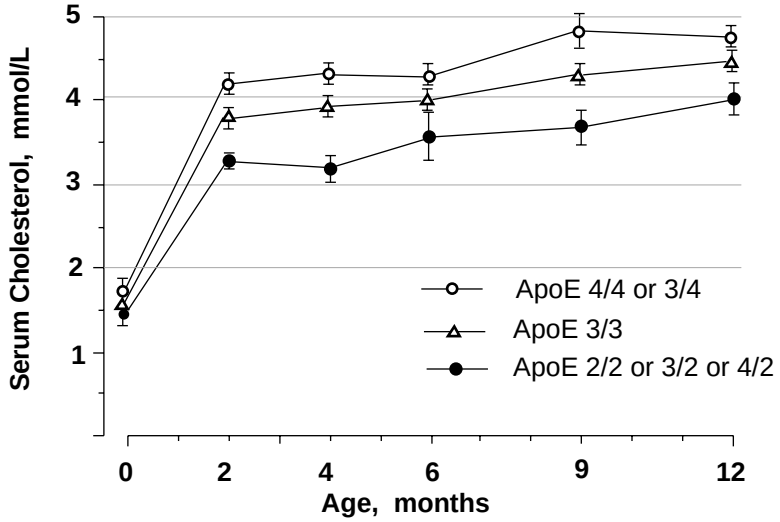


Figure 8. Serum cholesterol levels (mean±SEM) during the first year of life in exclusively breast-fed infants according to the apoE phenotype.

In the formula-fed infants, the apoE phenotype also strongly influenced the cholesterol levels (Fig. 9). Their cholesterol levels increased by 6 months, as in the exclusively breast-fed infants, although less strongly. At the age of 6 months, the E4 group had the highest cholesterol concentration (3.6 ±0.18 mmol/L) compared with the E3 group (3.5 ±0.12 mmol/L) and the E2 group (2.8 ±0.10 mmol/L)($p<0.01$). The differences between the groups were greatest at the age of 9 months. In the apoE4 group the high cholesterol, high-saturated-fat diet of breast-feeding increased total cholesterol significantly more than the formula diet. The difference between the apoE3 and apoE2 groups was also clear.

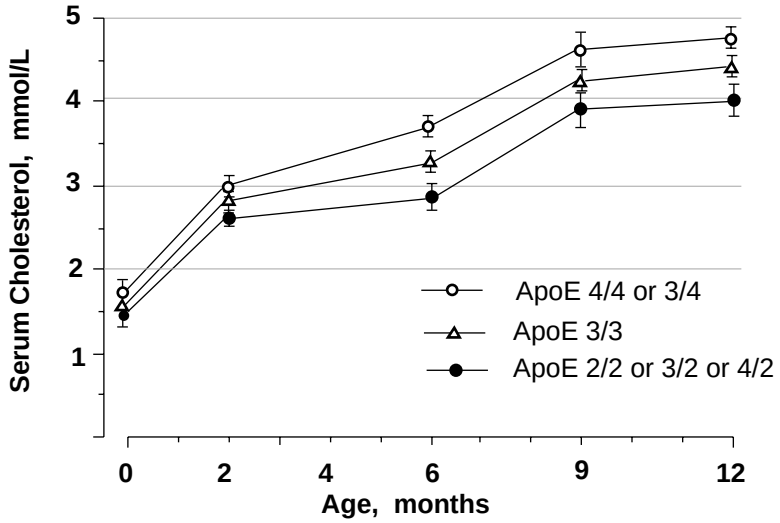


Figure 9. Serum cholesterol levels (mean±SEM) during the first year of life in formula-fed infants according to the apoE phenotype.

In the exclusively breast-fed and formula-fed infants the difference between the apoE4 and E2 groups was also evident when increments in serum cholesterol from birth were assessed (Table III).

The LDL cholesterol concentrations of exclusively breast-fed infants differed similarly after the age of 2 months. The E4 group had the highest and the E2 group the lowest values (Table IV). The LDL apoB level showed similar differences at 9 and 12 months. The total triglyceride, and total HDL, HDL2-, and HDL3-cholesterol concentrations were not related to the apoE phenotype.

Table III. The increase of serum cholesterol levels (mean±SEM) from the birth values at different ages in exclusively breast-fed and formula-fed infants by their apoE phenotypes.
The values are expressed as mmol/L.

	apoE phenotype					
	4/4 and 4/3		**3/3**		**2/3 and 2/4**	
Age	**breast**	**formula**	**breast**	**formula**	**breast**	**formula**
2 mo	2.6±0.2	1.4±0.5	2.3±0.1	1.7±0.1	2.0±0.2	1.4
6 mo	2.6±0.2	2.0±0.4	2.5±0.14	2.0±0.2	2.2±0.3	1.5
9 mo	3.5±0.3	2.6±0.4	2.8±0.1	2.5±0.2	2.3±0.1	2.3±0.1
12 mo	3.6±0.3	3.0±0.2	2.9±0.6	2.9±0.1	2.7±0.2	2.7±0.2

Table IV. Serum LDL-cholesterol and apoprotein B levels (mean±SEM) during the first year of life in exclusively breast-fed infants (up-to 9 months) according to their apoE phenotype.
The values of LDL-cholesterol are expressed as mmol/L, and for apoprotein B as mg/dL.

	apoE phenotype		
	4/4 and 4/3	**3/3**	**2/3 and 2/4**
LDL-cholesterol			
2 months	2.4±0.2	2.4±0.1	2.7±0.1
6 months	3.0±0.3**	2.8±0.2	2.4±0.3
9 months	3.1±0.3**	2.7±0.2	2.2±0.3
12 months	3.1±0.2**	3.0±0.2	2.3±0.3
Apo B			
2 months	82±6	76±5	87±5
6 months	108±7	107±6	89±15
9 months	94±8*	83±6	61±4
12 months	101±7*	93±5	71±10

* = $p<0.05$, ** = $p<0.01$ between phenotype (4/4, 4/3) and phenotype (3/3) or phenotype (2/3, 2/4)

Tracking of serum cholesterol and lipoprotein levels (II)
and the effect of the apoE phenotype on tracking (IV)

Tracking of serum total cholesterol, as assessed by correlation analysis, appeared by the age of 2 months and became stronger with age, being highest between 5 and 11 years, r=0.61 (p<0.001), (Table V). There was no difference between boys and girls. Tracking was strongest for LDL cholesterol r=0.64 (p<0.001); it was also strong for HDL cholesterol r=0.51 (p<0.001), and also significant for other lipoproteins from the age of 5 years on (Table VI).

Serum cholesterol level is sensitive to the type of diet and therefore we analyzed correlations of serum cholesterol levels separately in exclusively breast-fed infants and in those infants that were about to be weaned. The serum cholesterol values of the exclusively breast-fed infants at the age of 2 months (n=129) correlated with values measured at 6, 9, and 12 months of age (r=0.43, r=0.41, and r=0.44, p<0.001), respectively. The values of the exclusively breast-fed infants (n=94) at the age of 6 months correlated with the values measured at 9 and 12 months of age (r=0.36, and 0.42, p<0.001), respectively. The correlations became even stronger when we examined the subgroup of children that received exclusive breast-feeding until the age of 9 months (n=34); the correlations between 2 and 6 or 9 months of age were r=0.51, and r=0.62, p<0.001, respectively, and between 6 and 9 months r=0.57, p<0.001. The correlation for the exclusively breast-fed children between 6 months and 5 years of age was r=0.37, p<0.0001, while that for the children receiving partially breast milk, with formula or solid foods was r=0.12, p=n.s. The correlation for the exclusively breast-fed children between 9 months and 5 years of age was r=0.38, p<0.01, while that for the children receiving partially breast milk, with formula or solid foods, it was r=0.28, p<0.05.

Table V. Spearman correlation coefficients for serum total cholesterol levels at intervals during the first 11 years of age (n=151). * = p<0.05, ** = p<0.01, *** = p<0.001

Age	0 months	2 months	4 months	6 months	9 months	12 months	5 years
2 months	0.27**						
4 months	0.10	0.41***					
6 months	0.12	0.45***	0.50***				
9 months	0.05	0.34***	0.32***	0.38***			
12 months	0.16*	0.43***	0.45***	0.40***	0.47***		
5 years	0.02	0.32***	0.30***	0.31***	0.33***	0.45***	
11 years	0.11	0.24***	0.29***	0.32***	0.23***	0.40***	0.61***

Table VI. Spearman coefficients for correlations of values for lipoprotein fractions and total triglyceride at 11 years of age with values at 2, 6, 9, and 12 months, and 5 years of age.

	2 months	6 months	9 months	12 months	5 years
LDL-cholesterol	0.29	0.12	0.32*	0.62***	0.64***
HDL-cholesterol	0.31	0.06	0.32*	0.24	0.51***
HDL2-cholesterol	0.43*	0.29	0.43*	0.23	0.45***
HDL3-cholesterol	0.19	0.10	0.09	0.21	0.18
VLDL-cholesterol	0.09	0.06	0.13	0.10	0.19*
Triglyceride	0.04	0.08	0.33*	0.10*	0.25**

Another way to assess tracking is to determine the proportion of children who are persistently in the highest quartile or above the 90th percentile. The percentages of the children whose serum total cholesterol level was above the 90th percentile at birth, or at 2, 4, 6, 7.5, 9, or 12 months of age and remained above the 75th or the 90th percentile at the age of 5 years are shown in Table VII. Of the children who had cholesterol levels exceeding the 90th percentile at 12 months, 45% had levels above the 90th percentile (Fig. 10), 65% above the 75th percentile, and 85% above the 50th percentile 4 years later.

Table VII. The percentages of children whose serum total cholesterol levels were above the 90th percentile at birth, or at 2, 4, 6, 7.5, 9, or 12 months of age and were also persisted above the 75th or the 90th percentile at the age of five years, when 162 of these children had a check-up.

Age, months	above the 75th percentile at 5 years	above the 90th percentile at 5 years
0 mo	17 %	6 %
2 mo	47 %	35 %
4 mo	41 %	29 %
6 mo	44 %	30 %
7.5 mo	50 %	31 %
9 mo	50 %	33 %
12 mo	65 %	45 %

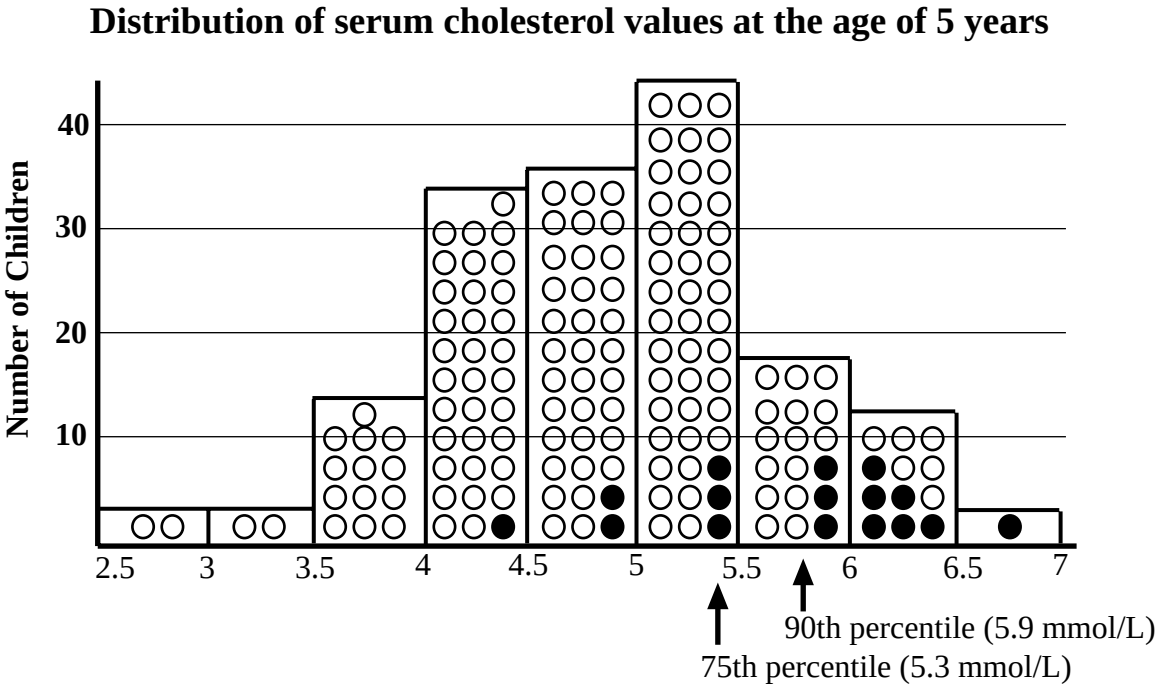


Figure 10. The frequency distribution of serum cholesterol at the age of 5 years.
The black dots indicate children whose levels were above the 90th percentile at the age of 12 months.

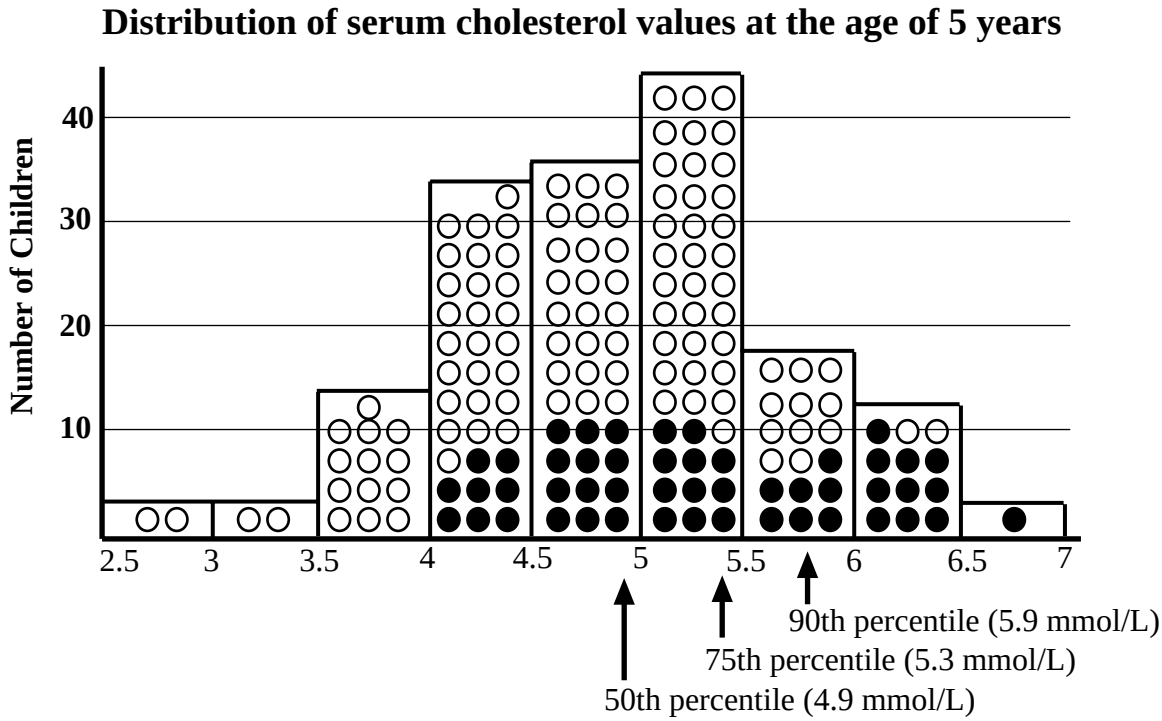


Figure 11. The frequency distribution of serum cholesterol at the age of 5 years.
The black dots indicate children whose levels were above the 75th percentile at the age of 12 months.

Of the children who had cholesterol levels exceeding the 90th percentile at 5 years of age, 65% had levels above the 90th percentile at the age of 11 years. Of the children who were above the highest quartile at 12 months, only 40% remained on the same level at the age of 5 years (Fig. 11). On the other hand, only 17% of the children with cholesterol levels below the 10th percentile at 12 months retained this position at the age of 5 years, and 40% of the children with cholesterol levels below the 10th percentile at 5 years retained this position at 11 years. In half of the children whose cholesterol levels were in the highest quintile at 12 months (above 5.2 mmol/L) these levels were still in the highest quintile (above 5.5 mmol/L) at the age of 5 years.

In retrospect, in 45% of the children whose cholesterol levels were above the 90th percentile at the age of 5 years, the levels had already been above this percentile at 12 months and in 80% they were above the 75th percentile. Correspondingly, 35% of the children whose serum cholesterol levels were above the 75th percentile at the age of 5 years had been placed similarily at the age of 1 year and 51 % of the children who had cholesterol levels in the highest quintile at the age of 5 years had already been included in this quintile at the age of 12 months.

When the groups of children with serum total cholesterol levels above the 90th percentile or below the 10th percentile at the age of 12 months were compared at the age of 11 years, their levels still differed markedly, with mean (±SD) values of 5.1±0.77 and 3.9±0.5 mmol/L ($p<0.001$), respectively. In the same way, when the groups of children with serum total cholesterol levels above the 90th percentile or below the 10th percentile at the age of 5 years were compared at the age of 11 years, their levels also differed markedly, with mean (±SD) values of 5.2±0.48 and 3.7±0.43 mmol/L ($p<0.001$), respectively.

Of the children whose total serum cholesterol levels were above the 90th percentile at the age of 5 years, 80% (12/15) also had an LDL-cholesterol level above the 90th percentile (3.95 mmol/L), and of the children whose total serum cholesterol level was above the 90th percentile at the age of 11 years, 88% (14/16) also had an LDL-cholesterol level above the 90th percentile (3.25 mmol/L). The remaining children had LDL-cholesterol values just below the 90th percentile.

Effect of the apoE phenotype

The infants had the following apoE phenotypes; 44 had E3/4 or 4/4 phenotypes (group apoE4), 94 had the E3/3 phenotype (group apoE3), and 13 had the E2/3 or 2/4 phenotype (group apoE2). Correlation coefficients of serum lipids and lipoprotein cholesterols between 5 and 11 years of age for the different apoE phenotypes are presented in Table VIII.

Table VIII. Correlation coefficients of serum lipids and lipoprotein cholesterols between 5 and 11 years of age in the different apoE phenotypes.

	apoE 2/3 or 2/4 (n=13)	**apoE 3/3** (n=94)	**apoE 3/4 or 4/4** (n=44)
Total cholesterol	0.72***	0.64***	0.42***
LDL-cholesterol	0.84***	0.70***	0.37*
HDL-cholesterol	0.40*	0.54***	0.41**
HDL2-cholesterol	0.24*	0.51***	0.23*
HDL3-cholesterol	0.08	0.17	0.19
VLDL-cholesterol	0.16	0.09	0.01
Triglyceride	0.13	0.16	0.14

These three groups had the following correlation coefficients for cholesterol at 4 months while (receiving breast-feeding), 12 months, or 5 years of age as compared with the level at 11 years; the group apoE2: r=0.65 ($p<0.01$), r=0.60 ($p<0.01$) and r=0.72 ($p<0.01$), group apoE3: r=0.27 ($p<0.01$), 0.43 ($p<0.001$) and r=0.64 ($p<0.001$), and group apoE4: 0.14 (p=n.s.), r=0.33 ($p<0.05$), and 0.42 ($p<0.01$) (Fig. 12). The effect of the apoE phenotype on the tracking of the LDL-cholesterol levels was strong; the correlation coefficients between 5 years and 11 years of age were for group apoE2 r=0.84 ($p<0.001$), for group apoE3 r=0.70 ($p<0.001$), and for group apoE4 r=0.37 ($p<0.05$) (Fig. 12).

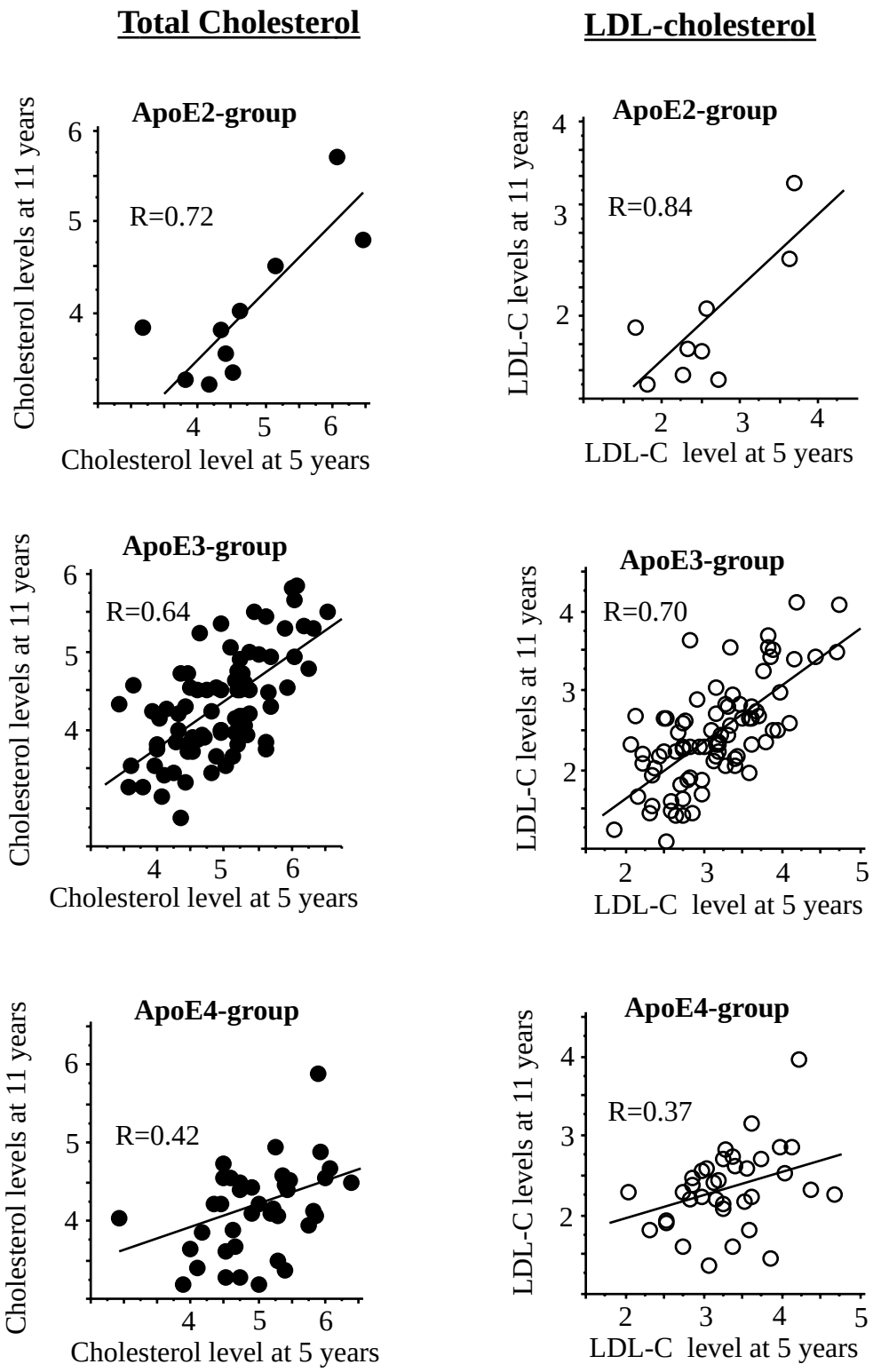


Figure 12. Correlation coefficients for total cholesterol and LDL-cholesterol at 5 years of age compared with the level at 11 years of age in apoE2, apoE3 and apoE4 groups.

The influence of the parents on the cholesterol level of the child (unpublished data) The parents had a strong influence on the serum cholesterol level of their child. The total serum cholesterol values for the children were closely correlated ($r=0.38$, $p<0.001$) with the mean of the cholesterol values for their parents (Fig. 13a); in the majority, this good correlation depended on the correlation between mother and child ($r=0.44$, $p<0.001$), the correlation between father and child being low ($r=0.16$, $p<0.05$). The majority of the mothers worked outside the home and their children were in day care outside the home (n= 94). Even so, and irrespective of the form of this day care, the correlation of cholesterol levels between the children and their mothers was higher than between the children and their fathers.

For analyzing and visualizing the relation between the serum cholesterol value for the child and the values for the parents, a three-dimensional figure is optimal (Fig. 13b). Such a figure makes it possible to see the effect of different combinations of values for the parents on the child's cholesterol level. In Figure 13b, it can be seen that the surface describing the child's cholesterol values leans markedly toward that of the mother but only slightly toward that of the father, which means that irrespective of the fathers' cholesterol level, the child's serum cholesterol level at the age of 5 years depends more strongly on that of the mother.

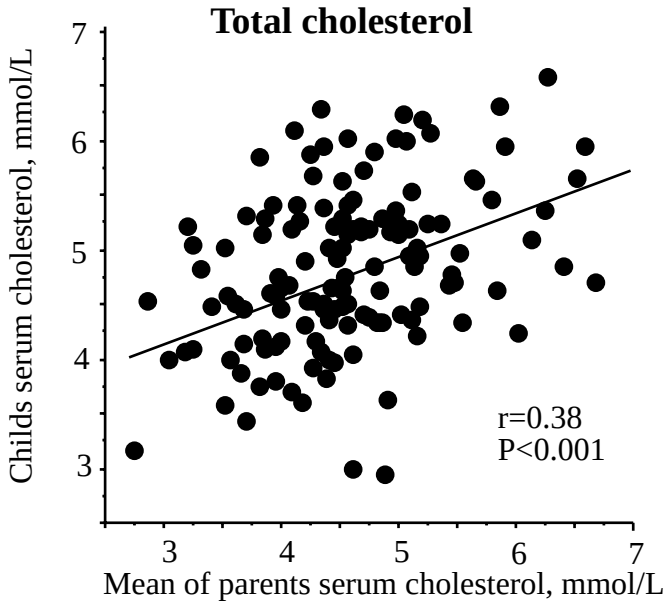


Figure 13a. Correlation between serum total cholesterol values for children and the mean of the values for their parents.

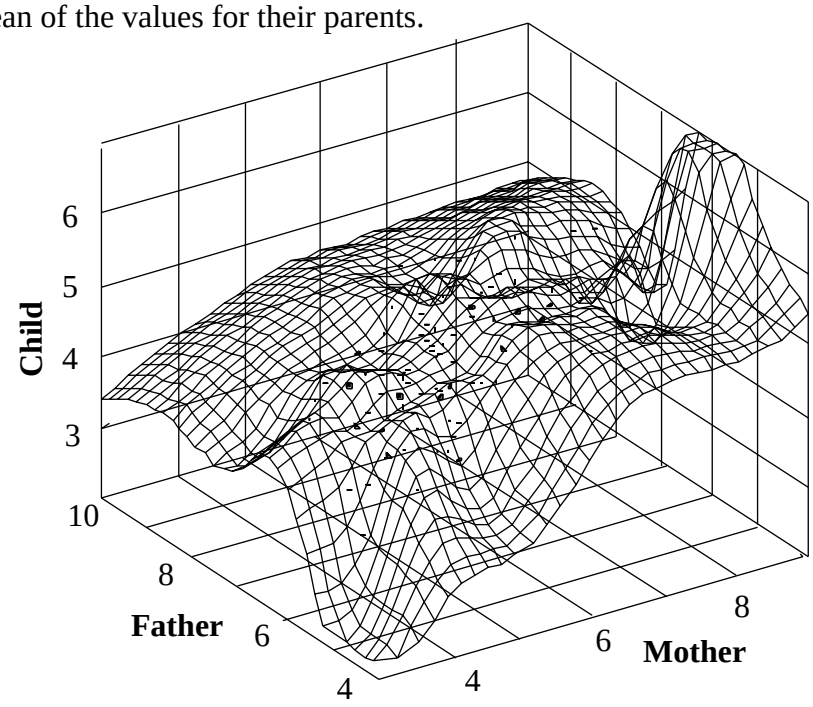


Figure 13b. Smoothed plot of the simultaneous dependence of childrens' serum total cholesterol levels on their mothers' and fathers' serum total cholesterol levels

The child's total HDL cholesterol correlated significantly with that of both parents (Fig. 14a). This correlation was also significant in those children that were in day care outside the home, the correlations of HDL cholesterol for these children and their parents being child vs. mother ($r=0.22$, $p<0.01$) and child vs. father ($r=0.27$, $p<0.01$).

With a three-dimensional figure it can be seen that, in contrast to total cholesterol, HDL cholesterol is symmetrically dependent on the values of both parents (Fig. 14b). In fact,when the children of fathers whose HDL cholesterol levels were above the 90th percentile were compared with the children of fathers whose levels were below the 10th percentile, these two groups of children differed highly significantly from each other, the HDL cholesterol concentrations for the two groups (mean±SEM) being 1.7±0.06 vs. 1.4±0.1 ($p<0.01$). When the children of mothers whose HDL levels were above the 90th percentile were compared with the children of mothers whose HDL levels were below the 10th percentile, there was a similar difference in HDL concentration: 1.7±0.08 vs. 1.3±0.05 ($p<0.01$).

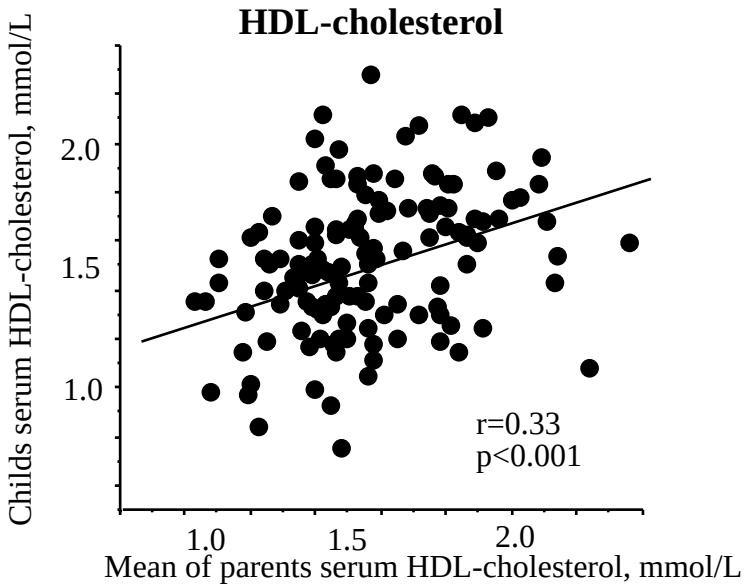


Figure 14a. Correlation between serum HDL-cholesterol values for children and the mean of the values for their parents.

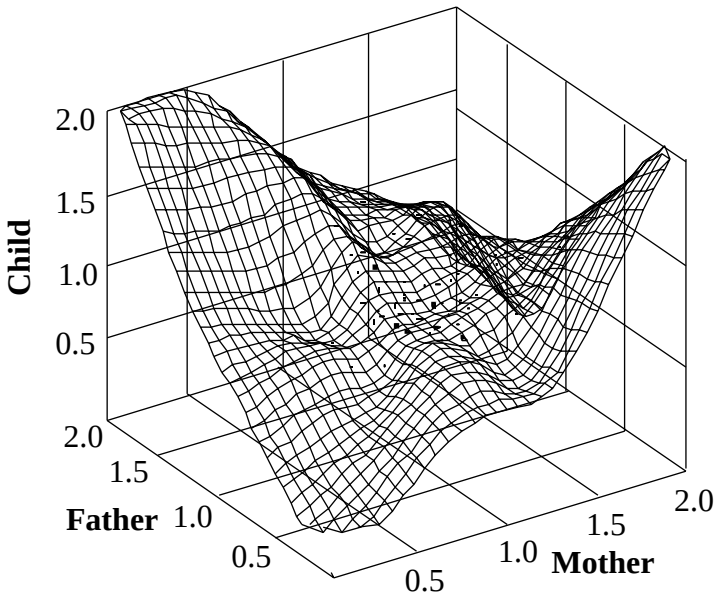


Figure 14b. Smoothed plot of the simultaneous dependence of childrens' serum HDL-cholesterol levels on their mothers' and fathers' serum HDL-cholesterol levels

We also compared the children from families in which both parents had lipid levels above +0.5 standard deviation (SD) (mother above +0.5 SD of the mothers' values and father above +0.5 SD of the fathers' values) with the children of families in which both parents had lipid levels below the respective -0.5 SD values. For serum total cholesterol, these two groups of children differed from each other highly significantly, the mean (±SD) value being 5.2±0.6 for the children whose parents were both above +0.5 SD compared with 4.3±0.56 for the children whose parents were both below -0.5 SD ($p<0.001$) (Fig. 15a).
For LDL cholesterol, the mean (±SD) value for the children of parents above +1/2 SD was 3.2±0.68 compared with 2.7±0.54 ($p<0.001$) for the children of parents below -1/2 SD (Fig. 15a). For HDL cholesterol, the respective values for the children were 1.6±0.29 vs. 1.3±0.2 ($p<0.001$), for HDL2 cholesterol 0.78±0.25 vs. 0.53±0.27 ($p<0.001$), for HDL3 cholesterol 1.0±0.12 vs. 0.84±0.12 ($p<0.001$), and for VLDL cholesterol 0.15±0.08 vs. 0.081±0.07 ($p<0.01$) (Fig. 15b). The total triglyceride of the corresponding groups of children coming from families whose parents' total triglyceride values were above +1/2 SD compared with the children from families below -1/2 SD differed only slightly, the respective mean values being 0.72±0.16 vs. 0.58±0.16 ($p<0.05$).

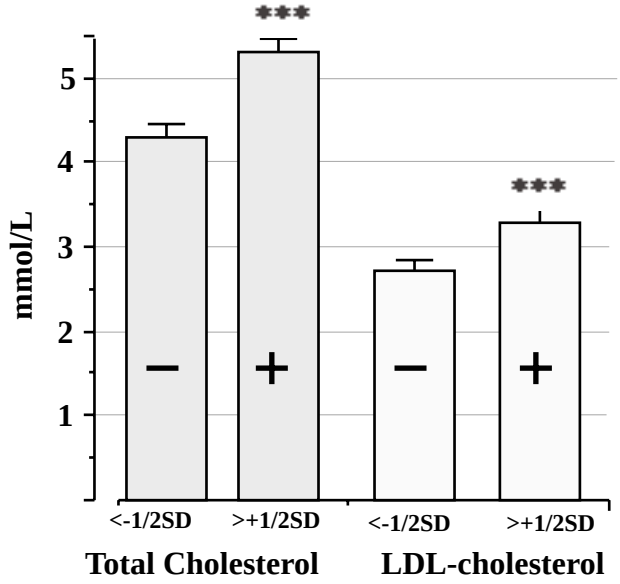


Figure 15a. Total cholesterol and LDL-cholesterol (mean±SEM), in children whose both parents lipid values are either under -1/2 standard deviations or over +1/2 standard deviations of respective values of all parents. ******* = $p<0.001$

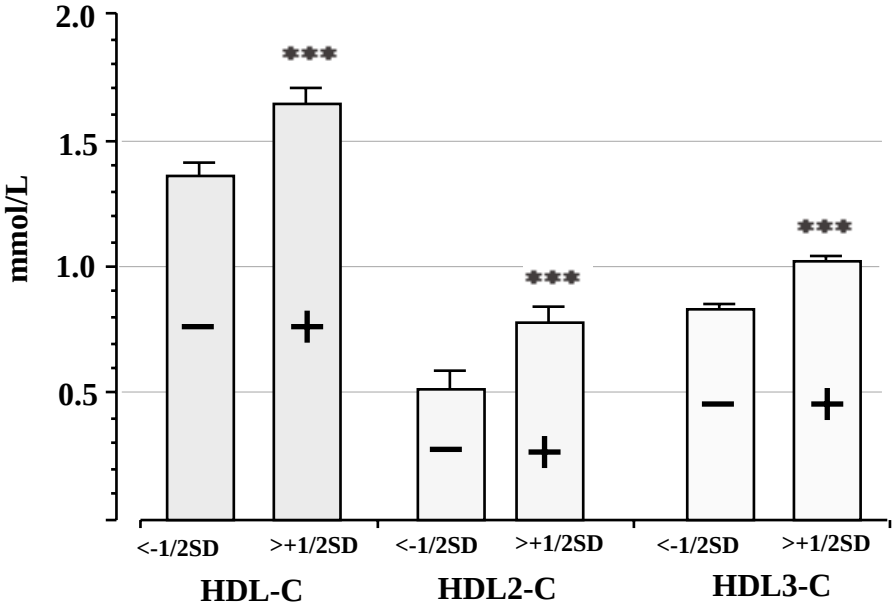


Figure 15b. Total HDL-cholesterol, HDL2- and HDL3-cholesterol (mean±SEM), in children whose both parents lipid values are either under -1/2 standard deviations or over +1/2 standard deviations of respective values of all parents. ******* = $p<0.001$

DISCUSSION

Effects of the breast- and formula-feeding on serum cholesterol (I, III)

In infancy dietary regimen already has a strong influence on serum total cholesterol. During the first months of life, breast-fed infants rapidly develop higher total cholesterol concentrations than formula-fed infants, but the difference gradually diminishes before the age of one year (Huttunen. Atherosclerosis-83). Compared with some formulas, human milk is rich in cholesterol and saturated fatty acids, but poor in unsaturated fatty acids (Lane. Pediatr Res-86), and thus is likely to raise the serum total cholesterol concentration, while diets poor in cholesterol and rich in polyunsaturated fatty acids lower the serum total cholesterol concentration (Grundy. Am J Clin Nutr-88). Changes in total serum cholesterol concentration are primarily due to LDL-cholesterol, whereas the concentrations of HDL2-, HDL3-, and VLDL-cholesterol are less sensitive to dietary changes.

Weaning

Infants receiving exclusive breast-feeding during the first few months of life go through a remarkable change in their diet when they are weaned. Human milk, rich in cholesterol, is replaced with formula, poor in cholesterol. We found a reduction in the serum cholesterol levels of the infants after weaning, as compared with those of the infants that were continuing exclusive breast feeding. Exclusive breast feeding had also a strong effect on mothers lipid levels, exclusive prolonged lactation decreased the serum levels of cholesterol and triglycerides, which rose significantly after the end of lactation (Kallio. Metabolism-92). The effect of nutrition on the blood lipid levels has been studied intensively in adults by giving them diets containing different amounts of saturated and unsaturated fatty acids and cholesterol (Grundy. Am J Clin Nutr-88). Our results regarding the effect of the weaning process on the serum cholesterol of the exclusively breast-fed infants agree well with the results obtained in the experimental studies of adults, in whom diets poor in cholesterol and rich in unsaturated fatty acids reduce the serum cholesterol levels (Grundy. Am J Clin Nutr-88).

Daily intake of lipids

There was a great difference in the daily intake of cholesterol between the breast-fed and the formula-fed infants; the breast-fed infants received 15-20 mg/kg and the formula-fed infants received only 2-5 mg/kg of cholesterol per day (Kallio. Am J Clin Nutr-89). The intake of cholesterol by breast-fed infants is very high, even when compared with that of adults (Friedman. Am J Clin Nutr-75). Dietary cholesterol can suppress the activity of LDL receptors by increasing the hepatic content of cholesterol and thereby causing a high level of LDL-cholesterol in the serum. Also, saturated fatty acids inhibit receptor-mediated uptake of LDL-cholesterol in the liver, arising the serum LDL-cholesterol level (Spady. PNAS-85), while unsaturated fatty acids reduce the LDL-cholesterol level.

Cholesterol absorption

In our study, the exclusively breast-fed infants' serum cholesterol levels did not correlate with the cholesterol intake in the mothers milk. However, the sample size was relatively small. With the same cholesterol intake, the efficiency of cholesterol absorption from the gut may vary considerably between individuals, which may be important in the regulation of hepatic cholesterol synthesis and and LDL receptor activity, and thus explain in part interindividual differences in the serum cholesterol response to dietary cholesterol. An association has been observed between cholesterol absorption and the apolipoprotein E phenotype. Subjects with the E3/E4 phenotype were found to absorb cholesterol more efficiently and to have a higher serum LDL-cholesterol level than than subjects with other apolipoprotein E phenotypes (Kesäniemi. Am Heart J-87, Lehtimäki. J Lipid Res-90).

HDL-cholesterol

Polyunsaturated fatty acids reduce HDL-cholesterol levels (Vega. J Lipid Res-82), probably by inhibiting the production of its major apoprotein A-I. This might be the reason for our finding that HDL2-cholesterol tended to be lower in the formula-fed than in the breast-fed infants throughout the first year of life, though the difference was not statistically significant. LDL-cholesterol was even lower at the same time and so the HDL- / LDL-cholesterol ratio was higher in the formula-fed than in the breast-fed infants. HDL3-cholesterol, on the other hand, was not sensitive to the type of feeding.

Effect of the apoprotein E phenotype on serum cholesterol (III)

The present data show clearly that the apoE phenotype strongly influences the serum total and LDL cholesterol levels during the first year of life both in exclusively breast-fed infants and in infants weaned to a diet of formula and solids. During exclusive breast feeding, the serum total cholesterol concentration was highest in the E4 group and lowest in the E2 group. The LDL cholesterol and apoB levels appeared to follow the same pattern. In the weaned infants, the influence of the apoE phenotype was also clear. The absolute values for each apoE group were up to 1 mmol/L lower than in the exclusively breast-fed infants, especially at the age of 2-9 months. In the apoE4 group this difference tended to persist even at the age of 12 months. During the first 6 months of life, the weaned infants of the E4 group had cholesterol levels similar to those of the exclusively breast-fed infants of the E2 group.

The increment in serum cholesterol in the exclusively breast-fed infants above the level of the formula-fed infants was higher in the E4 group than in the E2 group. The difference between the two diets was significant mainly in the E4 group. Why, then, did the E4 group respond more strongly than the apoE2 group to exclusive breast-feeding and, to some extent, to the formula diet? Saturated fatty acids may down-regulate LDL apoB receptor activity more sensitively in the E4 group than in the E2 group; in adults such mechanisms appear significant. In addition, removal of remnants (of both chylomicrons and VLDL) by the liver may have been faster in the E4 group (Weintraub. J Clin Invest-87), resulting in a greater accumulation of LDL cholesterol. On a high-cholesterol diet, such as breast feeding, LDL removal might be effectively inhibited by down-regulation of the LDL apoB receptor. Furthermore, intestinal cholesterol absorption might be more efficient in the E4 subjects than in the apoE2 subjects (Kesäniemi. J Clin Invest-87). Adults of the E4 group, as compared with the E2 group, have more efficient intestinal absorption, and slower elimination and synthesis of cholesterol associated with slower removal of LDL apoB (Miettinen. Arterioscler Thromb-92). In addition, adults of the E2 group are usually nonresponders to cholesterol feeding because, as compared with the E4 group, they have more efficient compensatory mechanisms for regulating cholesterol metabolism, so that their level of serum cholesterol remains low.

In infants consuming mother’s milk, which is rich in cholesterol (15-20 mg/kg/day) and saturated fat, the high serum cholesterol levels may be explained by efficient cholesterol absorption especially in the E4 group. The apoE-related regulation of the serum cholesterol level was less clearly expressed in the weaned infants than in the exclusively breast-fed infants. Similarly, the apoE-related regulation of the serum cholesterol level is usually less strongly expressed during a cholesterol-lowering diet than during the high-saturated fat, high-cholesterol Western diet.

Our results confirm that the relation of apoE polymorphism to serum lipoprotein concentrations noted in adults can already be seen in children (Lehtimäki. J Lipid Res-90, Srinivasan. Metabolism-93). The apoE4/4 phenotype, together with a diet high in cholesterol and saturated fat, may be a significant inducer of hypercholesterolemia in this subpopulation.

Tracking of serum lipids (II)

Tracking of serum cholesterol levels has been well documented in older children and adolescents (Bao. Arch Intern Med-96), but little has been known about trends during the first year of life (Freedman. Pediatrics-87, Webber. J Chron Dis-80). In this study a slight tracking of the serum total cholesterol level appeared when levels in cord blood were compared with levels in blood samples measured at intervals during the first 12 months. However, there was no correlation between the levels at birth and at 5 years of age. This is in accord with other observations (Kwiterovich. Lancet -73, Webber. J Chron Dis -80, Freedman. Pediatrics-87). As the children grew older, tracking became significant. There was an appreciable correlation between serum cholesterol levels at the ages of 6 months and 5 years, and the correlation appeared strongest between the levels at 12 months and 5 years of age (r=0.47). These results resemble those reported by others (Webber. J Chron Dis-80, Freedman. Pediatrics-87).

Among the children with high or low initial serum cholesterol levels, the differences in lipid concentrations were still evident at re-examination 5 years later, indicating that relatively high levels of serum cholesterol in childhood are likely to persist. Our results agree well with those obtained in a study in which re-examinations were made up to 8 years later (Garn. Am J Clin Nutr-80).

The effect of diet on tracking

Diet has a strong influence on the serum cholesterol level during the first year of life; the exclusively breast-fed children received a fairly homogeneous diet compared with the children who received partially breast milk, solid foods or formula, and therefor we analyzed these children separately. The correlations in the breast-fed children were higher than those for the group as a whole. The correlations were strongest in the subgroup of children who were exclusively breast-fed until the age of 9 months, ranging from r=0.51 to r=0.62 at different intervals during the first year of life. These results are similar to those found in older children and young adults, in whom correlations between serum cholesterol levels over time have been found to range from r=0.52 to 0.64 (Porkka. Prev Med-91). In contrast, the children who received partially breast milk, solid foods or formula at the age of 6 or 9 months had markedly weaker correlations of serum cholesterol levels measured at the age of 5 years. The weaker correlation was due to the change in diet; human milk, which is rich in cholesterol, was replaced with formula and solid foods, which caused reductions in childrens serum cholesterol levels.

Tracking analyzed with percentiles

When tracking was defined as the proportion of children persisting in high the serum cholesterol percentiles, 45% of those with a total cholesterol above the 90th percentile at 12 months remained at the same relative level at 5 years. In earlier studies 44-46% of the children initially in the highest decile for cholesterol remained there over time (Frerichs. J Chronic Dis-79, Laskarzewski. Pediatrics-79, Webber. J Chronic Dis-83). In the study from Muscatine of children whose initial cholesterol levels were above the 90th percentile, 43% had values above the 90th percentile, 62% were above the 75th percentile, and 81% above the 50th percentile as young adults 12 to 16 years later (Lauer. Pediatrics-88). In the present study the corresponding percentages between 12 months and 5 years of age were 45%, 65%, and 85%, respectively.

Tracking analyzed with quintiles

When the cholesterol values were divided into quintiles, 50% of those children who had cholesterol levels in the highest quintile at the age of 12 months, still had cholesterol levels in the highest quintile at the age of 5 years. In the Bogalusa study the corresponding figure was 43% between 1 and 7 years of age (Freedman. Pediatrics-87), and for older children the proportions persisting in the highest serum cholesterol quintile 4, 6, and 9 years after the initial cholesterol determination were 54%, 45%, and 55%, respectively. In Beaver County, children were examined at the age of 12 years and re-examined 9 years later; 49% of the subjects in the highest cholesterol quintile at baseline were similarly placed at follow-up (Orchard. J Pediatr-83).

Tracking of LDL- and HDL-cholesterol

In our study, a strong correlation was observed between the LDL-cholesterol levels measured in children aged 12 months and 5 years. The corresponding correlation for HDL-cholesterol was less significant. In the Bogalusa study the correlation of LDL-cholesterol was 0.56 and of HDL-cholesterol 0.31 between 1 and 7 years of age (Freedman. Pediatrics-87). In older children, the correlation observed between the initial and follow-up levels of LDL-cholesterol was between 0.47 and 0.65 at different ages (Freedman. Prev Med-85, Lauer. Pediatrics-88) and for HDL-cholesterol between 0.33 and 0.58 (Freedman. Prev Med-85, Porkka. Prev Med-91). In children with familial hypercholesterolemia, the correlations between follow-up measurements were 0.73 for LDL-cholesterol and 0.55 for HDL-cholesterol (Mellies. Metabolism-85).

A high serum total cholesterol level (above the 90th percentile) was associated with a high LDL-cholesterol level. Of the children with serum total cholesterol levels above the 90th percentile at the age of 5 years, 81% had an LDL-cholesterol level above the corresponding 90th percentile and even the remaining 19% of these children were close to this level. This observation agrees with the results of a study which found that 78% of a population of 500 children whose total cholesterol level was above the 95th percentile had an LDL-cholesterol level above the corresponding 95th percentile (Garcia. Am J Dis Child-91). These results differ from a study which reported that approximately half of the adolescents with serum total cholesterol levels above the 95th percentile did not have elevated LDL-cholesterol levels (Dennison. Pediatrics-90).

Stability of high cholesterol values

In our investigation, 45% of the children who had cholesterol levels above the 90th percentile at the age of 12 months remained above this same percentile 4 years later; this is four and half times more than the expected prevalence if the distribution had been random. Further, 65% of the children who had cholesterol levels above the 90th percentile at the age of 5 years remained above this same percentile at the age of 11 years, which is about six times more than the expected prevalence (=10%) if the distribution had been random. Studies of older children and adolescents have detected similar percentages (Laskarzewski. Pediatrics-79, Webber. J Chronic Dis-83, Lauer. Pediatrics-88).

On the other hand, only 40% of the children who were above the 75th percentile at 12 months remained above the 75th percentile at the age of 5 years, - this is only one and a half times more than would be expected by chance alone. And later on, only 53% of the children who were above the 75th percentile at 5 years, remained above the 75th percentile at the age of 11 years, - this is only twice the number that would be expected by chance alone. These findings imply that high serum cholesterol values are more stable over time than lower values. The reason for the stability of high serum cholesterol concentrations in some of these children is probably genetic, for example familial hypercholesterolemia or familial combined hypercholesterolemia.

Short-term variation of serum cholesterol levels

However, the serum cholesterol level is not stable from day to day; it is sensitive to the considerable changes in the diet during the first year of life. Breast-fed infants receive much cholesterol and saturated fat (Kallio. Am J Clin Nutr-89) and have markedly higher cholesterol levels than formula-fed children. This difference diminishes after 6 months of age and practically disappears by the age of 12 months. The variation among individual cholesterol levels is due partly to the phenomenon of regression toward the mean, i.e. the shift toward less extreme values of serum cholesterol at a second sampling of children initially at the extremes of the distribution (Morrison. Pediatrics-79), and partly to intraindividual variation in serum cholesterol, some subjects showing extreme variability from one blood sample to the next (Hegsted. PNAS-87). The fact that for some of these children the serum cholesterol level at the next measuring point was lower than some threshold value, i.e., below the 90th percentile, does not necessarily mean that these children run no risk of CHD in future. The relationship between serum cholesterol and CHD is continuous and not confined to values over a certain threshold (Stamler. JAMA-86).

Tracking and the screening recommendations in children

Selective cholesterol screening on the basis of family history alone has been found to be neither sensitive nor specific for predicting elevated cholesterol levels in children (Dennison. J Pediatr-89, Garcia. Pediatrics-89), which is why cholesterol screening has been recommended for all children after 3 years of age (Garcia. Am J Dis Child-91). The screening recommendation suggests that a blood sample should be taken any time after the age of 2 years from those children who have a family history of premature cardiovascular disease or at least one parent with high blood cholesterol (NCEP. Pediatrics-92). Our results indicate that tracking of serum cholesterol during the first year of life is stronger in children receiving a relatively homogeneous diet such as exclusive breast feeding, and that tracking becomes weaker when the children are weaned to formula and solid foods. After the weaning process is completed, the childrens relative serum cholesterol levels have become established and the tracking of serum cholesterol has developed and is of the same magnitude as for older children and adolescents. Thus the cholesterol values determined in children at the age of 12 months are already predictive of subsequent cholesterol values later in childhood, especially for children who have high serum cholesterol levels.

The effect of the apoE phenotype on tracking of serum lipids (IV)

The effect of the apoE phenotype on tracking of serum cholesterol has been studied less (Srinivasan. Atherosclerosis-96). In our study, covering the first 11 years of life, tracking of serum cholesterol levels was already evident during the first year and was strongest between the values measured at 5 and 11 years of age. The apoE phenotype had a strong effect on the tracking of cholesterol; those children that belonged to group apoE2 had the strongest tendency ($r=0.72$) to maintain their position relative to the rest of the children between 5 and 11 years of age, compared to the children of group apoE3 ($r=0.64$) and those of group apoE4 ($r=0.42$), our results agree well with the results obtained by other investigators (Srinivasan. Atherosclerosis-96). This phenomenon was already seen when the values at the age of 12 months were compared with the values at 11 years. The explanation is that the different apoE phenotypes are associated with different responses of serum cholesterol to dietary cholesterol modifications (Miettinen. Lancet-88, Tikkanen. Arteriosclerosis-90).

The apoE phenotype and tracking of LDL- and HDL-cholesterol

Among the lipoproteins studied, the strongest correlation was observed between the LDL-cholesterol levels measured in these children between the ages of 5 and 11 years. The apoE phenotype had a strong influence on this correlation; the children in group apoE2 had a high correlation coefficient ($r=0.84$), while the children in group apoE3 had a weaker correlation ($r=0.70$) and those in group apoE4 had the lowest correlation ($r=0.37$). The sensitivity of the correlation coefficient between LDL-cholesterol and the apoE phenotype reflects the findings that the apoE 4 phenotype is associated with higher levels of LDL-cholesterol than the apoE 2 phenotype (Utermann. Nature-77, Ehnholm. J Lipid Res-86), higher cholesterol absorption efficiency (Kesäniemi. J Clin Invest-87), and with a lower LDL catabolic rate (Miettinen. Arterioscler Thromb-92).

In contrast to LDL-cholesterol, HDL-cholesterol was not related to the apoE phenotype. HDL-cholesterol is not sensitive to the type of diet, and so the different binding properties of the E alleles, which, for instance, lead to differences in postprandial fat clearance, do not affect the HDL levels. In Bogalusa, the correlations for LDL-cholesterol were r=0.74 in the apoE2 group and r=0.48 in the apoE4 group, respectively (Srinivasan. Atherosclerosis-96). There are, however, some significant differences between our studies; we had only whites, while they had blacks and whites in their study group and, although these groups differ systematically in many lipid values, they were combined in the analyses. Further, we followed our children for 11 years, measuring lipid values at same age, which is very important in tracking studies, because lipid values change during childhood and especially during puberty, while in the Bogalusa study all the children, irrespective of age or stage of puberty, were grouped together in the analyses. In our study, the apoE phenotype explained the tracking of total and LDL-cholesterol to equal extents, while in Bogalusa study the apoE phenotype was retained as a predictor variable only in the case of LDL-cholesterol (Srinivasan. Atherosclerosis-96). In Bogalusa, in terms of persistence in rank over time, none of the individuals in the apoE2 group, who were in the highest quartile of LDL cholesterol at baseline, none of those maintained this high rank at follow-up, while the corresponding values for persistence in ranking in the lowest quartile over time were 82% for the apoE2 group (Srinivasan. Atherosclerosis-96). We have only a limited number of children in this group, so we are unable to divide this number reliably into subgroups and then further analyze their persistence over time.

Parental influence on the cholesterol level of the child

A number of studies have investigated the influence of familial factors both genetic and those arising from the shared environment, on cholesterol levels. The genotype determines the range within which the individual's phenotype is located, and environmental factors determine where the phenotype appears within that range. Children's lipid and lipoprotein levels are related to levels in members of their family (Hennekens. Pediatrics-76, Schrott. Circulation-82, Moll. Circulation-83, Freedman. NEJM-86, Lee. Pediatrics-86), and there is a strong familial aggregation of total cholesterol, LDL-cholesterol, and HDL-cholesterol in children and parents (Beaty. Am J Med Genet-83). That genetic factors play a substantial role in determining lipid levels among relatives has been shown in studies of twins, nuclear families, and multigenerational kindreds (Namboodiri. Am J Epidemiol-84). Heritability is the fraction of the total variance caused by these factors. Within different populations the genetic heritability of the total cholesterol level was 0.57-0.65, for LDL-cholesterol 0.62-0.68, and for HDL-cholesterol 0.45-0.51, and the cultural (environmental) heritability for total cholesterol level was 0.03-0.08, for LDL-cholesterol 0.05-0.07, and for HDL-cholesterol 0.12-0.15 (Dahlen. Int J Epidemiol-83, Namboodiri. Arteriosclerosis-83, Hamsten. Atherosclerosis-86). Thus genetic heritability makes a significant contribution and environmental heritability a relative marginal contribution toward explaining the variation in lipid levels within the population. The genetic contribution to lipoproteins is largely polygenic with little evidence for major monogenic effects (Hasstedt. Am J Med Genet-86).

Familial aggregation of adverse levels of lipoproteins appears partly to account for the aggregation of CHD in families. The close association between lipid risk factors of parents and offspring in hypercholesterolemic families in a shared household environment facilitates within-family identification of dyslipoproteinemia; first-degree relatives of individuals with high levels of total cholesterol have higher levels of total cholesterol than first-degree relatives of individuals whose cholesterol levels are not above normal (Morrison. JAMA-83). When both parents have lipid levels above the median, the levels in 67-83% of their offspring are also above the median (Rosenbaum. Genet Epidemiol-86).

When we used simple linear regression, the total serum cholesterol values for the children were closely correlated with the mean of the cholesterol values for their parents. In the majority, this good correlation depended on the correlation between mother and child, the correlation between father and child being low. Several other studies have likewise shown correlations to be significantly higher for mother-pediatric offspring than for father-pediatric offspring, a phenomenon suggested to be due to closer sharing of the environment by pediatric-offsprings with their mothers (Friedlander. Am J Epidemiol-87, Laskarzewski. Am J Epidemiol-81, Morrison. Prev Med-80, Morrison. Metabolism-82). In our investigation, however, the majority of the mothers worked outside the home and their children were in day care outside the home. Irrespective of the form of this day care, the correlation of cholesterol between the children and their mothers was higher than between the children and their fathers. These intrafamilial correlations do not permit the separation of genetic contribution from that of shared environment.

In the present study a three-dimensional figure was used to describe the child’s serum cholesterol, and thus a smoothed plot was produced to depict the simultaneous dependence of the children’s cholesterol on their mothers’ and fathers’ cholesterol. For analyzing and visualizing the relation between the serum cholesterol value for the child and the values for the parents, a three-dimensional figure is optimal, the surface describing the child’s cholesterol values leans markedly toward the mother but only slightly toward the father, which means that, irrespective of the fathers’ cholesterol level, the child’s serum cholesterol level depends more strongly on that of the mother.

SUMMARY AND CONCLUSIONS

I. Breast-fed infants rapidly developed higher total cholesterol concentrations than formula-fed infants during the first months of life, but the difference gradually diminished before the age of one year. Human milk is rich in cholesterol and saturated fatty acids compared to formulas, and thus is likely to raise serum total cholesterol concentration, while diets poor in cholesterol and rich in polyunsaturated fatty acids, lower the serum total cholesterol concentration. Changes in total serum cholesterol concentration are primarily due to LDL-cholesterol, while the concentrations of HDL2-, HDL3, and VLDL-cholesterol are less sensitive to dietary changes. Breast milk is the natural food of an infant and the cholesterol level in breast-feeding must be considered physiologic, so it has to be asked if artificially made formulas are sufficient in this respect at the moment.

II. Tracking of serum cholesterol during the first year of life is stronger in children receiving a relatively homogeneous diet, such as exclusive breast feeding, and tracking becomes weaker as children are weaned to formula and solid foods. After the weaning process is completed, the childrens' relative serum cholesterol levels become established and the tracking of serum cholesterol develops and is of the same magnitude as for older children and adolescents. Thus, the cholesterol values determined in children at the age of 12 months are predictive of subsequent cholesterol values later in childhood, especially for children with high serum cholesterol levels.

III. Healthy infants on prolonged exclusive breast feeding offer a unique physiologic dietary model for assessing the effects of high-cholesterol, high-saturated fat diet on serum lipid levels. Our results confirm earlier findings that the relation between apoE polymorphism and serum lipoprotein concentrations noted in adults can already be seen in children. The apoE 4/4 phenotype together with a diet high in cholesterol and saturated fat may be a significant inducer of hypercholesterolemia in this subpopulation. Because of the greater sensitivity of E4 individuals to dietary cholesterol and saturated fat, dietary intervention in this group may be more effective in reducing serum total cholesterol and LDL-cholesterol concentrations, and further in reducing the risk of CHD in these high-risk individuals in future. However, dietary intervention may be postponed for some years, because infants need the increased caloric density provided by human milk and also the other nutrients contained in breast milk.

IV. The apoE phenotype of the child should be taken into consideration when blood samples are taken for cholesterol analyses and conclusions are drawn from the child's original cholesterol value to predict future values. Cholesterol values determined in children at the age of 12 months or 5 years are predictive of subsequent cholesterol values later in childhood, especially for children in the apoE2 or apoE3 groups. In contrast, children with the apoE phenotype 3/4 or 4/4 are sensitive to the diet and their cholesterol levels are less stable during the course of time. These results suggest the importance of analyzing the apoE phenotype in children with high cholesterol levels. Better prediction of future cholesterol values in at least one group of these children could lead to the development of more concrete intervention strategies in pediatric practice.

V. A smoothed plot can be produced of the simultaneous dependence of one variable on two other variables, e.g. children's serum cholesterol on their mothers' and fathers' cholesterol by using the three-dimensional model building, locally weighted regression smooth. The surface describing the child's cholesterol values leans markedly toward the mother but only slightly toward the father, which means that, irrespective of the fathers' cholesterol level, the child's serum cholesterol level at the age of 5 years depends more strongly on that of the mother. Such three-dimensional model building and figures could be used more often to present the simultaneous dependence of the children's values on their mothers' and fathers' values for other parameters besides serum cholesterol.

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