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COMMON DYSLIPIDEMIAS, HIGH LIPOPROTEIN(a) CONCENTRATIONS AND ENDOTHELIAL FUNCTION IN THE CHILDREN OF THE STRIP STUDY FAMILIES

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ABSTRACT

Jouni Lapinleimu. **Common dyslipidemias, high lipoprotein(a) concentrations and endothelial function in the children of the STRIP study families**. University of Turku, Faculty of Medicine, Internal Medicine, University of Turku Doctoral Programme of Clinical Investigation, the Research Center of Applied and Preventive Cardiovascular Medicine, University of Turku and Division of Medicine, Turku University Hospital, Salo hospital, Finland. Turun yliopiston julkaisuja – Annales Universitatis Turkuensis Turku, 2016.

Extreme lipid values predisposing on illnesses are dyslipidemias. Dyslipidemias evolve in early childhood, but their significance or persistency is not well known. Common dyslipidemias may aggregate in the same families.

This thesis is a part of the longitudinal randomized Special Turku coronary Risk factor Intervention Project STRIP, in which 1054 families with six months old children were randomized to a control or to an intervention group. The family lipid data from the first 11 years was used. Fasting samples at the age of five years defined the lipid phenotypes. The dyslipidemias coexisting in the parent and the child were studied. At the age of 11 years 402 children participated artery ultrasound studies. The significance of the childhood dyslipidemias and lipoprotein(a) concentration on endothelial function was evaluated with the flow mediated arterial dilatation test.

Frequently elevated non-HDL cholesterol concentration from one to seven-year-old children associated to similar parental dyslipidemia that improved the predictive value of the childhood sample. The familial combinations were hypercholesterolemia (2.3%), hypertriglyceridemia (2.0%), familial combined hyperlipidemia (1.8%), and isolated low HDL-cholesterol concentration (1.4%). Combined hyperlipidemia in a parent predicted most frequently the child's hyperlipidemia. High lipoprotein(a) concentration aggregated in some families and associated to childhood attenuated brachial artery dilatation. Hypercholesterolemia and high lipoprotein(a) concentration at five years of age predicted attenuated dilatation.

This study demonstrated that parental dyslipidemias and high lipoprotein(a) concentration help to find early childhood dyslipidemias. The association of hypercholesterolemia and lipoprotein(a) concentration with endothelial function emphasizes the importance of the early recognition of the dyslipidemias.

Keywords: atherosclerosis, children, endothelium, familial dyslipidemias, lipoprotein(a), risk factors, ultrasound

TIIVISTELMÄ

Jouni Lapinleimu. **Yleiset dyslipidemiat, suuri lipoproteiini(a) pitoisuus ja endoteelin toiminta lapsilla STRIP tutkimuksen perheissä**. Turun yliopisto, Lääketieteellinen tiedekunta, Sisätautioppi, Turun yliopiston kliininen tohtoriohjelma, Sydäntutkimuskeskus, Turun yliopisto ja Medisiininen toimialue, Turun yliopistollinen keskussairaala, Salon sairaala. Turun yliopiston julkaisuja – Annales Universitatis Turkuensis Turku, 2016.

Poikkeavat lipidiarvot, jotka altistavat sairauksille ovat dyslipidemioita. Dyslipidemioita kehittyy varhaislapsuudessa, mutta silloin niiden merkitys ja pysyvyys ovat epäselviä. Yleiset dyslipidemiat saattavat kasautua samoihin perheisiin.

Tämä väitöskirja on osa pitkittäistä satunnaistettua SepelvaltimoTaudin Riskitekijöiden InterventioProjekti STRIP tutkimusta missä 1054 kuuden kuukauden ikäisen lapsen perhettä satunnaistettiin kontrolli- tai interventioryhmiin. Tämä tutkimus käytti perheiden ensimmäisten 11 vuoden rasva-arvojen seurantatietoja. Lapsilta viiden vuoden iässä otetuista paastonäytteistä määriteltiin lipidi-aineenvaihdunnan ilmiasut. Dyslipidemioiden esiintymistä tutkittiin yhdessä perheen vanhemmilla ja lapsella. Lasten ollessa 11-vuotiaita 402 lasta osallistui valtimoiden ultraäänitutkimuksiin. Lapsuuden dyslipidemioiden ja suuren lipoproteiini(a) pitoisuuden merkitystä arvioitiin virtauksen lisääntymisen välittämällä olkavaltimon laajentumisen kokeella.

Toistuvasti suurentunut yhden ja seitsemän ikävuoden väliltä mitattu non-HDL kolesteroli pitoisuus liittyi vanhempien samankaltaiseen dyslipidemiaan, mikä paransi lapsen lipidinäytteen ennustavuutta. Perheiden yhdistelmät olivat hyperkolesterolemia (2,3 %), hypertriglyseridemia (2,0 %), familiaalinen kombinoitu hyperlipidemia (1.8 %), ja itsenäinen pieni HDL-kolesteroli pitoisuus (1.4 %). Vanhempien kombinoitu hyperlipidemia ennusti suhteellisesti eniten lasten hyperlipidemiaa. Vanhempien kohonnut non-HDL kolesterolin pitoisuus lisäsi lapsen dyslipidemian pysyvyyttä. Suuri lipoproteiini(a) pitoisuus kasaantui muutamiin perheisiin ja ennusti lapsilla heikentynyttä valtimolaajentumista. Hyperkolesterolemia ja suuri lipoproteiini(a) pitoisuus viiden vuoden iässä mitattuna ennustivat vaimentunutta laajentumista.

Tämä tutkimus osoitti, että kun käytetään vanhempien dyslipidemioita ja suurta lipoproteiini(a) pitoisuutta, voidaan löytää varhaislapsuuden dyslipidemioita. Hyperkolesterolemian ja suuren lipoproteiini(a) pitoisuuden yhteys heikentyneeseen endoteelin toimintaan puoltaa dyslipidemioiden varhaista seulontaa.

Avainsanat: ateroskleroosi, lapset, endoteeli, familiaaliset dyslipidemiat, lipoproteiini(a), riskitekijät, ultraääni

CONTENTS

ABBREVIATIONS

AAP	American Academy of Pediatrics
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
ApoAI	apolipoprotein AI
$ApoB_{100}$	apolipoprotein B [100 (%)]
$ApoB_{48}$	apolipoprotein B [48 (%)]
ApoC	apolipoprotein C
ApoE	apolipoprotein E
BMI	body mass index
CETP	cholesterol ester transferring protein
CHD	coronary heart disease
CM	chylomicron
CV	coefficient of variation
CVD	cardiovascular disease
FCHL	familial combined hyperlipidemia
FFA	free fatty acids
FH	familial hypercholesterolemia
FMD	flow mediated dilatation
HDL	high density lipoprotein
HDL-C	high density lipoprotein cholesterol
HMG-CoA	3-hydroxy-3-methylgutaryl coenzyme A
HL	hepatic lipase
IMT	intimal media thickness
LCAT	lecitin-cholesterol-acyl-transferase
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
Lp(a)	lipoprotein-a
LPL	lipoprotein lipase

LRC	lipid research clinic
NPV	negative predictive value
NCEP	national cholesterol education program
non-HDL-C	non-high density lipoprotein cholesterol
OR	odds ratio
PCSK9	pro-protein convertase subtilisin/kexin type 9
PPV	positive predictive value
SR-B1	scavenger receptor B type 1
STRIP	Special Turku coronary Risk factor Intervention Project
TC	total cholesterol
TG	triglyceride
USF1	upstream transcriptor factor -1
VCAM-1	vascular cell adhesion molecule-1
VLDL	very low density lipoprotein
VLDL-C	very low density lipoprotein cholesterol

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to with Roman numerals in the text.

I Lapinleimu J, Lapinleimu H, Nuotio IO, Rönnemaa T, Simell OG, Viikari JSA. Expression of common familial dyslipidemias in early childhood. Atherosclerosis 2009; 204: 573-9.
DOI: http://dx.doi.org/10.1016/j.atherosclerosis.2008.10.004

II Lapinleimu J, Nuotio IO, Lapinleimu H, Simell OG, Rask-Nissilä L, Viikari JSA. Recognition of familial dyslipidemias in 5-year-old children using the lipid phenotypes of parents. The STRIP project. Atherosclerosis. 2002; 160: 417-23.
DOI: http://dx.doi.org/10.1016/S0021-9150(01)00593-7

III Lapinleimu J, Raitakari O, Lapinleimu H, Pahkala K, Rönnemaa T, Simell OG,Viikari JSA. High lipoprotein(a) concentrations are associated with impaired endothelial function in children. The Journal of Pediatrics 2015; 166: 947-52.
DOI: http://dx.doi.org/10.1016/j.jpeds.2014.12.051

Additional results that are previously unpublished are also presented.

1. INTRODUCTION

Atherosclerosis is a continuous life-long process that has its inception in infancy (Strong and McGill 1962; Pesonen et al. 1975; Berenson et al. 1992). Clinical, atherosclerosis-related morbidity has shifted in the high income countries from the middle age to the oldest age groups while coronary heart disease (CHD) mortality has dropped by about 60% in 40 years since 1969 in the Finnish population (Official Statistics of Finland 2015). The favorable mortality trend may also slow down as many risk factors, such as high total cholesterol (TC) level, obesity and type 2 diabetes, are becoming more frequent (National Institute for Health and Welfare 2015). Prevention should involve influencing the general population, and early intervention of high risk individuals. Intervention in childhood includes implementing a healthy diet, preventing smoking and obesity and promoting sufficient physical activity. Lowering non-high density-lipoprotein cholesterol (non-HDL-C) concentrations and low density-lipoprotein cholesterol (LDL-C) concentrations of an individual child together with the whole family can be done safely with an individualized diet (Kelley et al. 2004; Niinikoski et al. 2007).

Dyslipidemias are extreme levels of the lipids and lipoproteins that may be harmful. Dyslipidemias may occur in several family members and over several generations. Hypercholesterolemia with high LDL-C concentrations, hypertriglyceridemia, combined hyperlipidemia, isolated low high density-lipoprotein-cholesterol (HDL-C) concentrations and high lipoprotein(a) [Lp(a)] concentrations can be regarded as such deviations. Environmental factors affect the expression of the dyslipidemias. A family history of CHD is not sensitive enough to identify dyslipidemias in children (Ritchie et al. 2010). Dyslipidemia aggregations are described as familial dyslipidemias: familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCHL), familial hypertriglyceridemia and familial low HDL-C concentrations (Genest et al. 1992) and, furthermore, high Lp(a) concentrations have a strong familial link (Sveger et al. 2000; Guardamagna et al. 2011). High Lp(a) concentrations are also associated with atherosclerotic disease (Kamstrup et al. 2008).

The stability, evolution, distinction and significance of an abnormal lipid value of a child are not well known (Tolfrey et al. 1999; Friedman et al. 2006; Magnussen et al. 2011), with the exception of monogenetic FH. Although there is a lack of knowledge, there are recommendations to screen for dyslipidemias in early childhood. Selective targeted screening of childhood lipids has been recommended if there is a family history of premature coronary heart disease or if the parents have dyslipidemia (American Academy of Pediatrics 2011; Catapano et al. 2011; Working group. 2013; Dixon et al. 2014; Watts et al. 2014)

Flow mediated dilatation (FMD) is a sensitive method used universally to study endothelial function in childhood (Celermajer et al. 1992). Endothelial dysfunction and a low FMD value precede atherosclerosis progression (Halcox et al. 2009) and predict cardiovascular mortality in a population with high risk for cardiovascular disease (CVD), (Aggoun et al. 2008).

The STRIP (Special Turku coronary Risk factor Intervention Project) -study comprised 1054 families with 1062 index children, who were randomized at age 6 months together with their parents to either a control group or an intervention group. The latter received intensive dietary advice aimed at a total fat consumption (30 to 35% of the total energy), of which one third saturated, one third monounsaturated and one third unsaturated fat, and at a daily cholesterol intake below 200 mg (Lapinleimu H et al. 1995). Over time, the study also included other aspects of CVD prevention, e.g., physical activation and avoiding smoking and obesity. The children in the intervention group, and especially the boys in that group, achieved significantly lower non-HDL-C levels than the children in the control group during childhood (Niinikoski et al. 2007). The boys in the intervention group had also higher FMD values at age 11 years than the boys in the control group, which indicates better endothelial function in the intervention group (Raitakari et al. 2005).

This thesis used mainly the first 11 years of the family lipid data of the STRIP study. To learn about the value of repeated annual serum lipid measurements in childhood, the non-HDL-C and HDL-C concentrations were analyzed from study point 1 to 7 years in the control group and their parents. The concentrations were used to identify persistently dyslipidemic children based on the values of the children and their parents.

The lipid phenotypes were based on fasting samples of LDL-C, triglyceride (TG) and HDL-C when the child was 5 years old. The phenotypes and their intra-familial associations were used to evaluate the prevalence of the common dyslipidemias in the nuclear families of at least one parent and the child. The associations between the Lp(a) concentrations and FMD values at age 11 years were studied. Finally, we studied the associations between predefined dyslipidemias and high Lp(a) concentration (> 500 mg/l) at age 5 years and subsequent endothelial function at age 11 years.

2. REVIEW OF THE LITERATURE

2.1 Atherosclerotic process and its consequence

2.1.1 Epidemiology of atherosclerosis

2.1.1.1 Cardiovascular disease and coronary heart disease

CVD is a complication of advanced atherosclerosis and constitutes a major global health burden. In 2008 CVD accounted for 48% of the non-communicable deaths worldwide (World Health Organisation 2014). The atherosclerotic process begins early in life and lasts lifelong (Strong and McGill 1962; Pesonen et al. 1975; Berenson et al. 1992). Atherosclerosis causes structural and functional changes and defects. The final outcome of atherosclerosis is clinical CVD, such as CHD, ischemic cerebral disease, arterial kidney disease and intermittent claudication. CHD is the most apparent and frequent of these diseases. The TC levels of males aged 22 years (mean) predict CHD 30 years later (Klag et al. 1993). Mortality and morbidity related to CHD or CVD are used for statistical comparisons between time periods and geographical regions. Figures show that, globally, over 80% of all cardiovascular deaths occur in low and middle income countries (World Health Organization 2014; GBD 2013 Mortality and Causes of Death Collaborators 2015). Currently, the highest prevalences are encountered in the countries that once belonged to the former Soviet Union or belong to eastern Europe, India and Middle East. In high income countries, including Finland, CVD and especially CHD have declined drastically during the last 30 years from the highest prevalence in the world to the average European prevalence level (Nichols et al. 2014). There is a demographic shift of the epidemiology of CVD from middle age to the elderly and from men to women. CVD may be considered the result of a combination of structural and functional pathological changes resulting from several known and unknown risk factors (Hopkins and Williams 1981). Usually, the time for CVD to develop covers decades and involves not only atherosclerosis, but also pathological blood clotting.

2.1.1.2 Atherosclerosis and autopsy studies

Structural changes can be traced by autopsy studies of young casualties of violence and war. These studies reveal that atherosclerosis develops over the entire life span. Autopsy studies display the result of the overall risk (Table 1). In an autopsy study of 150 victims aged 6 to 30 years, the *ante mortem* levels of TC, LDL-C and the ponderal index correlated with the degree and spread of atherosclerotic changes (Berenson et al. 1992). Later, larger cohorts confirmed these results (Strong et al. 1999).

Table 1. Atherosclerosis in the autopsy studies of the children and young adults with accidental deaths.

Year	Author	Location	N	Age (years)[c]	Site	Major findings
1925	Zinserling	St.Petersburg	320	1 to 15y	aorta	All children had fatty streaks by 9y
1948	Eskola	Finland	1203[a]	1 to 19y	aorta	Fatty streaks 0 to 9y: 8%, 10 to 19y: 23%
1992	Berenson et al.	U.S.A.	93/204[b]	2 to 39y	aorta and coronary arteries	The extent of the plaques correlated to the number of the risk factors.
1999	Strong et al.	U.S.A.	2876	30 to 34y	right coronary artery	The extent of the plaques correlated to the non-HDL-C and inversely to HDL-C concentrations. Raised lesions 15 to19y: 10%, 19 to 30y: 30%

[a)] Number in the group of children and adolescents.
[b)] Number of the autopsies with antemortem data/ all autopsies
[c)] Range, y: years of age

Table 2. Atherosclerosis in the autopsy studies of the war casualties.

Year		Casualties from	N	Age (years)[a]	Site	Main Findings
1915	Mönckeberg	First world war	140	27.7	aortic intima	fatty streaks increased by age
1953	Enos et al.	Korean war	300	22.1	coronary artery	77% modest and 15% severe lesions
1971	McNamara et al.	Vietnam war	105	22.1	coronary artery	45% modest and 5% severe lesions
2012	Webber et al.	Afghanistan and Iraqi wars 2001 - 2010	3832	25.9	coronary artery	8.5% modest and 2.3% severe lesions

[a)] mean

Mönckeberg reported in 1915 was an increasing prevalence of fatty streaks in the aortic intima over age among autopsied German war casualties (Mönckeberg 1915). Subsequent studies showed a similar but less pronounced trend in the prevalence of atherosclerosis, but this may partly have been due to variable definitions varied and different age distributions (Table 2). In 2001–2011 Webber et al. reported the autopsy results of 3,832 U.S. service members aged 18–59 years (mean 25.9 years), 98.3% were male. The overall prevalence of atherosclerosis in the coronaries was 8.5% and of severe atherosclerosis 2.3%. Comparisons of the occurrence of risk factors associated with atherosclerosis showed that dyslipidemias, hypertension and obesity are the most frequent predictors with prevalence ratios of 2.09, 1.88 and 1.47, respectively (Webber et al. 2012).

2.1.1.3 Atherosclerosis imaging studies

Structural atherosclerosis can be studied in children non-invasively by measuring the intimal media thickness (IMT) with ultrasound. The method is predicts CVD events with moderate accuracy (Polak et al. 2011). It is usually conducted from the carotid bulb, but can be measured from the abdominal aorta of children, as well. IMT results support the validity of risk factors operative already in early childhood as predictors of CHD (Magnussen et al. 2009; Juonala, Magnussen et al. 2010). Arteries and arterial calcifications can be imaged and scored by computed tomography or magnetic resonance imaging (Mahoney et al. 1996; Hartiala et al. 2012). Interestingly, computed tomography has been used in the Horus study of the coronary arteries. In that study 137 ancient mummies of different cultures were imaged. The study revealed that significant atherosclerosis including calcified plaques existed even 4000 years ago in less than half of the members of the royal families of Egypt and Americas, as well as among preserved hunter-gatherers (Thompson et al. 2013). This shows that the western life style is not the only environment disposing on atherosclerosis.

2.1.1.4 Estimation of functional atherosclerosis

Functional studies of atherosclerosis assess the properties of the arteries and especially endothelial function. In these studies, the arterial pressures of the brachium and the ankle are compared (ankle-brachial index), the velocity of the pulse wave from the heart to the limb is measured or the stiffness of arterial dilatation on pulse pressure is measured.

The endothelial function of the coronary arteries has been tested with intra-coronary infusion of acetylcholine followed by imaging of the dilatation of the more distal segment. This dilatation has been held as a gold standard of flow-mediated dilatation (Miner et al. 2010). Paradoxical vasoconstriction occurs in obstructed atherosclerotic coronaries (Ludmer et al. 1986). Attenuated dilatation is associated with certain conditions, such as premature CHD in the family (Clarkson et al. 1997), cigarette smoking (Lavi et al.

2007), insulin resistance (Shinozaki et al. 2001), young men with hypertriglyceridemia (Lundman et al. 2001), small LDL-particle size (Vakkilainen et al. 2000) and high Lp(a) concentration (Tsurumi et al. 1995; Schachinger et al. 1997). This method is able to predict CHD events (Schachinger et al. 2000). However, it is invasive, expensive and not very suitable to study people without apparent disease.

FMD of the brachial artery after temporary occlusion is a widely used method and has replaced the acetylcholine method. Attenuation of the brachial artery FMD was first reported in FH and CHD patients (Celermajer et al. 1992). Later, coronary acetylcholine testing and flow-mediated vasodilation in the brachial artery were shown to correlate strongly (Anderson et al. 1995). Halcox et al. showed that an attenuated FMD predicts thickening of the carotid IMT in adults (Halcox et al. 2009). Finally, attenuation of the FMD of the brachial artery predicts CHD events (Hafner et al. 2014). Subsequently attenuation of the FMD in childhood associates with low birth weight (Leeson et al. 1997), premature CHD or CHD event history in the family (Clarkson et al. 1997; de Jongh, Lilien, Bakker et al. 2002), FCHL (Mietus-Snyder and Malloy 1998), high LDL-C concentration (Jarvisalo, Rönnemaa et al. 2002), hyperlipidemia (Engler et al. 2003), type 1 diabetes (Järvisalo et al. 2004), passive and active smoking (Kallio et al. 2007), physical inactivity (Pahkala et al. 2008), prepubertal obesity (Aggoun et al. 2008), markers of the inflammation (Järvisalo, Harmoinen et al. 2002; Glowinska-Olszewska et al. 2007). Furthermore, lowering of high LDL-C concentrations with statin treatment seems to improve FMD in children with FH (de Jongh, Lillen, op't Roodt et al. 2002; Ferreira et al. 2007).

High triglyceride or low HDL-C concentrations were not associated with attenuation of the FMD in most studies (Järvisalo, Rönnemaa et al. 2002; Juonala et al. 2008), but Toikka et al. did report attenuated FMD in young men with persistently low HDL-C concentrations (Toikka et al. 1999). A low HDL-C concentration is associated with endothelial dysfunction in Asian Indians (Packard et al. 2005).

2.1.2 Pathology of atherosclerosis

2.1.2.1 Endothelial dysfunction

The medium sized and large arteries are lined luminally by a one-cell thick layer: the endothelium. The endothelium functions as a selective barrier between the compounds of the blood and tissues, regulates hemostasis by preventing contact between tissue and blood and acts as a site for coagulation and thrombolysis. The endothelium also regulates inflammation and vascular tone. Endothelial dysfunction may be due to several reasons and contributes to atherosclerosis. An epigenetic local response to shear stress may explain why permeability may increase locally (Dunn et al. 2014). The endothelium regulates local blood perfusion according to oxygen demand. An optimal vessel tone

requires that the vessel can constrict and dilate adequately. The endothelium reacts to internal stimuli, e.g., shear stress, transmural local pressure and temperature and to several external stimuli, including temperature and mental stress. Local needs of vascular dilatation are signalized from the endothelium to the smooth muscle layer primarily by the release of nitric oxide. Nitric oxide gas is synthetized from L-arginine by endothelial nitric oxide synthetase (Yang et al. 1991).

2.1.2.2 LDL-C in the macrophages

Locally increased endothelial permeability enables LDL-particles to penetrate the endothelium and access deep into the intima. Oxidative modification of the LDL-C moiety has been considered as a prerequisite for its internalization into the macrophages. Radiolabeled LDL accumulates in foam cells within atherosclerotic plaques (Iuliano et al. 2000). The severity of the CHD events is associated with the degree of LDL-C oxidation (Ehara et al. 2001; Tsimikas et al. 2003).

A high LDL-C concentration activates a subclass of circulating monocytes (Kelley et al. 1988; Wu et al. 2009). These monocytes adhere easily the endothelium, transmigrate into the intima and transform there into macrophages. One group of these macrophages is prone to initiate atheroma build-up, whereas another group is associated with atheroma regression. The atheroma-prone macrophages express receptors on their membrane, e.g., scavenger receptors A1 and CD36, which recognize and gather oxidized LDL-C into the cells (Podrez et al. 2000; Kunjathoor et al. 2002), (Figure 1).

Macrophages separate cholesterol and re-esterify it in their vacuoles thus macrophages become gradually foam cells. In this way, esterified cholesterol is stored and neutralized, but an excessive load of intracellular free cholesterol and cholesterol crystals have been found to initiate an inflammatory process, which may lead to foam cell apoptosis (Rajamäki et al. 2010; Seimon et al. 2010). Inflammation is induced by crystal-sensitive intracellular inflammasomes which initialize the cleavage and secretion of interleukine-1 family cytokines and further inflammation (Duewell et al. 2010). Foam cell apoptosis releases cholesterol and toxic compounds and promotes the inflammatory process with further formation of crystalline cholesterol as a result. Then the lipid core or multiple cores of the atheroma arise (Figure 1). Medial smooth muscle cells begin to proliferate into the intima and may constrict the vessel. In the advanced stage, connective tissue fibrosis is added to the atheroma and tissue calcification occurs. Between the atheroma plaque and the vessel lumen there remains a roof of connective tissue that is vulnerable to rupture. When a rupture occurs, the consequence is that plasma thrombosis factors gain contact with thrombogenic factors and acute thrombus formation starts in the complicated atheroma (Figure 1). The emergence of a clinical cardiovascular event depends on the site and size of the thrombus.

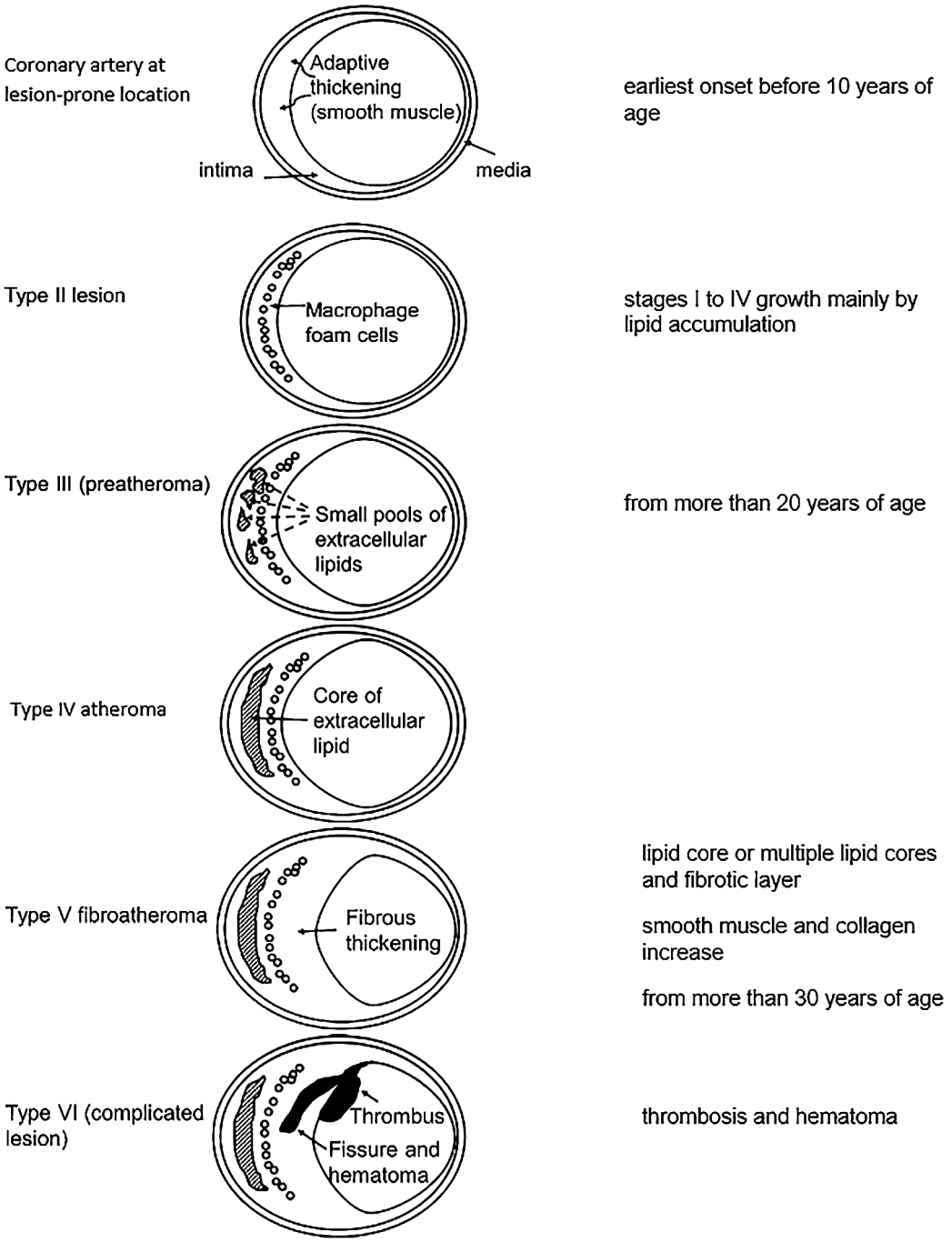

Figure 1. Stages and typical age of advancing atherosclerosis (modified from Stary 1995).

A macrophage can get rid of its cholesterol load by reverse cholesterol transportation and efflux. An intracellular cholesterol load stimulates nuclear receptors to increase the ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) to shift the cholesterol to the HDL-particles. The capacity of the cholesterol efflux from the macrophage is inversely associated with the degree of atherosclerosis and CVD events (Khera et al. 2011; Rohatgi et al. 2014). In turn, a high HDL-C level may result from a strong efflux capacity which may be genetically mediated (Scherrer et al. 2015).

2.1.3 Common atherosclerosis risk factors

Mendelian randomization governing gene polymorphism regulates LDL-C levels and is associated with CHD. This naturally suggests that LDL-C is a causal factor for the development of the atherosclerosis (Ference et al. 2012). Since there were already 246 suggested CHD risk-factors back in 1981 (Hopkins and Williams 1981), this figure must have increased since. Nevertheless, there are only a few classic risk factors which are strong predictors of CHD (Berenson et al. 1998). Age, gender and the family history of premature CHD are untreatable risk factors, but five risk factors are and should be targeted: hypertension, smoking, obesity, insulin-resistance, sedentary life style and dyslipidemia.

2.1.4 Lipid metabolism

2.1.4.1 Cholesterol metabolism

Cholesterol synthesis is a multiple stage process which may be considered to start from two carbon acetyl-coenzyme A moieties to 3-hydroxy-3-methylgutaryl-coenzyme A (HMG-CoA) and further to mevalonate and through multiple steps to the 30-carbon compound squalene and finally to cholesterol, which has 27 carbon atoms (Figure 2). The enzyme that reduces HMG-CoA conducts the rate limiting step in the synthesis of cholesterol. Cholesterol is an essential constituent of lipid membranes and a precursor of steroid hormones, including vitamin D. In addition to *de novo* synthesis, cholesterol is absorbed from dietary source from the intestine into the systemic circulation and this constitutes another source of cholesterol to the body.

When the liver receives a high quotient of cholesterol either from the diet or from tissues through reverse transportation, cholesterol is taken up through its receptors and ultimately excreted into the bile. It is also made to the bile. Most of the bile acids and about half of the cholesterol content is taken up again from the jejunum, and thus cholesterol is partly recycled to the liver.

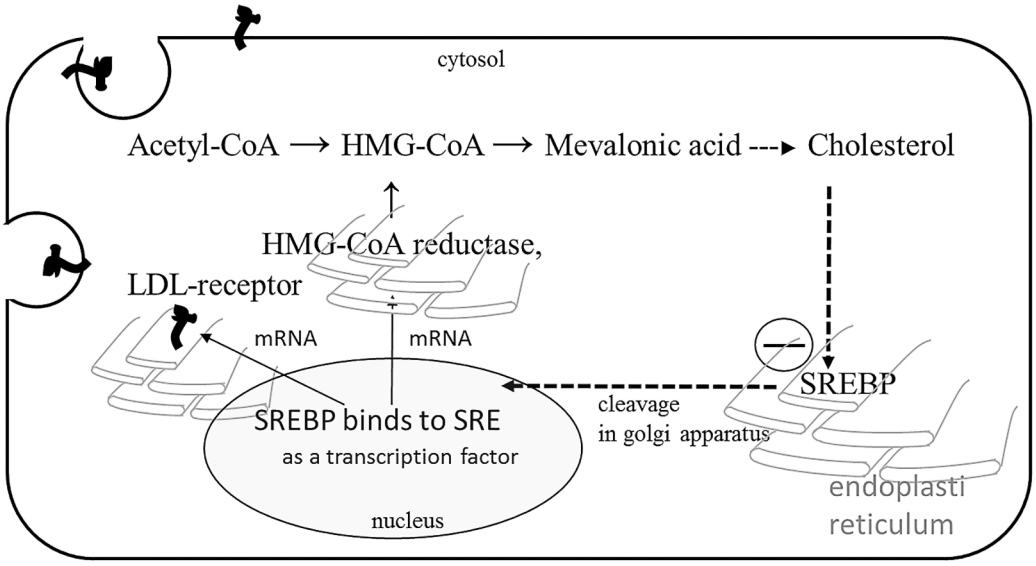

Figure 2. Cholesterol synthesis and its intracellular regulation. Cholesterol is synthetized from Acetyl-CoA through HMG-CoA to Mevalonic acid. The latter is a cholesterol production rate limiting step and regulated by HMG-CoA reductase. HMG-CoA reductase is inhibited by statins. Mevalonic acid gives negative feedback to reduce the HMG-CoA reductase activity. Cholesterol synthesis is regulated by negative feedback by SREBP on the genes for the HMG-CoA reductase synthesis and other genes. Low intracellular level of cholesterol increases the synthesis of enzymes in cholesterol production from Mevalonate and production of LDL-receptors. In addition, there is sterol- independent phosphorylation activation of HMG-CoA reductase and hormonal regulation to SREBP. (Modified from Williams textbook of endocrinology, 12th ed. 2012 and Lippincott's Illustrated reviews of biochemistry 3rd ed. 2005)

2.1.4.2 Triglycerides and triglyceride rich lipoprotein-particles

TG:s are formed from the dietary fats and TG metabolites: free fatty acids (FFA), glycerol and 2-monoacylglycerol in the intestinal enterocytes. When the lipids, cholesterol, phospholipids and TG, fuse in the cytosol of enterocytes with apolipoprotein-B48, chylomicrons (CM) are formed. CM are large 200 to 600 nm sized lipoproteins. Their core consists of more than 90% TG. Proprotein convertase subtilisin kexin type 9 (PCSK9) stimulates TG-rich CM synthesis (Rashid et al. 2014). Post prandially, CM are transported to the mesenteric lymph before entering the circulation where they contribute to the postprandial increase in TG concentrations. Subsequently lipoprotein lipase (LPL) hydrolyzes FFA from the CM to produce fuel for the heart and skeletal muscles and for storage in the adipose tissue. CM remnants are finally bound via apoE-ligand to the LDL-receptors of the liver and removed from the circulation (Figure 3). If the cholesterol content of a cell is high due its own cholesterol production or dietary supply, LDL-receptors are downregulated (Kovanen et al. 1981).

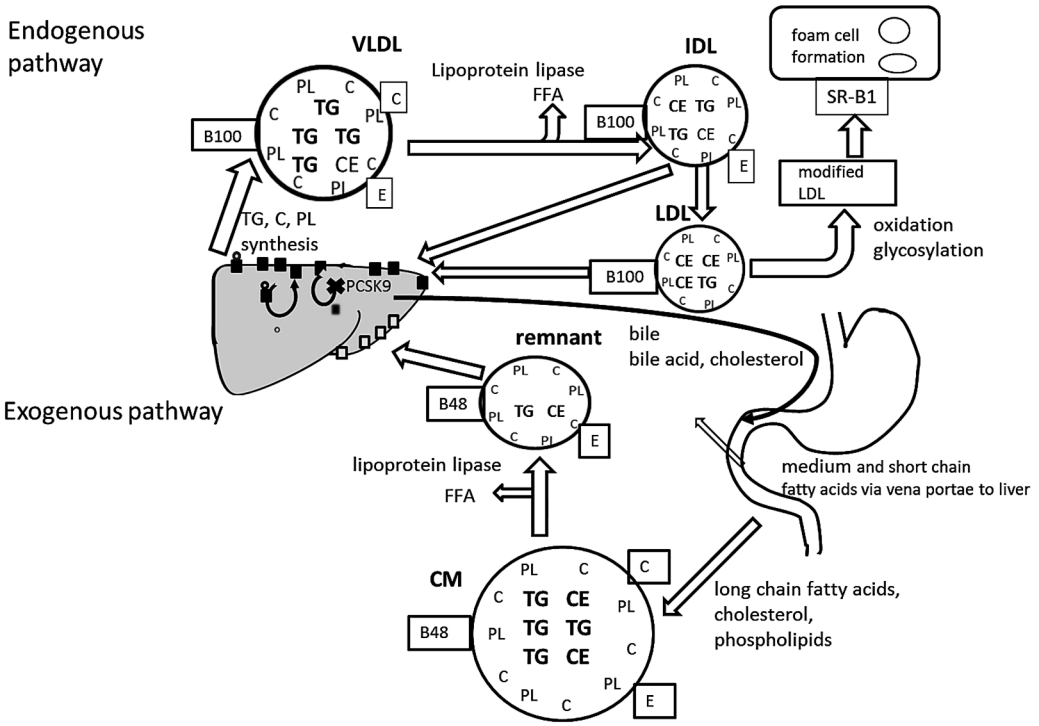

Figure 3. Chylomicron, endogenous and exogenous lipid and lipoprotein pathways. Endogenous pathway: VLDL from the liver is metabolized to IDL and finally to LDL. LDL-receptors bind to the LDL-B_{100} and IDL-apolipoprotein E and they are internalized into the liver. The LDL-receptors are recycled to the surface of the cells. There, the receptors may be bound to PCSK9 which prevents LDL-receptor recycling. Exogenous pathways: CM are formed in the intestinal mucosa, CM transport long-chain fatty acids, cholesterol, cholesterol esters, phospholipids and TG and supply fatty acids from TG to the tissues. They form CM remnants and enter the liver through the CM remnant receptor. PL: phospholipids, C: free cholesterol, CE: cholesterol esters, TG: triglyceride, B_{100}: apolipoprotein B100, B_{48}: apolipoprotein B_{48}, C: apolipoproteins C (C2 and C3), CM: chylomicrons, E: apolipoprotein E, FFA: free fatty acids, IDL: intermediate density lipoprotein, LDL: low density lipoprotein, SR-B1: scavenger receptor B1, PCSK9: proprotein convertase subtilisin/ kexin type- 9, VLDL: very low density lipoprotein, ■ : LDL-receptor, □ : CM remnant receptor.

The liver produces very low density lipoprotein (VLDL) particles (sized 30– 90 nm) to supply fats for the tissues in a two-step process. First, partially lipidated apolipoprotein-B (apoB or $apoB_{100}$) is formed in the endoplasmic reticulum and next TG, cholesterol, cholesterol esters and phospholipids are added to the particle before rate-controlled release of VLDL. The tissues that use fatty acids as energy secrete LPL to catalyze FFA from the TG within CM and VLDL. LPL production is highly regulated by apoC2 and other apolipoproteins and the proteins of the adipose tissue (Meyers et al. 2015). The tissues express also VLDL-receptors which bind VLDL. This process leads to the formation of VLDL remnants that are, as are the CM remnants, inflammatory. This leads to macrophage recruiting to the arterial wall; these macrophages may pass the

endothelium (in contrast to the large lipoprotein particles). These inflammatory particles, if in high concentration, activate a specific subset of circulating monocytes which shift from the circulation into the endothelium (Ting et al. 2007). They also upregulate Tumor Necrosis Factor-α (TNF-α) which leads to increased expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) in the aortic endothelial cells (Saja et al. 2015).

2.1.4.3 Low density lipoprotein

The VLDL residues lose TG, apolipoproteins E and C2 and thus LDL-particles (size 20 nm) are formed. LDL-particles consist of cholesterol esters and some TG in the core, phospholipids and cholesterol on the surface and one apoB molecule. The task of the LDL-particles is to supply cholesterol to the tissues. A high proportion of TG in relation to the cholesterol esters in VLDL (as the consequence of the TG load in the liver) would lead to a low content of cholesterol per LDL-particle. Thus, a load of TG leads to a small LDL-particle size and an unfavorable lipid profile that facilitates the traffic of these particles through the endothelium and to initiate atheroma building (Taskinen et al. 1986) (Phillips and Perry 2015).

2.1.4.4 High density lipoprotein

Apolipoprotein A1 (apoA1) is produced and secreted from the liver and intestine. ApoA1 interacts with the cholesterol-phospholipid transporter ABCA1 to receive lipids from the peripheral tissues and nascent primordial HDL is formed (Sahoo et al. 2004), (Figure 4). Cholesterol esters catalyzed by lecithin-cholesterol-acyl transferase (LCAT) accumulate into the core of the discs to form mature, spherical HDL_3-particles. Gradually HDL_3-particles receive cholesterol esters from CM or VLDL particles and thus become buoyant HDL_2 particles (size 10 nm). Physical activity, insulin effect and female gender increase the HDL_2 concentration through increased LPL activity (Gordon et al. 1996, Despres et al. 2000).

2.1.4.5 Cholesterol reverse transport and efflux capacity

The main function of HDL-C is reverse transport of cholesterol from the tissues to the liver (Miller and Miller 1975). Since ABCA1 acts in the liver, the tissue cholesterol efflux from the macrophages to the mature HDL-particles is promoted by ABCG1. Scavenger receptor type B1 (SR-B1) is an alternative receptor-gate for reverse transportation. Cholesterol ester transfer protein (CETP) catalyzes the exchange of acquired cholesterol from HDL to VLDL for TG. When mature HDL_2 returns to the liver carrying a load of cholesterol, hepatic lipase triggers HDL_2 to release non-esterified cholesterol and surface rafts to the liver and thus it regenerates HDL_3 from HDL_2. Subsequently, attached apoE enables the HDL-particle to be internalized into the liver. In addition, some HDL_2 may be disassembled as it binds to the SR-B1 receptor of the liver. The residual is recycled to

the plasma and may be removed by the kidneys (Horowitz et al. 1993). The cholesterol efflux capacity from the macrophages to the HDL-particles correlates inversely with CHD events (Khera et al. 2011; Saleheen et al. 2015).

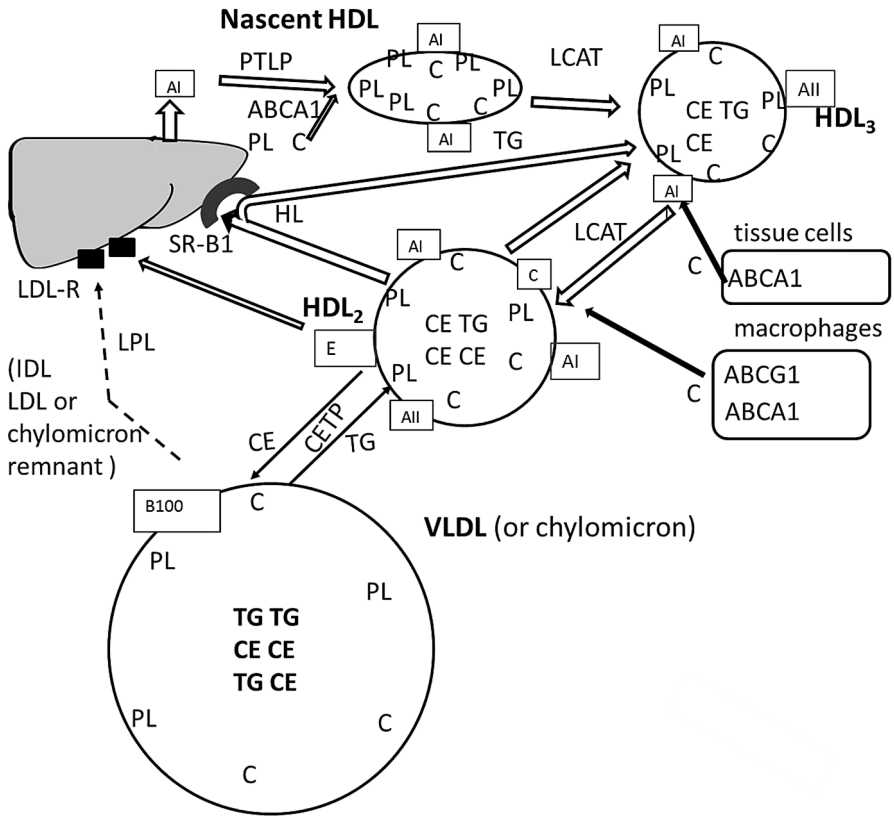

Figure 4. High density lipoprotein (HDL) metabolism in reverse cholesterol transportation. A1 produced by the liver or intestine receives a surface of cholesterol and phospholipids to form nascent HDL. Maturing HDL_3 accumulates esterified cholesterol in its core as excess macrophage cholesterol effluxes to HDL. LCAT mediates buoyant HDL_2. CETP mediates release of cholesterol from HDL in exchange for triglycerides with VLDL or chylomicrons. These particles convert to smaller particles and return cholesterol to the liver. HDL_3 may also bind to SR-B1 on the liver and selectively release cholesterol to the liver to recirculate as HDL_3. Some HDL may enter liver catabolism through apoE-mediated binding to the LDL-receptor. PL: phospholipids, C: free cholesterol, CE: cholesterol esters, TG: triglyceride, B100: apolipoprotein B100, FFA: free fatty acids, SR-B1: scavenger receptor-B1, VLDL: very low density lipoprotein, IDL: intermediate density lipoprotein, LDL: low density lipoprotein. AI: apolipoprotein-A I, AII: apolipoprotein-A II, PTLP: phospholipid transferring protein, LCAT: lecitin cholesterol acyltransferase, CETP: cholesterol ester transferring protein, HL hepatic lipase, ABCA1: adenosine triphosphate-binding cassette protein A1, ABCG1: adenosine triphosphate-binding cassette protein G1. LPL: lipoprotein lipase.

2.2 Dyslipidemias

2.2.1 Phenotypical classification of dyslipidemias

The hyperlipidemia classification according to Fredrickson is based on the electrophoretic mobility of the lipoproteins (Fredrickson et al. 1967). Normally, four different bands can be separated: CM at the origin, pre-beta-lipoprotein, beta-lipoprotein and alpha-lipoprotein. Beta-lipoprotein corresponds to LDL, pre-beta-lipoprotein to VLDL and alpha-lipoprotein to HDL. The classification of the dyslipidemias was based on the increased intensity of the bands. The Fredrickson classification is shown in Table 3. In type III there is a broad beta-fraction in electrophoresis and IDL in the ultracentrifugation. Most dyslipidemias can be classified as types IIA, IIB and IV while I and III andV are rare. The alpha band representing the position of HDL was omitted from the original classification types although the band was readily visible by electrophoresis. The main purpose of Friedewald's classification was to describe different distributions of TG metabolism.

Table 3. Fredrickson (1967) classification of the hyperlipidemias

Phenotype	Electrophoretic band emphasized	Ultrcentrifugate lipoprotein fraction elevated	Lipid concentration(s) elevated
I	CM	CM	very high TG, TC
IIA	β-LP	LDL	TC
IIB	β-LP and pre-β-LP	LDL and VLDL	TC and TG
III	broad β-LP	IDL	TC and TG
IV	pre-β-LP	VLDL	TG
V	CM and pre-β-LP	VLDL and CM	very high TG, TC

CM: chylomicron, β-LP: β-lipoprotein, pre-β-LP: pre-β-lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, IDL: intermediate-density lipoprotein, TG: triglycerides, TC: total cholesterol

2.2.2 Secondary vs primary dyslipidemias

Before a diagnosis of primary dyslipidemia is justified, secondary reasons must be excluded and treated. Secondary elevation of TC, LDL-C and TG can be caused by hypothyroidism, anorexia nervosa, diabetes, chronic liver disease or kidney disease with proteinuria (Daniels et al. 2011). Hyperlipidemia can be associated with high cortisol or steroid hormone levels due to medication or an endocrine disease. Retroviral medication causes marked hypercholesterolemia (Kumar et al. 2006). The survivors of the childhood cancer may suffer from dyslipidemia (Taskinen M. 2000). Severe hypertriglyceridemia has been associated to the treatment with asparaginase and steroids (Bhojwani et al 2014). A low HDL-C level can be associated with obesity, and isolated low HDL-C concentrations with untreated celiac disease. In addition, substantial continuous alcohol use may reduce cholesterol levels. Childhood obesity is associated with high LDL-C, TG or low HDL-C concentrations in various combinations (Ylitalo 1981; Korsten-Reck et

al. 2008). For practical purposes, most treatable secondary dyslipidemias are routinely screened by clinical examination and thyroid, liver and kidney function testing plus exclusion of diabetes and proteinuria.

2.2.3 High low density lipoprotein cholesterol concentration, type IIA

LDL-C is an essential and necessary requirement for atherosclerosis and CHD. TC may be considered as a surrogate to LDL-C. Cholesterol is present in the core of atheromas, and plaques are not formed unless cholesterol is available in animal models (Anitschkow and Chalatow 1913). Results of the seven countries study (Keys et al. 1986) show that cardiovascular mortality and TC concentration are associated among males. LDL-C level predicts CHD events in the multifactorial risk models of the Framingham study (Castelli 1984). Decreasing LDL-C concentrations by medical treatment decreases the incidence of CVD in relation to the LDL-C concentration reduction (Baigent et al. 2010). Mendelian randomization of the LDL-C level gene polymorphism suggests that LDL-C is a causal factor in the development of atherosclerosis (Ference et al. 2012). However, a single measurement of LDL-C in middle aged subjects does not predict CHD events as well as the cumulative exposure to dyslipidemia measured either with non-HDL-C or LDL-C (Navar-Boggan et al. 2015).

2.2.3.1 Monogenetic autosomal dominant hypercholesterolemia

The most severe structural hypercholesterolemia is FH. It is caused by impaired re-uptake of LDL-C from the circulation. FH is usually caused by a defective LDL-receptor (Brown and Goldstein 1974; Vuorio, Turtola, Piilahti et al. 1997). The LDL-receptor defects vary in significance from functional defects and defective externalization of the receptor to the cell surface to agenesis of the receptor. The degree of the defect correlates with the cholesterol level and is associated with the occurrence of CHD (Guardamagna. Restagno et al. 2009). In the heterozygotic form of FH one allele is usually intact, but in the rare homozygotic form of FH both alleles, and thus all LDL-receptors, are defective, which leads to a very high cholesterol concentration and extraordinary premature atherosclerosis. Not only the receptor itself may be deficient, but there are gain-of-function mutations of the genes governing PCSK9 (Chen et al. 2005). This hyperfunction results in a reduced number of LDL-receptors on the cell surface. Thus, PCSK9 gain-of-function, which is genetically rare, may impair cholesterol removal and lead to hypercholesterolemia reminding of the situation of functional LDL-receptor defects. Finally, apoB may be defective and unable to bind properly to the LDL-receptor (Rauh et al. 1992).

The prevalence of the severe heterozygotic FH in Finland is assumed to be about 1/600 (Lahtinen et al. 2014), but based on prevalence figures of homozygotes of many

other populations, this may be an underestimation. Most of the heterozygotic FH cases remain undiagnosed and untreated. However, the impact of FH on CHD and the LDL-C concentration depends on environmental factors which attenuate the expression and hazardousness of FH. This was exemplified in urban China of the 1990's, where physical work and limited nutrition were a part of daily life (Pimstone et al. 1997).

2.2.3.2 Common hypercholesterolemia

In the polygenic forms of hypercholesterolemia no single gene can be identified as the culprit. Since the condition is polygenic, wide family screening to second degree relatives or further is not useful, but intra-familial heritability of LDL-C may explain about 66% of the total variance explained by heredity, according to a report from Sardinia (Pilia et al. 2006). A number of single nucleotide polymorphisms mark hypercholesterolemia. Kathiresan et al. studied a population of 5,414 persons in the Malmö Diet and Cancer study using a score encompassing 11 common gene variants to explain common hypercholesterolemia and low HDL-C concentrations (Kathiresan et al. 2008). The score predicted a 0.5 mmol/l difference in LDL-C levels and was associated with later CHD events. The concept "polygenic FH" has been used for structural hypercholesterolemia that has features "classical" of FH, but which does not extend over first degree relatives (Talmud et al. 2013). No single mutation alone affecting LDL-C removal has been identified, but polymorphism of the genes that cause FH, such as the LDL-receptor, PCSK9 and apoB explain this type of hypercholesterolemia partly. Futema et al. developed a weighted score of six single nucleotide polymorphisms of the LDL-C regulating genes that explained partially hypercholesterolemia among single-mutation negative families where FH was suspected (Futema et al. 2015).

2.2.4 Low high density lipoprotein cholesterol concentration

The alpha-lipoprotein or HDL concentration associates negatively with CHD, as shown by many studies over the years (Barr et al. 1951; Nikkilä 1953; Miller and Miller 1975; Gordon et al. 1977; Assmann and Schulte 1992). Among young people a low HDL-C concentration predicts advanced atherosclerosis in adulthood especially the low HDL-C is associated with obesity (Magnussen et al. 2009). HDL levels in childhood associate negatively with the degree of the coronary artery atheroma. (Berenson et al. 1998; McGill et al. 2000). Among Finnish families with low HDL-C (selected by probands with low HDL-C concentrations and premature CHD) low HDL-C concentrations were associated with larger IMT than normal HDL-C concentrations (Alagona et al. 2002). A dyslipidemia characterized by low HDL-C concentrations is atherogenic and has been claimed to be a risk for CHD (Medina-Urrutia et al. 2011). Although HDL-C subunit concentrations predict even better atherosclerotic events than the total HDL-C concentration, the latter has not been replaced – obviously because it is a practical and

relatively cheap measurement (Alagona et al. 2002). Low HDL-C concentrations have also been associated with impaired endothelial function (Li et al. 2000) in young adults and adolescents (Kuvin et al. 2003; Sezgin et al. 2006).

2.2.4.1 Monogenetic low high density lipoprotein concentration

Monozygotic, familial low HDL-C concentrations are rare and extreme traits, but they serve as models to enhance our understanding of the much more common metabolic alternations of HDL. A mutated apoA1 gene may impair apoA1 binding to ABCA1 which will reduce the assembly of nascent HDL-C, leading to a reduced HDL-C concentration. Some of the low A1 concentration traits are associated with increased susceptibility to atherosclerosis. There are also mutations that protect against CVD in the A1 gene which produce a phenotype of very low apoA1 and HDL-C concentrations. These genes, e.g., $A1_{Milano}$, may lead to high HDL-C catabolism (Weibel et al. 2007). LCAT deficiency is a rare autosomal recessive condition associated with extremely low HDL-C and apoA1 concentrations. In the Finnish population, 5% of the lowermost HDL–C concentrations are explained by the $LCAT_{Fin}$ -mutation (Miettinen et al. 1998). These defects cause defective esterification of cholesterol in lipoproteins that leads to accumulation of cholesterol in the cornea, red blood cells, glomeruli and vessel walls. However, these deficiencies are not associated with an increased risk of CHD. Another autosomal codominant and extremely rare deficiency is the Tangier disease which is caused by a deficiency in ABCA1. This pattern has led to the recognition of more common familial deficiencies in ABCA1 function which associate with moderately low HDL-C concentrations (Wang et al. 2000).

2.2.4.2 Common low high density lipoprotein concentration

Common low HDL-C without high concentrations of VLDL-TG or LDL-C (isolated hypo-HDL) is a phenotype that is apparently related to the interaction of genetic and environmental factors or genetic factors alone. Familial and non-familial primary hypo-HDL may be linked to the polymorphism of apoA1, apoC3 or apoA4 and its prevalence is about 5% in the population. In a study of 63 non-obese, non-diabetic children no cases of defective apoA1 were identified, but the variations in LPL explained about 15% of the traits (Montali et al. 2015). Polymorphism of the endothelial lipase and ABCA1 genes explain the HDL-C variation reported from Japanese school children (Yamakawa-Kobayashi et al. 2003; Yamakawa-Kobayashi et al. 2004). Epigenetic mechanisms may impact on non-hereditary persistent dyslipidemia. A link between HDL-C concentrations and the degree of the methylation in the CETP and LPL genes has been described. The methylation status depicts the activity of gene reading (Guay et al. 2013).

2.2.5 Hypertriglyceridemia types

In a pediatric lipid clinic 76 children were referred for hypertriglyceridemia. The cause was in 42% FCHL, in 33% type IV, in 17% secondary to the life-style and obesity, in 4% familial lipoprotein lipase deficiency and in 2% type III dyslipidemia (Manlhiot et al. 2009). The genetic defects causing the most marked hypertriglyceridemia may be overrepresented among lipid clinic referrals.

2.2.5.1 Types I and V

Extreme hypertriglyceridemia associates with hyperchylomicronemia and may not be expressed fully in children although severe manifestations of type I and V have been detected before adulthood (Viikari et al. 1974). Type V may be associated to a defect in LPL or apoC3. CM-remnants are inflammatory which may explain the risk of pancreatitis associated with marked hypertriglyceridemia.

The gene variants of the *LPL* gene were studied in149 Italian patients with severe or moderate hypertriglyceridemia. In the group of severe hypertriglyceridemia with serum TG concentrations over 10 mmol/l 17.4% were homozygotes, 6% compound heterozygotes and 10% simple heterozygotes for the defective *LPL* gene, whereas of those with moderate hypertriglyceridemia only 5.6% were heterozygotes. In the severely high TG group almost half had a history of pancreatitis (Rabacchi et al. 2015). Carriers of loss-of-function mutations in *ANGPTL4,* which regulates LPL, had lower triglyceride levels than noncarriers and less CHD (Myocardial Infarction Genetics and CARDIoGRAM Exome Consortia investigators, 2016).

2.2.5.2 Type III

Rare type III dyslipidemia is associated with the homozygous allele E2 of the *apoE* gene. However, environmental factors, acquired diseases (such as hypothyroidism) and other genetic factors (such as the polymorphism of PCSK9) are needed to interact with the apolipoprotein E2-E2-genotype to exhibit the phenotype (Brouwers et al. 2014).

2.2.5.3 Type IV, common hypertriglyceridemia

Type IV dyslipidemia is characterized by mild to modest hypertriglyceridemia with no significant increases of LDL-C or CM. The common triglyceridemia phenotype is typically heterogenetic and it is a result of the interaction between genes and environmental factors (De Castro-Oros et al. 2014). The TG rich lipoprotein remnants are considered to be atherogenetic, as they enter the intima and are engulfed by lymphocytes that transform into foam cells forming the nucleus of the atheroma (Nordestgaard et al. 1992). High TG concentrations in young subjects are associated with CAD later in life

(Berenson et al. 1998). High TG concentrations are associated with impaired endothelial function of the coronary arteries (Sezgin et al. 2006), but the association is contentious (Schnell et al. 1999). The predictive value of high TG concentrations in childhood with respect to atherosclerosis in adulthood is poor.

2.2.5.4 Type IIB, combined hyperlipidemia

Combined hyperlipidemia is a form of dyslipidemia, where both LDL and VLDL are elevated at the same time in one person. This phenotype is associated with obesity and insulin resistance (Paramsothy et al. 2009). It has a genetic predisposition, but it can also associate with type 2 diabetes as such which is characterized by high fat consumption and VLDL efflux from the liver (Brouwers et al. 2008).

2.2.6 Familial combined hyperlipidemia

Familial combined hyperlipidemia (FCHL) was first described in 1973 independently by Goldstein et al and by Nikkilä and Aro as an autosomal dominant trait that was expressed over several generations and increased the occurrence of premature CHD. The expressed phenotypes were either IIA, IIB or IV or no hyperlipidemia. IIB was held as the central feature of the familial trait (Rose et al. 1973; Namboodiri et al. 1975). With these criteria the prevalence of FCHL in the healthy population has been estimated to be about 1–2% (Goldstein et al. 1973; Nikkilä and Aro 1973; Grundy et al. 1987) and among survivors of premature CHD around 10% (Genest et al. 1992).

Combined hyperlipidemia is characterized by modest to high TG, high TC, high non-HDL-C, high apoB, and low HDL-C concentrations as well as small LDL-C particle size (Nuotio I et al. 1996). This lipid phenotype is regarded as typical for the cardiometabolic syndrome, or syndrome-X, is often accentuated by obesity and is associated with insulin resistance. The phenotype is structural and genetic, and it is very typical characteristic for FCHL (Porkka et al. 1997).

FCHL is hyperlipidemia typically with high LDL or total cholesterol and TG concentrations. Elevated TG concentrations emphasize the dyslipidemic nature of the hypercholesterolemia. Hypertriglyceridemia also signifies the presence of small dense LDL which may be especially atherogenetic (Vakkilainen et al. 2002). Mortality is increased among affected family members (Austin et al. 2000; Voors-Pette and de Bruin 2001; Hopkins et al. 2003).

No general etiology for FCHL has been established despite the fact that there are apparent structural metabolic factors underlying FCHL: 1. Adipose tissue turnover is attenuated. 2. The liver may take in excessive amounts of FFA resulting in fatty liver and overproduction of VLDL (Brouwers et al. 2007). 3. Clearance of VLDL and its products

from the plasma may be impaired (Castro Cabezas 2003). This leads to excess VLDL remnants and small dense LDL. 4. LDL-C removal from the plasma is impaired. Indeed, polymorphism of the LDL-receptors and PCSK9-genes has been reported among affected FCHL family members (Brouwers et al. 2014). Several genetic defects in support of all these models have been reported.

Multiple enzymes that regulate lipid metabolism between the lipoproteins, e.g., LPL and hepatic lipase (HL), are defective and this results in the FCHL phenotype (Babirak et al. 1992; Pihlajamäki et al. 2000). There are defects in the apolipoprotein receptors, among them defects in the LDL-receptors (Civeira et al. 2008), in the apolipoproteins (e.g., apoE) and in the clearance of LDL-cholesterol from the plasma. Circulation of the TG to the liver is impaired. In chromosome 11 q 24–25, there is an *A1/C3/A4/A5* gene cluster that has also been widely studied and found to be mutated in some families (Wojciechowski et al. 1991), but this is not a common trait. These findings suggest that FCHL is a heterogeneous trait without common genetic aberrations.

A deviant form of the *upstream transcriptor factor-1 (USF1)* gene was first identified with genome wide scanning and associated with FCHL in 1998 (Pajukanta et al. 1998; Pajukanta et al. 2004) and has subsequently been found to be associated with FCHL in some Mexican, Dutch and U.S. populations (Huertas-Vazquez et al. 2005; Lee et al. 2007). USF1 is a transcription factor that regulates the reading of more than 40 genes regulating metabolism. Gain-of-function of the *USF1* gene may lead to fatty liver disease by stimulating fatty acid synthetase, which is the major factor for *de novo* lipogenesis and expression of glucokinase (Naukkarinen et al. 2005). However, *USF1* is only one, albeit ubiquitous, gene that can explain many of the characteristics of FCHL. *USF1* polymorphism has an impact on the polymorphism of fat tissue cathecolamine-induced lipolysis (Hoffstedt et al. 2005). The *USF1* gene at SNP rs2516839 has a deviant allele polymorphism (T versus C). In an autopsy study, the TT genoype compared to the CC was more strongly associated with advanced atherosclerosis and coronary calcification, and carried a 2.2-fold risk of sudden cardiac death compared to the CC genotype (Kristiansson et al. 2008).

To unify the abnormalities found among affected family members, new phenotype criteria for FCHL were adopted: the ApoB concentration is to be over the 90th percentile and the concentration of TG in the serum /plasma above 1.5 mmol/l. If this phenotype is identified in at least two first degree relatives as a primary disorder and if an affected kindred also has premature cardiovascular disease, then the criteria for familial FCHL are fulfilled. In 2004, a nomogram was produced to refine the diagnosis of the FCHL phenotype (Veerkamp et al. 2004). Using this nomogram Wiesbauer et al studied patients with myocardial infarction before age 40 years. In the control population, the prevalence

of the FCHL-phenotype was 2.5%, and among the relatives of CHD patients it was 28% (Wiesbauer et al. 2009).

FCHL can be regarded as a heterogeneous group of genetic and gene-driven metabolic disorders, where one or several disorders together lead to the clinical FCHL phenotype. Years back, it was thought that FCHL is incompletely expressed until the 3rd decade of age (Rose et al. 1973). Later it was shown that by taking the age and possibly weight of the subject into account proper cut-points can be used and the diagnosis can be made also during childhood (Cortner et al. 1990; Shamir et al. 1996; Porkka et al. 1997). In children, FCHL is associated with thickened IMT and markers of chronic inflammation (Guardamagna, Abello et al. 2009). Members of families with FCHL who have hyperlipidemia have impaired FMD as adults (Engler et al. 2003; Karasek et al. 2006) and also as children (Mietus-Snyder and Malloy 1998). However, this claim has been challenged, since one study did not find any difference in FMD between 98 dyslipidemic and 230 non-dyslipidemic FCHL relatives (Ter Avest et al. 2007).

2.2.7 High lipoprotein(a) concentration

Lp(a) was originally described by Berg (Berg 1963) and was then identified as sinking pre-beta lipoprotein (Albers et al. 1975). The Lp(a)-particle is similar in size as the LDL-particle. ApoB is covalently bound in Lp(a) to form a large glycoprotein apo(a) containing five cysteine-rich domains called kringles (Steyrer et al. 1994). In kringle IV-2 there is polymorphism with a variable number of tandem repeats which determine the size of the apo(a). The size of the apo(a) correlates inversely with the concentration of the Lp(a), (Bowden et al. 1994). There are also other polymorphic sites in kringle IV that contribute to ethnic differences reflected as differences in the size and concentration of Lp(a), (Ogorelkova et al. 2001).

The Lp(a) concentration is individual already in childhood (Routi et al. 1997), whereas the inter-individual distribution of the concentrations is skewed towards high concentrations. Up to 90% of the inter-individual variation in the Lp(a) concentration may be under genetic control, mostly by *Lp(a)*-gene locus polymorphism located in chromosome 6q22–23 (Boerwinkle et al. 1992). The isoform size of the apo(a) appears also to be individual (Perombelon et al. 1994). The Lp(a) concentration is very stable and tracks well from the early childhood, although its level rises after infancy within the same individual (Bailleul et al. 1995; Routi et al. 1997). Familial occurrence of high Lp(a) concentrations is common and associates with premature CHD among relatives (Genest et al. 1992; Marquez et al. 1993; Routi et al. 1996; Guardamagna et al. 2011).

The Lp(a) levels are refractory to dietary intervention and most environmental effects (Erqou et al. 2009), but estrogen in postmenopausal hormone replacement therapy and

oral contraceptives with desogestrel reduce the levels of Lp(a) (Porkka, Erkkola et al. 1995). Female gender is associated with a slightly raised and African-American ethnicity with a markedly raised Lp(a) level (Srinivasan et al. 1991). Interestingly, treatment with evolocumab, a PCSK9 antagonist, has resulted in an up to 30% reduction of the Lp(a) concentration. The reduction was greatest in the patients who also were on statin treatment which as monotherapy did not effect the Lp(a) concentration (Raal et al. 2014).

A high Lp(a) concentration is an established CVD risk factor (Dahlen et al. 1986; Kamstrup et al. 2008; Clarke et al. 2009). It may also describe the residual CVD risk after counting for LDL-C and conventional risk factors (Khera et al. 2013). The risk of CHD and other arterial thromboembolic events is associated with the genetically regulated number of kringle IV-2 repeats (Kraft et al. 1996; Helgadottir et al. 2012). Hopewell et.al. compared the genetic regulation of KIV-2 repeats and reported that the highest and lowest quintiles of the Lp(a) concentration correlated with CHD. This study suggested that the Lp(a) concentration is regulated by the apo(a) size. However, the size was not a more powerful CHD risk factor than the Lp(a) concentration (Hopewell et al. 2013). Evidence has accumulated that assessing the Lp(a) concentration in parallel with the conventional risk factors would improve prediction of the CHD risk, especially in the moderate risk population (Kamstrup et al. 2013; Willeit et al. 2014). FH patients have often high Lp(a) concentrations, which is dangerous in combination with a high risk for premature CHD (Nenseter et al. 2011). Alonso et al. studied FH patients, their Lp(a) levels and CHD events (Alonso et al. 2014). They found that the most severe FH genotypes, i.e. those leading to deletion of the expression of the LDL-receptor, associated strongly with the highest Lp(a) levels (>500 mg/l). An Lp(a) concentration of 500 mg/l has been recommended as a cut-off point for high Lp(a) concentrations in adults (Nordestgaard et al. 2010).

The primary reason why Lp(a) promotes CVD is not known, but there are some hypotheses. The fourth apo(a) kringle is homologous with the fibrin-binding domain of the plasminogen molecule and it may thus inhibit plasminogen activity (McLean et al. 1987; Loscalzo et al. 1990). As plasminogen is essential for dissolving blood clots, the Lp(a) concentration is associated with a propensity of arterial thrombosis formation. Another feature of the Lp(a) molecule may also explain how it contributes to CHD. Lp(a) seems to induce monocyte activation in endothelial cells (Poon et al. 1997). Lp(a) binds to macrophages and hence promotes foam cell formation (Zioncheck et al. 1991). Lp(a) affects directly the vascular endothelium and the cytoskeleton of the epithelial cells and increases the amount of endothelial reactive oxygen species – this promotes atherosclerosis (Cho et al. 2012; Iwabayashi et al. 2012). Furthermore, it has been shown that oxidized LDL-C, oxidized phospholipids and Lp(a) promote advanced CHD in an interactive way (Tsimikas et al. 2005). Thus, high Lp(a) concentrations may be a direct risk factor for CHD.

2.3 Recognition and screening of early dyslipidemia

The benefits of childhood screening for dyslipidemia are apparent. Early identification of a dyslipidemia in a child enables early intervention and adaptation of the child to life-style adjustments. In adults, the earlier the lowering of the TC level is achieved, the less CHD events are to be expected (Law et al. 1994; Lloyd-Jones et al. 2003). Intervention into the style of life of a child involves the whole family, and this may improve adherence of the parents to the life-style changes. If a dyslipidemia is manifested early in life, the reason may be mainly genetic and early childhood dyslipidemia may thus be used to trace a familial trait.

The disadvantages of childhood lipid screening include a poor specificity of the dyslipidemic finding, cost, possibly a poor efficiency of the screening and a fear for labeling a child ill which may disrupt the harmony of the family (Magnussen et al. 2008). The conditions required to make a finding of childhood dyslipidemia useful are a distinct expression of the dyslipidemia, tracking of the dyslipidemia to adulthood, the harm caused by the dyslipidemia and early intervention must have been proven effective. If these premises are fulfilled, early effective prevention is beneficial.

2.3.1 Expression and tracking of dyslipidemia

FH-children have proportionally high LDL-C levels soon after birth and their cholesterol level stabilizes on a high level after weaning by age 1 year and tracks well to the adulthood (Vuorio, Turtola, Kontula 1997). Children with a familial propensity to high TG concentrations may have incomplete expression of the TG concentration before adulthood, but this is not certain. It is important that gender and age specific cut-points are used before a diagnosis of familial hypertriglyceridemia is entertained (Cortner 1990).

FH-children keep their rank of hypercholesterolemia over the years, but the same may not be the case for other familial dyslipidemias. Still, common knowledge about childhood lipid tracking should be applied. Thus, TC, non-HDL-C and LDL-C levels have reasonable persistency: Spearman's correlation coefficients between childhood and adulthood lipid values are 0.6–0.7, for HDL slightly less. TG concentrations, on the other hand, correlate rather poorly in this respect, about 0.2–0.4 (Porkka and Viikari 1995; Magnussen et al. 2011). If the lipid values were expressed as z-scores by gender and age, this would improve their information value (Porkka 1991). However, the overall stability individual lipid values over time and age may not be of main importance, but rather the persistency of the most extreme values. Due to the nature of common distributions, the inter-individual differences are higher at the extremes than in the midrange (Porkka and Viikari 1995).

2.3.2 Familial dyslipidemia screening based on premature atherosclerotic disease

Premature familial CHD burden is often defined as a primary CHD event occurring in a male first-degree relative before age 55 and a female first degree relative before age 60 or 65. Grandparents, uncles and aunts with premature CHD imply also a CHD risk for the child. Familial dyslipidemias, such as FH and FCHL, are associated with premature CHD, but a premature CHD family history is an insensitive indicator of childhood dyslipidemias (Muratova et al. 2001; Ritchie et al. 2010). However, premature CHD does have a high predictive value and therefore it remains an indicator for selective screening of dyslipidemias.

2.3.3 Familial dyslipidemia screening

Recognition of FH as early as possible in an individual's life allows early identification and intervention, and provides a strong argument in favor of screening of the family for dyslipidemia and CVD risks. In FH hypercholesterolemia is usually pronounced and diagnosis is possible and feasible already in infancy. Genetic or molecular diagnosis of the FH is most useful. Other dyslipidemias may not have such a clear expression in the childhood. The EAS is campaigning actively for an increased awareness of FH, its gene recognition and cascade screening (Wiegman et al. 2015).

Familial dyslipidemias can be recognized on the basis of the lipid values, if they exhibit the characteristics of monogenetic dyslipidemia with dominant heritance. If associated, in general, with premature CHD or to other morbidity, this type of dyslipidemia is likely to be harmful.

Since the phenotype of a dyslipidemia may not be diagnostic enough, genetic diagnoses would be useful. Until today there are no practical tests available for this, apart from those for FH and type III dyslipidemia (Guardamagna, Restagno et al. 2009). In Europe, there are more than 1,600 known genetic defects that are used interactively with phenotypical diagnostics for instance in cascade screening. The Finnish population has a very strong founder effect. Therefore, the number of the defective LDL-receptor genes is small and 7 of the most common defective LDL-receptor genes cover about 90% of all clinical cases of FH (Lahtinen et al. 2014).

The current guidelines on the screening of childhood dyslipidemia in the US and Europe, including Finland, are listed in Table 4. The guidelines are very similar and there is a trend towards combining an early selective strategy with later universal screening. The main target is to identify the children with FH, but screening will identify also other types of childhood dyslipidemias.

Table 4. Current guidelines for screening for dyslipidemia and familial hypercholesterolemia in children

Year	Organisation (published in)	Target population	Selective screening Universal screening	Aim to find	Drug indication
2011	NHLBI, AAP, AHA (Daniels et al. 2011)	pediatric high risk	from 2y onwards 9 to 11y and 17 to 21y	high risk child	age >10y, depending on risk and LDL-C
2011	NLA Expert panel (Goldberg et al. 2011)	FH adults and children	from 2y onwards 9 to 11y	FH	age >8y LDL>4.8, non-HDL>5.5 mmol/l
2013	Current Care guideline (Working group, 2013)*	adults and children	by 10y against	FH, FCHL, type-III	age 8 to 10y, LDL > 4.0 mmol/l . FH, T1DM
2013	Physician's Handbook (Salo, 2013)	dyslipidemic children	2 to 10y against	high cholesterol in childhood	age 8y, TC>6, LDL> 4.5 mmol/l
2014	International FH Foundation (Watts et al. 2014)	FH adults and children	5 to 10y consider	FH	age 8 to 10y, FH with LDL>4,0 mmol/l
2015	EAS consensus statement (Wiegman et al. 2015)	FH children and adolescents	from 5y onwards consider	FH	age 8 to 10y, target LDL <3,5 mmol/l

All guidelines recommend diet or lifestyle change before a statin treatment is considered.
NHLBI: National Heart, Lung and Blood institute (United States), AAP: American Academy of Pediatrics
AHA: American Heart Association (United States), NLA: National Lipid Association (United States),
EAS: European Atherosclerosis society
working group: working group set up by the Finnish Medical Society Duodecim and Finnish Society of Internal Medicine
FH: Familial hypercholesterolemia, FCHL: Familial Combined Hyperlipidemia. Type-III: type-III dyslipidemia
TC: total cholesterol concentration, T1D: Type 1 diabetes mellitus, LDL: Low density lipoprotein cholesterol concentration, y: years
*Including background materials (Kovanen 2013; Kovanen and Salo M 2013)

2.4 Treatment of early life dyslipidemia

2.4.1 Life-style intervention

Life-style intervention is an elementary part of dyslipidemia treatment. Prevention of illness includes also prevention of smoking, obesity and a sedentary lifestyle. Successful dietary treatment of childhood hypercholesterolemia lowers the LDL-C level in FH by about 10% (Negele et al. 2015). Dietary intervention is recommended from age 2 years, although, according to the STRIP study, safe intervention may be initiated even one year earlier (Lapinleimu H et al. 1995; Rask-Nissilä et al. 2000; Niinikoski et al. 2007). The key points are to reduce the proportion of saturated fats, to restrict the contribution of fat to about 30% of the total energy intake and to increase the proportion of the soluble fiber. Nutrients containing sitostanol and sitosterol, which reduce the LDL-C concentration, appear to be safe and effective for children (Rask-Nissilä et al. 2000; Tammi et al. 2001). Children with hypertriglyceridemia should reduce, if possible, the intake of simple carbohydrates and prefer fish to meat. Professional repeated dietary counselling of the child and the parents is required for full effect of dietary modifications (Niinikoski et al. 2007).

2.4.2 Pharmacologic and invasive treatments

Wide consensus holds statins as the main treatment from age 8–10 years of children with FH (Table 4). With a 10-year span of experience of this treatment one can state that treatment of children with statins is efficient and safe (Kusters et al. 2014). Children with a high CVD risk and hypercholesterolemia, but not FH, may also be suitable for treatment with statins. Bile-acid sequestrates and ezetimibe are second-line drugs, while nicotinic acid is no longer recommended for children or adults. For the treatment of homozygotic FH new treatment modalities are emerging, e.g., PCSK9 inhibiting antibodies (alirocumab and evolocumab) which provide a reduction of LDL-C among homozygote FH patients of around 31% in 12 weeks (Raal et al. 2015).

Severe, life-threating hypercholesterolemia, as homozygous FH, requires aggressive treatment. FH homozygote patients may undergo liver transplantation in an attempt to cure the condition (Huang et al. 2015). LDL-apheresis, used with homozygous FH, reduces angina, slows IMT progression and prevents new CHD events in adult patients with high Lp(a) concentration (Ezhov et al. 2015; Sampietro et al. 2015). Gene therapy has been studied for the treatment of patients with homozygous FH. However, these studies have not yet yielded effective results. Gene therapy studies were initially promising for treating FH, but further development was not successful, because the viral vectors needed to carry the DNA were eliminated by host. Recently, a model has been reported, where LDL-receptor defective mice received healthy genes in the form of non-viral minicircle vectors able to replace the defective gene and sustain long-time control of LDL-C concentration (Hou et al. 2016).

3. AIMS OF THE STUDY

This study aimed at finding diagnostic clues for the identification of children with common dyslipidemias, which would associate with the corresponding parental dyslipidemias and to examine the significance of the dyslipidemias with regard to endothelial function. For this, data on the STRIP study children from infancy to prepubertal age was used. The specific aims were:

1. To study, if repeatedly deviant non-HDL-C and HDL-C values of children aged 1–7 years associate with a similar parental dyslipidemia and if the associations improve the identification of the actual dyslipidemia of the child. (I)

2. To find and describe the associations of the common familial dyslipidemias especially between 5-year-old children and their parents, and to clarify when dyslipidemia in the family justifies studying the lipids of the offspring (II).

3. To study if high lipoprotein(a) concentrations are associated with changes in endothelial function of children aged 11 years, when endothelial function is measured with flow mediated dilatation of the brachial artery. (III).

4. To examine if dyslipidemia phenotypes and high lipoprotein(a) concentrations of children aged 5 years predict endothelial function at age 11 years, when endothelial function is measured with flow mediated dilatation of the brachial artery. (Unpublished data)

4. MATERIALS AND METHODS

4.1 Study design

The base study, from which the sub-study populations were drawn, is a randomized, prospective, controlled intervention trial, the Special Turku coronary Risk factor Intervention Project (STRIP). The STRIP cohort profile has been described in detail (Simell et al. 2009). From 1990 to 1992 altogether 1054 families with 1062 6-month-old babies (the index children) were recruited to the STRIP study from the municipal well-baby clinics of the city of Turku. The study sample comprised 56.5% of the eligible age cohort (Lapinleimu et al. 1995). The families participating the study were randomized to either an intervention or control group. The intervention group received intensive individualized dietary counselling to reduce their TC concentration. The aim was also to reduce the impact of known environmental cardiovascular risk factors, such as smoking, obesity, a sedentary life-style and hypertension. The control group was followed according to the standards of the Finnish well-baby clinics and school youth care.

The first visit was scheduled when the child was 7 months old. In the intervention group the subsequent visits were arranged every one to three months and in the control group every four to six months until the child was 2 years old. Thereafter the visits were set twice a year until the child was 7 years old. After that the visits were annual. At the visits the families were met by a dietitian, a registered nurse and a physician. At the visits, anthropometric data and laboratory data were obtained, as were data on food records, and a clinical examination was made by the physician. The parents and the siblings of the index child provided similar data, when they attended the visit. The lipid samples were drawn without a preceding fast when the child was 7 and 13 months and then annually until the age of 4 years. Subsequently, the samples were drawn at ages 5, 7, 9, 10 and 11 years after an overnight fast. At the age of 11 years, ultrasound studies including FMD were offered for the children (Järvisalo, Rönnemaa et al. 2002; Raitakari et al. 2005). Figure 5 shows the setting of studies I, II and III for the index children.

4.1.1 Intervention

Dietary counselling began when cow milk was introduced to the diet of the child at age 10 months. The aim was to provide 30–35% of the energy from fats until age 2 years and thereafter 30% and to restrict the proportion of the saturated fats to a maximum of one-third. The families met a dietitian who counseled to provide an individualized diet. The intervention group was also instructed to avoid excessive salt and to favor berries and fruits in the diet. Otherwise, nutritional counselling was based on the Nordic recommendations 2004 (Becker et al. 2004). The nutritional intervention led into lower TC, non-HDL-C and LDL-C cholesterol concentrations among the boys in the intervention group boys than in the control group (Lapinleimu H et al. 1995; Niinikoski et al. 2007; Niinikoski et al. 2012).

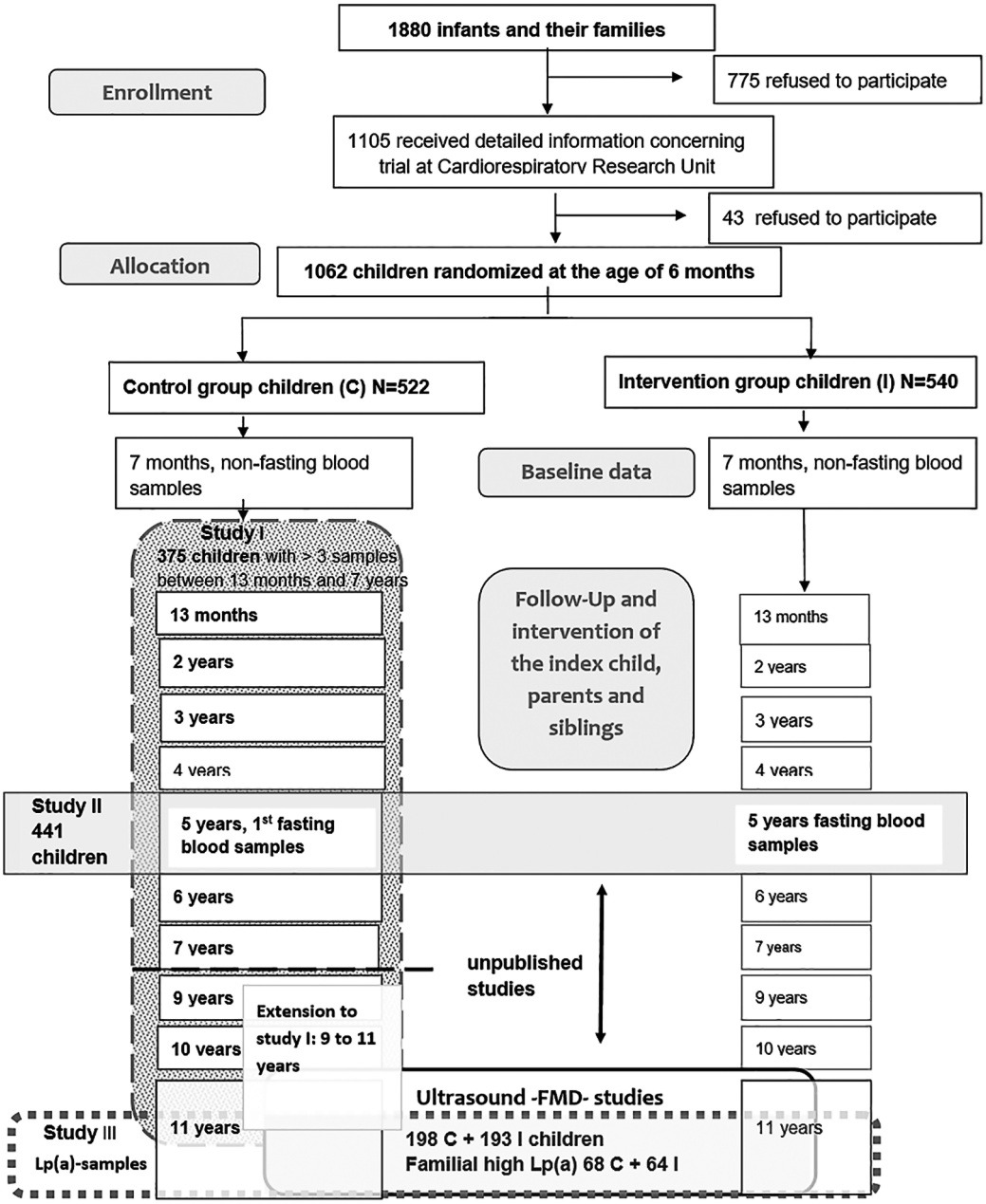

Figure 5. The flowchart of the substudies on the STRIP-study. C: control group, I: intervention group, Familial high Lp(a): children of the parents with high Lp(a) concentration. Study I used longitudinal data of the control group 1 to 7 years and additionally an extension of 9 to 11 years. Study II used crossectional data of the first fasting samples from 5-year age point combined from the control and intervention groups. Substudy III used children who provided flow mediated ultrasound-study and lipoprotein (a) concentrations at the age 11 years. Study III used FMD and Lp(a) data obtained at age 11 years from both control and intervention groups.

4.2 Subjects

4.2.1 Study I

The study group consisted ultimately of 522 control group index children and their families. Of them, 375 (72.8%) children provided four to six blood samples between age 1 and 7 years. Both parents of 353 (68.5%) of these children provided repeated lipid data. The predictive values of their lipid measurements was estimated. Subsequent 9, 10 and 11-year data was used as an extension, where 281 (55%) children provided two or three measurements.

4.2.2 Study II

Study II used cross-sectional data of the 5-year age point; there were 441 nuclear families of the index child and both parents. The families were taken from both the intervention and the control group of the main study. In addition, samples were available from 218 siblings aged 1–12 years of the index child. Only the eldest available sibling of the offspring was chosen. The fasting serum TG value of 13 fathers was higher than 4.4 mmol/l, which invalidated the use of the Friedewald formula for them, and therefore TC was used for lipid phenotyping instead of LDL-C. Obesity of the index child was defined among the 5-year-old children as a weight-for-height more than 20% above the gender-specific standard mean. Obesity of the parent was defined as a body mass index (BMI) of more than 29.9 kg/m^2.

The repeatability of the quintile level dyslipidemias was further studied. This concerned non-HDL-C and HDL-C of children aged 1–7 years of age, and LDL-C or TG of children aged 5–7 years according to the chosen parental dyslipidemias.

4.2.3 Study III

In Study III 198 index children in the control group and 193 children in the intervention group aged 11 years provided samples for measurement of the Lp(a) concentrations and FMD-values. A further sub-study was performed with 68 control group and 63 intervention group children, whose parents' Lp(a) concentration was more than 250 mg/l.

4.2.4 Unpublished studies

The Lp(a) concentrations in the longitudinal cohort of the STRIP index-children and the cross-sectional Lp(a) concentration of the parents were studied. The parents had provided Lp(a) values in the beginning of the study and data was grouped and combined according to the age to the children. The number of samples obtained from the children varied from 524 to 738, and the number of parental blood samples by 3-year intervals varied from

10 to 217 among the mothers and 14 to 213 among the fathers. The lipid phenotypes of the index children were defined as in Study II and they were combined with the Lp(a) defined at the same age. In 362 children healthy and dyslipidemia phenotypes were defined at the age of 5 years and subsequently FMD at the age of 11 years what allowed to study the significance of the early dyslipidemia phenotypes.

4.2.5 Exclusions

The study comprised healthy Caucasian children. In studies I and II, only the elder twins of the eight twin pairs was included to make one child to represent each family. Pregnancy was considered to be a strong confounding factor and it prevented the lipid values of the mothers to be used at the time of pregnancy and the year after in studies I and II. In Study II, eight children had turbid samples and were excluded. In the beginning of the STRIP study, FH was recognized in four families. They were excluded from the analyses. However, the ranges of the non-HDL-C values of the children within the FH-families were presented for comparison in one analysis.

4.3 Physical examination

Weight (± 0,1 kg) was measured using an electronic scale (until age 15 months Seca 725 Hamburg, Germany and thereafter Soehnle, Murrhardt, Germany) and the standing height (± 0,1 cm) was measured from age 2 years with the Harpenden Stadiometer (Holtain, Crymych, Great Britain). Weight-for-height was expressed as the fractional deviation from gender-specific standard means of Finnish children (Saari et al. 2011). BMI was determined as weight/height^2 (kg/m^2) and it was calculated on each visit. Since 159 parents only gave blood samples at the 5-year control without having provided data on weight or height, we substituted the lacking BMI values with the preceding. Weight-for-height is the relative weight in relation to the height according to gender specific Finnish standard growth curves (Sorva et al. 1984). From age 9 years onwards pubertal stage was estimated according to Tanner (Tanner and Whitehouse 1976) and it was included into the comparisons in Study III when the children were 11 years old.

4.4 Interview and questionnaires

The family history of CHD or CVD of the parents or grandparents to the index child was inquired annually. The year of birth of the grandparents was also registered; this allowed recognition of grandparents who were over 55 years or 65 years for males and females, respectively, with or without a history of CHD or CVD. Cumulative data on the occurrence of CHD was used until the 7-year study point.

4.5 Laboratory methods

Lipids and Lp(a) were measured at the Research and Development Unit of the Social Insurance Institution, Turku, Finland. The laboratory continuously compared the measured lipid values with the World Health Organization reference laboratory in Prague, Czech Republic, from two to four times a year. Blood samples were allowed to clot at room temperature for 30–60 min. Serum was separated with low-speed centrifugation at 1500 g over 10 min at 5° C and stored at –25° C for 2 weeks (Simell et al. 2000). The TC concentration was measured by a fully enzymatic method using cholesterol oxidase-p-aminophenazone (CHOD-PAP, Merck. Darmstadt, Germany) (Richmond 1973). The analyzer first used was an automatic Olympus AU 510 and next from January 2001 Olympus AU 400.

The HDL-C concentration was measured after precipitation of the non-HDL fraction of the TC with dextran sulphate 500,000 and $MgCl_2$ (Kostner 1976). The interassay and intra-assay coefficients of variation were 2% and 1.5% for TC and 1.9% and 1.2% for HDL-C, respectively. The serum TG concentration was measured with a colorimetric method (GPO-PAP, Merck) on an Olympus AU 400 analyzer. The inter-assay and intra-assay coefficients of variation were 4.3–4.5% and 3.1– 4.1%, respectively, depending on the triglyceride level (Salo P et al. 1999).

The serum Lp(a) concentration was measured with a solid state immunoradiometric assay with a direct sandwich technique (Pharmacia/Mercordia, Uppsala, Sweden; Marz et al. 1993). The Lp(a) concentration of the children ranged from the threshold value 12 mg/l to 1513 mg/l. The inter-assay and intra-assay coefficients of variation were 2.3% and 4.9%, respectively, at 45mg/l and 1.9% and 4.4% at 180 mg/l (Marz et al. 1993). Non-HDL-C was calculated as the TC – HDL-C concentration. The LDL-C concentration was estimated by using Friedewald's formula LDL-C = TC – HDL-C – TG/2.2 concentrations (mmol/l) (Friedewald et al. 1972). The Lp(a) cholesterol concentration was calculated as 0.3 x (Lp(a) concentration) / 386.7 g/mol (Li et al. 1994).

4.6 Ultrasound studies

Assessment of the endothelial function with ultrasound was done when the index children were 11 years of age, if they volunteered. The technique has been described (Raitakari et al. 2005). FMD was measured from the brachial artery with the method described by Celermajer et al. 1992. The study was a sonographic study performed with the Acuson Sequoia mainframe (Acuson, Mountain View, CA, USA) with a 13 MHz linear-array transducer. The left brachial artery was measured in a silent and temperature controlled laboratory with a B-mode scan of the arterial lumen. The resting basal end diastolic diameter was recorded when the electrocardiogram produced the R-wave (Figure 6).

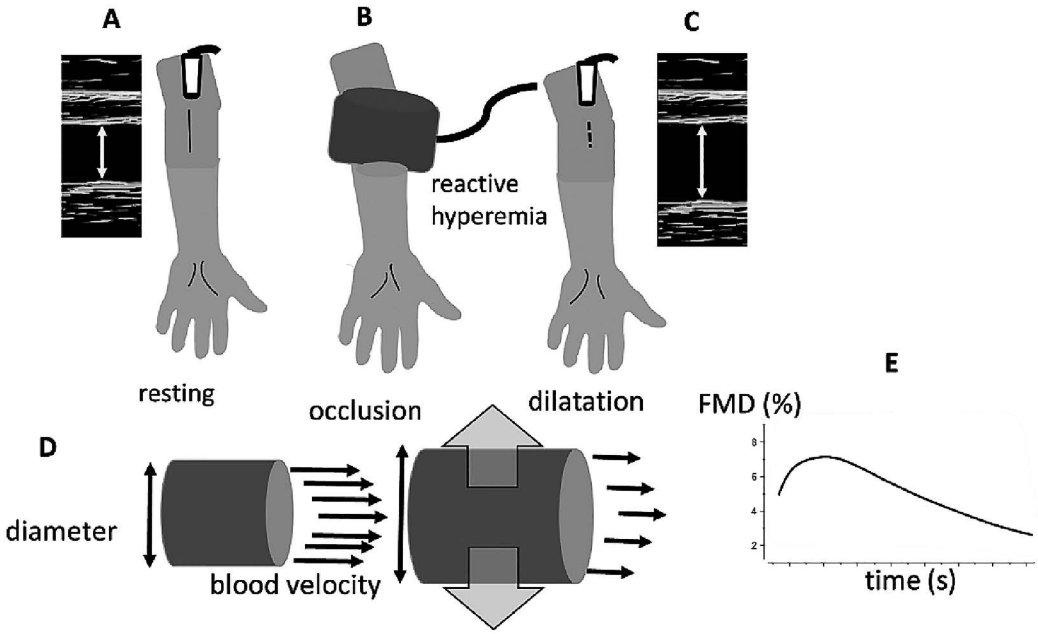

Figure 6. Schematic presentation of the measurement of the FMD according to Moens et al. 2005. In A, an ultrasound detector measures the resting diameter of the brachial artery with an M-mode image, the arrow indicates the diameter of the artery. In B, a cuff is inflated around the left forearm to 250 mmHg for 4 min 30 s. When the cuff is deflated (C), the increasing flow causes shear stress to the endothelium and this initiates nitric oxide synthesis, which is released and causes dilatation of the brachial artery (D). In E, the artery dilatation relative to baseline is graphed as a function of time after cuff release.

Arterial flow was determined with the Doppler signal. The brachial artery was occluded with an adult-sized cuff at the forearm, distal to the scanning site. The cuff was inflated. When the cuff was released the reactive hyperemia caused shear stress on the vascular wall and this dilatant the brachial artery. The dilatations were recorded continuously in B-mode from 40 s to 180 s after the cuff release. The arterial diameter was measured at 10 s intervals. Endothelium-independent dilatation was then measured by administration of 250 µg nitroglycerin sublingually. The recordings were read and measured afterwards by an experienced reader blinded to the study.

The between-study coefficient of variation (CV) was 9.3% and the between-observer CV 8.6%. Based on the to the raw data we measured also the maximal dilatation relative to the baseline diameter of the brachial artery – this occurs about 50 to 100 s after cuff release. This variable was used as a single measure of the dilatation.

4.7 Statistical analyses

All statistical analyses were performed with the SAS statistical software (Cary, North Carolina) version 9.1 for Study I, 6.12 for Study II and 9.4 for Study III.

4.7.1 Study I

4.7.1.1 Data management and dyslipidemia definitions

Linear regression was used to adjust non-HDL-C values of the mothers for age (Figure 7), and for the fathers quadratic regression was used (Figure 8). For HDL-C values no age adjustment was done. These models were in harmony with what is known about TC and HDL-C relative to age and gender (Schonfeld 1979). The individual residuals from each regression model were added to the non-HDL-C mean to obtain values standardized for age. The standardized values for non-HDL-C or the raw values for HDL-C were used to define quintile level percentile cut-points. A parent was defined as having a high non- HDL-C concentration, if more than half of the non-HDL-C values were above the 80th percentile and a low HDL-C concentration, if more than half of the individual HDL-C values were below the 20th percentile. The criteria were chosen to emphasize the persistency of the adulthood dyslipidemia. All childhood non-HDL-C or HDL-C values were corrected with the difference of the gender and age-point specific mean to the overall mean, so that all age-points and both genders shared one overall mean (Figure 9).

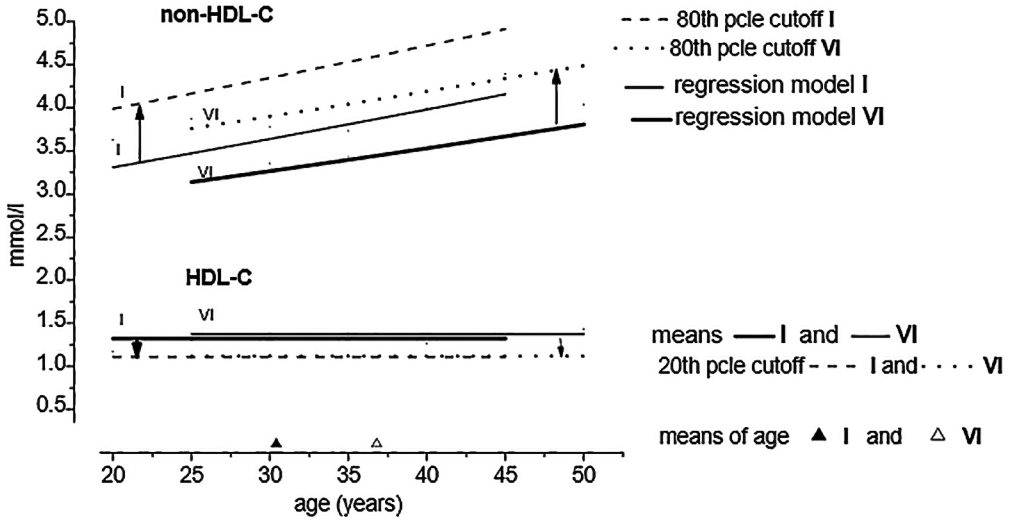

Figure 7. Regression models of the non-HDL-C and HDL-C concentration over age among control group mothers. The residuals of the non-HDL-C model were used to define the lipid status of the mothers. Two independent models are shown: first study-point (I) and the sixth (VI) six and half years later. pcle: percentile

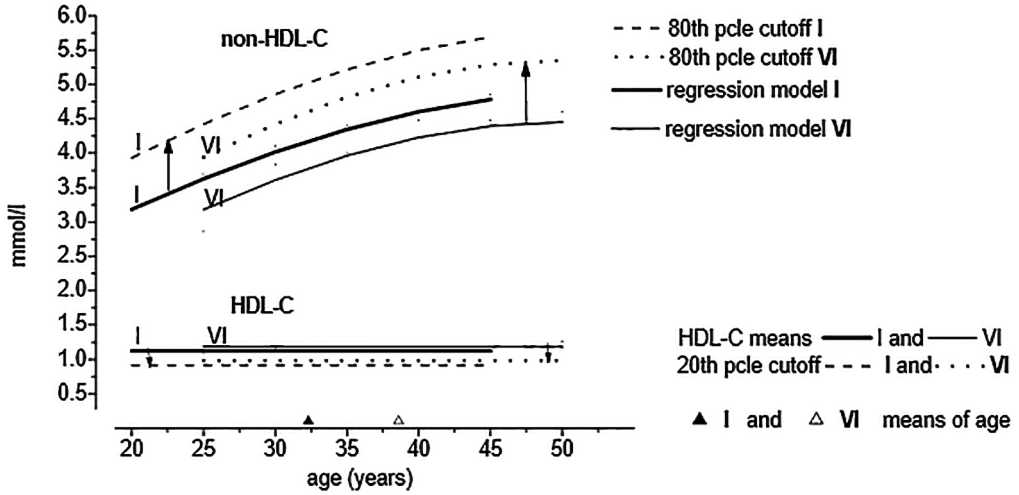

Figure 8. Quadratic regression models of the non-HDL-C concentration and HDL-C concentration over among control group fathers. The residuals of the models were used to define the lipid status of the fathers. Two independent models are shown: first study-point (I) and the sixth (VI) six and half years later. pcle: percentile

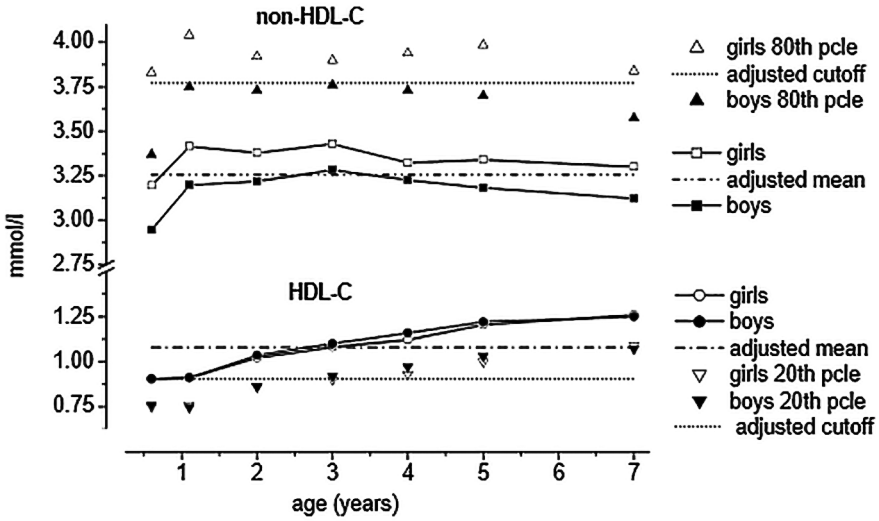

Figure 9. Non-HDL-C and HDL-C concentrations in control group children. The curves display mean and 80th or 20th percentile concentrations as raw data and as data adjusted for gender and age. Means are shown with connected curves. Vertical lines show the adjusted means and common percentiles. Raw data was standardized at each age- and gender-specific point to same means by calculating the difference of the mean of the point to the overall mean and by adding this difference to all individual values measured at the point.

In addition to the data of the first seven years, an extension of data on ages 9 to 11 years was taken to be used for the definitions of late childhood true dyslipidemias. Early and later childhood were combined for the definitions of true dyslipidemias over the whole childhood. If a child had a mean of the age-adjusted non-HDL-C concentration above the 80th percentile at early, later or his entire whole childhood, the child was defined as having a high non-HDL-C concentration in that interval. If a child had mean of the age-adjusted HDL-C concentration below the 20th percentile, the child was defined as having a low HDL-C concentration in the age interval.

4.7.1.2 Statistical tests

The continuous variables describing the lipid values and anthropometric variables in childhood were compared by the dyslipidemic state of the respective children's parents. Covariance analyses were used for continuous data with a general linear model for single dependent variables of the baseline data or a mixed model for repeated annual data from 1 to 7 years. The mixed models used a compound symmetry structure with age as the covariance pattern. The proportions of the dyslipidemias in the children were classified as sporadic or continuous and estimated according to their parents' state of dyslipidemia using Fisher's two-sided exact test.

4.7.1.3 Model to evaluate the value of single and repeated measures

Models were developed to estimate how one to three childhood samples above or below the defined cut-points would identify the actual mean non-HDL-C or actual mean HDL concentrations above or below the cut-points. The likelihood of the children to have deviant values was estimated by fitting a trivial normal distribution into the observed individual variance. The area of distribution of the individual mean to the estimated general cut-point in individual standard deviations resulted the individual probability of the child of having a value beyond the cut-point. This was also estimated for the mean of two or three samples by dividing the individual mean deviation by the square root of the two or three sample values according to the reduced variance. The individual probabilities of expression of actual high non-HDL-C or actual low HDL-C concentrations in one to three samples were summed as predictive statistics by the parental dyslipidemias. Since the predictive statistics and especially their differences would be non-parametric, the corresponding errors were estimated with bootstrap resampling technics using 10,000 replaced resamples. The predictive statistics was calculated separately for the children with and without parental a parental dyslipidemia burden. The significances of the differences between these groups were estimated with bootstrap permutation tests.

4.7.2 Study II

4.7.2.1 Data management and dyslipidemia definitions

The percentile cut-points were based on 746 index children and 306 siblings, 606 mothers and 559 fathers. The percentile cut-points for the index children were defined using the gender and intervention group specific 90th percentile for LDL-C and TG and the 10th percentile for HDL-C. The values of the siblings and parents were adjusted for age, gender and intervention or control group using the residuals of the lipid regressions on age. Among the fathers, TC and LDL-C the regressions were fitted on a quadratic model of the age. Then gender- and intervention group specific 90th and 80th percentile points were defined and the 10th percentile for HDL-C. The hyperlipidemia phenotypes were defined as follows: IIA: LDL-C > 90th percentile, IV: TG >90th percentile and IIB: LDL-C (and TC) > 90th percentile and TG > 90th percentile. Isolated hypoHDL was defined when there was no hyperlipidemia but HDL-C was below the 10th percentile.

The family dyslipidemias were defined as follows: Hypercholesterolemia families: both parent(s) and the index child share IIA. Hypertriglyceridemia families: both parent(s) and the index child share IV. Combined dyslipidemia family: a parent and the child shared hyperlipidemia and IIB was included. In mixed families the parent(s) and the child were either IIA and IV or IV and IIA. In an isolated hypoHDL family hypoHDL was shared by a parent and child. The recurrence of the childhood dyslipidemia was estimated between age 1 and 7 by parental high non-HDL-C, low HDL-C as in Study I, and a parental dyslipidemia phenotype in Study II.

4.7.2.2 Statistical tests

The significances in the characteristics between the lipid phenotypes among family members were analyzed with analyses of covariance, where the TG values were $\log_e$ transformed. $P < 0.05$ in the two-sided model was considered significant. Tukey's analyses were used to take multiple comparisons into account when phenotypes were compared.

The phenotypes of the index children and their parents were cross-tabled where the marginal means gave the expected rates of the lipid phenotype combinations. χ^2-tests estimated independently the likelihood of the excess or shortage of the family types. Logistic stepwise regression was used to analyze the dependencies of the childhood dyslipidemias on their characters and parental dyslipidemias, including hyperlipidemias, low parental HDL-C and mutually similar dyslipidemia in the parent pair. The recurrences of the dyslipidemia of the children was estimated according to the parent's dyslipidemia phenotype and compared to the children with healthy parents. Mann-Whitney's U test was then used to identify differences in trends.

4.7.3 Study III

4.7.3.1 Data management and definitions

198 index children in the control group and 193 children in the intervention group provided samples for Lp(a) concentrations and FMD measures when the children were 11 years old. An arbitrary cut-point of 250 mg/l for the Lp(a) concentration in either parent was used to delineate 68 control group children and 64 intervention group children with a high Lp(a) concentration burden among parents as familial risk children. Since the missing values of the adjusted total cholesterol concentrations in early years would have restricted the model, an early cholesterol variable was created by taking TC at the 3-year age point. For 20 children this value was missing, and it was substituted with a value from the closest available age point.

4.7.3.2 Statistical tests

Comparisons were done between the control group and intervention group and the parental Lp(a) concentration in both groups. A general linear model was used for the covariate comparison of the continuous values, but for Lp(a) and Lp(a) cholesterol, Mann-Whitney's U-test was used. Class variables were compared using Fisher's exact test. The covariates of the comparisons of the continuous variables were gender and, at age seven months, source of nutrition classified as breast milk, mixed or formula. The impact of the intervention group was allowed to differ by gender. Simple linear regression models and models with covariates were created to describe the relationship between FMD and Lp(a) concentration. The covariate candidates were selected from the lipid values drawn between ages 7 months to 11 years and from other measures obtained when FMD was measured. The values were entered into the covariate model if their Spearman correlation with the FMD was significant at $p < 0.15$. If the covariate in the linear model had a significance of $p < 0.05$, it was retained in the model. Thus systolic blood pressure and basal brachial artery basal diameter were held as covariates.

As it was possible that the relationship between FMD and Lp(a) concentration would be different between genders or between intervention groups, models were created for FMD on Lp(a) concentration, gender and Lp(a) concentration by gender interactively and for FMD on Lp(a) concentration, intervention group and Lp(a) concentration by intervention group interactively. Since there was a similar relationship and no interaction between Lp(a) concentration and FMD in both genders, the genders were combined. Heteroskedasticity and normality of the FMD and its residuals were assessed with plots.

4.7.4 Unpublished studies

The original lipid phenotypes of children in Study II were re-evaluated for the anticipated confounding effect of the Lp(a) cholesterol content measured at age 5 years. The LDL-C values were adjusted for Lp(a) concentration, as Dahlen originally suggested (Li et al. 1994), and the 90th percentile of the residuals was obtained and the phenotypes were redefined.

The intention was to use all 15 diameter estimates of one FMD study, and therefore a mixed model was used. The model was fitted for the brachial artery diameter as a function of the time. Log-likelihood criteria were used to select the autoregressive covariance pattern. Mixed models were used because of their ability to handle the marked within-individual random FMD variance or even their absent measures. Anthropometric variables, systolic and diastolic blood pressures and lipid values at age 11 years were taken into the models, and retained if their P-values were below 0.15. The possibility for additional confounding interactions between the dyslipidemias or hyper Lp(a) and gender or intervention group was checked as interactions were entered into the mixed models. The resulting least-square means of the dilatation on time after the cuff release were plotted. Finally, the childhood dyslipidemias were defined either as sporadic or familial and their number was measured according to the lowering peak FMD.

4.8 Ethics

The study was conducted according to the declaration of Helsinki. The STRIP study and the ultrasound studies were approved by the Joint Commission on Ethics of the Turku University and Turku University Central Hospital. Informed consent was obtained from the parents and later from the children. The STRIP study is registered as a clinical trial NCT0022360.

5. RESULTS

5.1 Screening and persistency of dyslipidemias in childhood (I)

Table 5 shows the inter-individual variances and their proportions of total, non-HDL- and HDL-C concentrations by family members. The table shows that the non-HDL-C concentration yielded the highest proportions of the variance between individuals, thus it may be used well to characterize an individual. The differences between children were smaller in the three lipid qualities than the differences in between their parents and especially in between their fathers. The results suggest reasonable capability of the lipid parameters to distinguish individuals as well children and adults.

Table 5. Inter- and intra-individual variance of 4 to 6 samples of the lipid variables in the family members (unpublished, Study I)

	girls	boys	fathers	mothers
TC concentration (mmol/l)	4,5	4,3	5,3	5,0
variance between individuals (%)	14	13	15	15
variance within individual (%)	10	11	9	11
variance between individuals of total variance (%)	**64**	**59**	**73**	**66**
non-HDL-C concentration (mmol/l)	3,4	3,2	4,2	3,6
variance between individuals (%)	18	17	19	20
variance within individual (%)	12	12	11	13
variance between individuals of total variance (%)	**70**	**65**	**77**	**70**
HDL -C concentration (mmol/l)	1,08	1,11	1,13	1,33
variance between individuals (%)	14	16	20	17
variance within individual (%)	14	12	11	12
variance between individuals of total variance (%)	**49**	**61**	**76**	**68**

Variances are given as coefficients of variance.
Proportions of the variance are calculated from the squares of the coefficients.
TC: total cholesterol, non-HDL-C: non-high density lipoprotein cholesterol,
HDL-C: High density lipoprotein cholesterol

The HDL-C, HDL-C, LDL-C and TG means recorded later in childhood (age 9–11 years) were plotted against the values recorded earlier in childhood (Figure 10). For LDL-C and TG, only fasting values recorded when the children were 5 and 7 years were used for the early samples. The plots show that the early lipid levels have reasonable stability and resemblance with later childhood. These plots show also the means of all children and the means of the children whose values were in an dyslipidemic quantile in the early childhood.

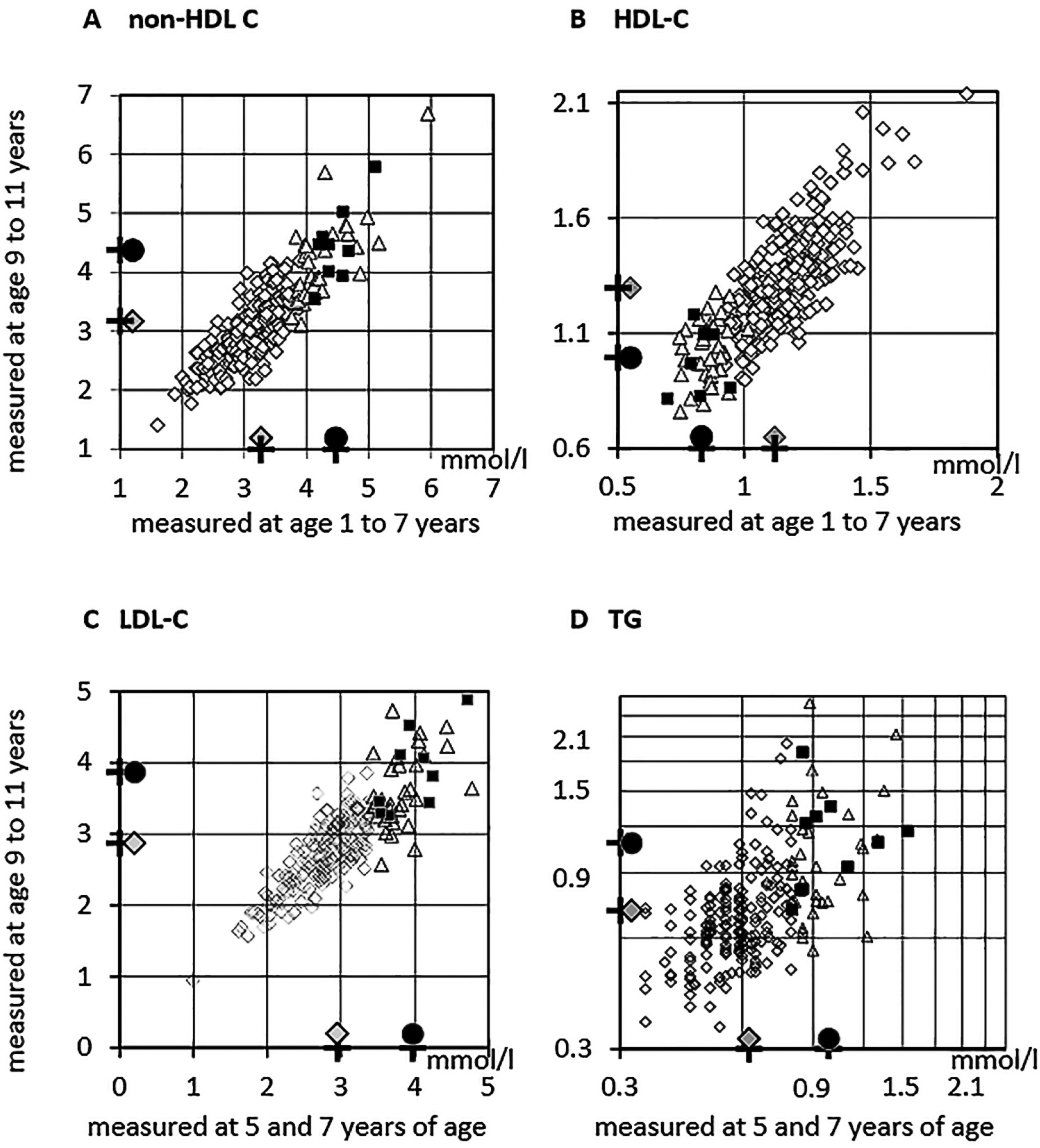

Figure 10. Individual lipid means measured in children aged 9–11 years related to early childhood values. All figures incluide dyslipidemic children from dyslipidemic families. Figures A and B are based on non-fasting blood samples drawn from children aged 1–7 years. Figures C and D are based on fasting blood samples drawn from children aged 5 and 7 years. Fastings samples were drawen after the child was 5 years old (for establishing fasting TG and LDL-C values). Geometric means were used for triglyceride values, logaritmic scales. ◊: individual value, △ individual value devaiting from mean, ■ individual value a child with familially associated dyslipidemia, ◊ overall mean ● means of familially associated dyslipidemia, non-HDL-C: non-high density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, TG: triglycerides (unpublished, Study I).

5.2 Significance of family history (I)

The challenge to screen selectively for high non-HDL-C children with dyslipidemic parents or with parents with premature CHD is illustrated by the data in Figure 11. However, as the ranges of a single sample overlap at the middle and in the high non-HDL-C medians, a single sample does not define the position of the median non-HDL-C concentration rank of the child. The frequency of dyslipidemic parents who expressed repeatedly elevated non-HDL-C or combined hyperlipidemia or the parents with dyslipidemia and a familial burden of premature CHD in their parents tended to be higher among the children with high non-HDL-C. Only hyperlipidemic parents select children with high median non-HDL-C values, but a history of CVD among the grandparents does not improve the selection. Another set of data of four children with an FH-parent is shown for comparison. The Non-HDL-C ranges of these children are either normal or higher than among other children.

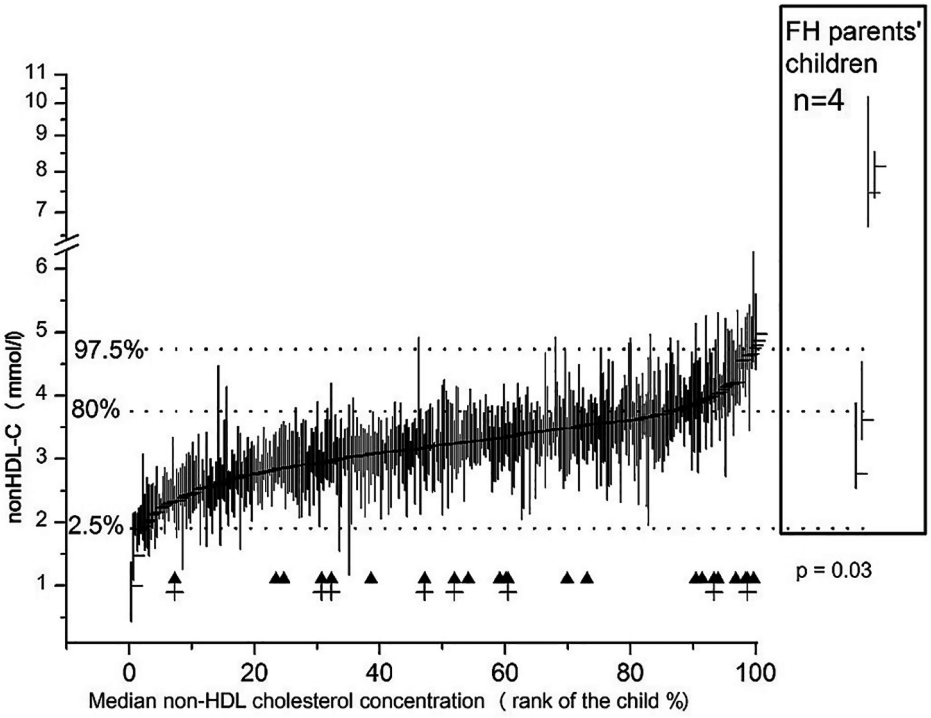

Figure 11. Non-HDL-C concentration ranges of the control group children at age 1–7 years (n=316). The data is ordered by medians. All values were age- and gender adjusted. Vertical bars are individual ranges of 5 to 6 samples, ▲: Dyslipidemic parents: either parent had non-HDL-C in the highest quintile in the 1–7-year study points or combined hyperlipidemia at the 5–7-year study point (n=22). Only parents whose parents' CHD status was defined are shown. +: Dyslipidemic parent with a parent with premature CHD (n=8). Premature CHD was either angina pectoris, heart infarction or invasive coronary treatment before age 55 years in males or 65 years in females. The P-value refers to the Mann-Whitney U-test. Only significant results with $p<0.2$ are marked. Right panel: Children to parents with familial hypercholesterolemia (FH) are from another cohort and shown for comparison. (unpublished, Study I)

The challenge to screen for low HDL-C children with low HDL-C parents or with parents with a history of premature CHD among their parents is illustrated by the data in Figure 12. Even the children in the lowest HDL-C medians could not be identified by a single measurement and children with medians above the middle range may express low HDL-C repeatedly low HDL-C and a familial burden of premature CHD in their parents do not provide information to identify these children.

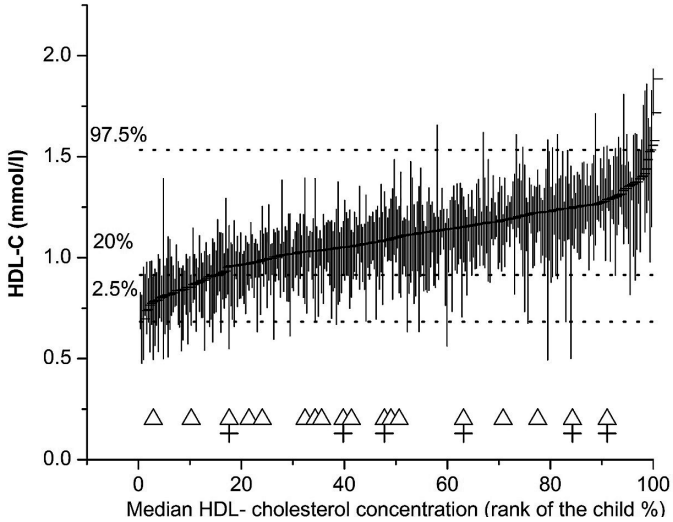

Figure 12. HDL-C concentration ranges of the children in the control group children ordered by medians (n=316). Vertical bars: individual ranges consisting of 5 to 6 samples. Δ: Low HDL-C parents (n=18): either one of the parents had all HDL-C values in the lowest quintile in 1 to 7-year study points **+:** A parent with low HDL-C with a parent with premature CHD (n=6), The definitions as in Figure 10. (unpublished, Study I)

The children with high non-HDL-C and low HDL-C are shown in Table 6 in relation to their parents' dyslipidemias. High non-HDL-C levels were related regardless of the age of values. The figure shows that parents who had repeatedly low HDL-C and parents with the children, but there was no association between low childhood HDL-C expressions until age 9–11 years in the low HDL-C group.

Table 6. High non-HDL-C or low HDL-C concentrations in children according to theparents' dyslipidemias (Modified from Study I)

		All children	Children by their parent's dyslipidemia			
Child age (y)	dyslipidemia in child		Children from high non-HDL-C parents	p	Children from low HDL-C parents	p
1 to 7		353 (100%)	44 (100%)		43 (100%)	
	high non-HDL-C	16.7 %	31.8 %	0.008	16.3 %	1
	low HDL-C	15.3 %	11.4 %	0.5	20.9 %	0.3
9 to 11		281 (100%)	32 (100%)		30 (100%)	
	high non-HDL-C	19.6 %	40.6 %	0.005	10.0 %	0.16
	low HDL-C	14.9 %	18.8 %	0.4	36.7 %	0.001
1 to 11		281 (100%)	32(100%)		30(100%)	
	high non-HDL-C	11.4 %	25.0 %	0.04	3.3 %	0.2
	low HDL-C	8.2 %	6.3 %	1	13.3 %	0.3

Percentages below 100% show the proportions of the dyslipidemic children in all and in each parent's dyslipidemia group. (y: years)

Table 7 shows the diagnostic value of the early childhood high non-HDL-C concentrations. The non-HDL-C values were set to identify the children with actual high non-HDL-C defined in three ways: children with actual high non-HDL-C in early childhood, later childhood and through the entire childhood. Since the early childhood high non-HDL children were defined as having at least half of their values above a cut-off, sensitivities, specificities and positive predictive values (PPV) were higher for the early childhood cohort than the later cohorts. Sensitivity increased when the number of the samples increased. The familial burden of the parent's high non-HDL-C increased the PPV, while adding more samples increased specificity. Low HDL-C concentrations in early childhood were not associated with parental low HDL-C concentrations (Table 8). An attempt to identify children with a low HDL-C early age through low HDL-C parents would only attenuate specificity and negative predictive value (NPV). Sensitivities, specificities and PPV in the prediction of early or early and later childhood low HDL-C values in children improved as the number of samples increased.

Table 7. Diagnostic value of early childhood high non-HDL-C concentrations (Modified from the table published in Study I).

Aim to find	Samples	Sensitivity			Specificity		
in child	of child	Non-HDL-C in family			Non-HDL-C in family		
		Healthy	High	p	Healthy	High	p
High non-HDL-C	1	72 (68 78)	85 (75 92)	0.001	90 (89 92)	92 (85 95)	0.2
in early childhood	2	77 (72 82)	89 (79 95)		94 (92 95)	95 (85 98)	
	3	79 (74 84)	91 (82 96)		95 (94 96)	96 (88 99)	
High non-HDL-C	1	53 (44 64)	60 (38 83)	0.3	88 (86 91)	91 (75 96)	0.3
in later childhood	2	53 (43 66)	60 (35 84)		91 (88 93)	93 (72 98)	
	3	52 (42 66)	60 (32 83)		92 (89 94)	93 (68 99)	
High non-HDL-C	1	82 (76 88)	92 (80 97)	0.05	88 (85 90)	90 (77 94)	0.3
in early and	2	87 (81 92)	95 (84 99)		91 (88 93)	93 (77 97)	
later childhood	3	89 (83 94)	97 (86 100)		92 (89 94)	94 (77 99)	

		Positive predictive value			Negative predictive value		
		Non-HDL-C in family			Non-HDL-C in family		
		Healthy	High	Ratio	Healthy	High	Ratio
High non-HDL-C	1	56	83	1.5 (1.2 1.9)	95	93	1.0 (0.9 1.0)
in early childhood	2	67	89	1.3 (1.1 1.6)	96	95	1.0 (0.9 1.0)
	3	73	91	1.3 (1.0 1.5)	96	96	1.0 (0.9 1.0)
High non-HDL-C in	1	50	82	1.6 (1.1 2.3)	89	77	0.9 (0.6 1.0)
later childhood	2	56	85	1.5 (1.0 2.1)	90	77	0.9 (0.6 1.0)
	3	59	86	1.5 (1.0 2.0)	90	77	0.9 (0.6 1.0)
High non-HDL-C in	1	43	76	1.8 (1.1 2.7)	98	97	1.0 (0.9 1.0)
in early and	2	51	82	1.6 (1.0 2.4)	98	98	1.0 (1.0 1.0)
later childhood	3	55	84	1.5 (1.0 2.2)	99	99	1.0 (1.0 1.0)

The values in the tables are probabilities (percentages and their 95% CI). non-HDL-C: non-high density lipoprotein cholesterol concentration.

Table 8. Diagnostic value of the early childhood low HDL-C concentrations (Modified from the table published in Study I).

Aim to find	Samples	Sensitivity			Specificity		
in child	of child	HDL-C in family			HDL-C in family		
		Healthy	Low	p	Healthy	Low	p
Low HDL-C	1	75 (72 79)	78 (66 89)	0.3	90 (88 92)	85 (79 90)	0.01
in early childhood	2	81 (77 85)	82 (69 93)		94 (92 95)	88 (82 93)	
	3	84 (80 88)	84 (71 94)		95 (94 96)	90 (84 95)	
Low HDL-C	1	56 (44 69)	50 (33 67)	0.3	86 (83 89)	80 (64 90)	0.1
in later childhood	2	57 (44 72)	48 (29 67)		89 (86 92)	81 (63 93)	
	3	58 (44 74)	48 (28 67)		90 (87 93)	82 (63 94)	
Low HDL-C	1	81 (74 87)	77 (55 97)	0.3	86 (84 89)	76 (64 85)	0.02
in early and	2	86 (79 92)	81 (57 98)		89 (86 91)	79 (65 87)	
later childhood	3	89 (82 94)	82 (51 99)		90 (87 93)	80 (65 89)	

		Positive predictive value			Negative predictive value		
		HDL-C in family			HDL-C in family		
		Healthy	Low	Ratio	Healthy	Low	Ratio
Low HDL-C	1	56	57	1.0 (0.6 1.4)	96	93	1.0 (0.9 1.0)
in early childhood	2	68	65	0.9 (0.6 1.3)	97	95	1.0 (0.9 1.0)
	3	74	70	0.9 (0.6 1.2)	97	96	1.0 (0.9 1.0)
Low HDL-C	1	35	59	1.7 (0.8 2.7)	94	73	0.8 (0.6 0.9)
in later childhood	2	41	60	1.5 (0.7 2.4)	94	73	0.8 (0.5 0.9)
	3	44	61	1.4 (0.6 2.3)	94	73	0.8 (0.5 0.9)
Low HDL-C	1	32	33	1.0 (0.3 2.3)	98	96	1.0 (0.9 1.0)
in early and	2	39	37	0.9 (0.2 2.0)	99	96	1.0 (0.9 1.0)
later childhood	3	44	39	0.9 (0.2 1.9)	99	97	1.0 (0.9 1.0)

The values in the tables are probabilities (percentages and their 95% CI). HDL-C: high density lipoprotein cholesterol concentration.

5.3 Familiality of dyslipidemia phenotypes (II)

The representativeness of the sub-study population was evaluated. Significantly more fathers (44%) than mothers (35%) did not attend at the 5-year visit (p=0.001). The mean ages of the parents were lower among the non-attenders than in the attenders (mothers 29.5 vs. 31.1 p=0.0001, fathers 32.1 vs. 32.9 p=0.02). There was no significant difference in TC or BMI at the beginning of the study between the parents who attended or did not attend at the 5-year study point.

The lipid phenotypes of the index children were paired to the phenotypes of their parents (Figure 13). There was a high phenotypic resemblance between parents and children which exceeded the random expectancy by 50% in the IIA-IIA, IV-IV, IIB-(IIA, IIB, IV) and hypoHDL-hypoHDL parent-child pairs, although a number of dyslipidemia

phenotypes of the children was identified in the families without parental dyslipidemia. There were dissimilar parent-child phenotypes, but not more than expected by chance. The frequencies of dyslipidemic families are presented in Table 9.

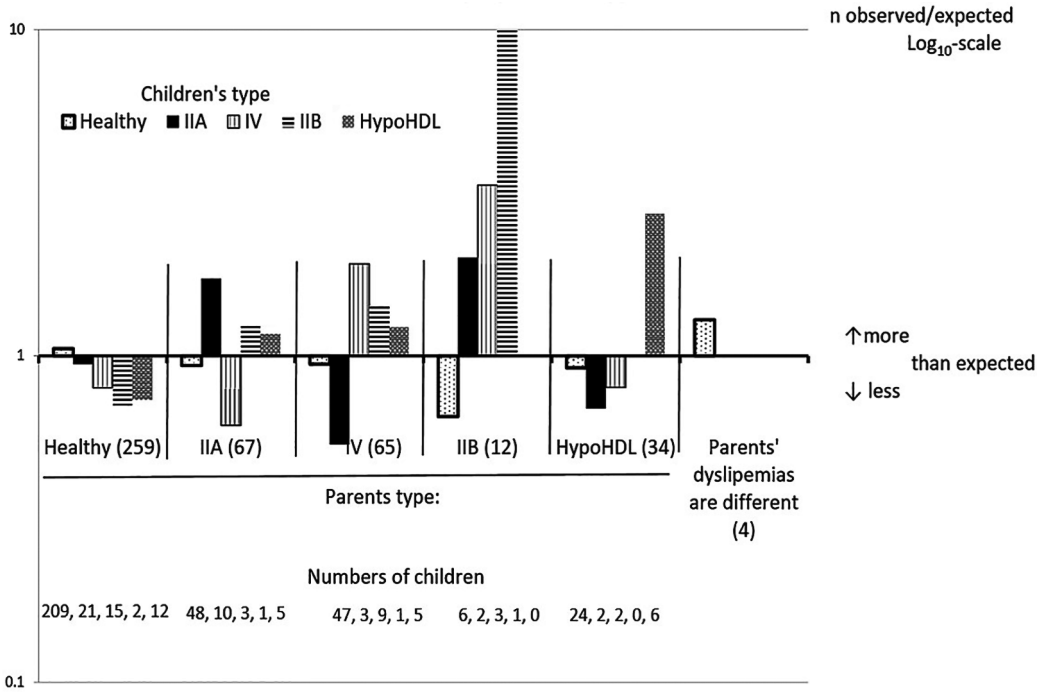

Figure 13. Overrepresentation and underrepresentation of dyslipidemias in 441 children according to parental dyslipidemia (one or both parents). Bars show the proportion of observed healthy and dyslipidemic children in relation to the proportion expected by random association. Parents were classified according to the parent with a dyslipidemic phenotype or according to the shared phenotype of parent pairs. The phenotypes of four parent pairs were discordant and the parents could not be classified. (unpublished, Study II).

Table 9. Family types (Modified from the table in Study II)

	n	Proportion
No familial dyslipidemia	402	91.2%
IIA parent and IIA child	10	2.3 %
IV parent and IV child	9	2.0 %
Multiple phenotypes in families		
Familial combined hyperlipidemia	8	1.8 %
Mixed phenotypes of parent and child[a]	6	1.4 %
HypoHDL in parent and child	6	1.4 %
Different phenotypes in parents	0	
Total	441	100.00 %

a: One parent-child pair had different phenotypes (IIA and IV) (Modified from study II).

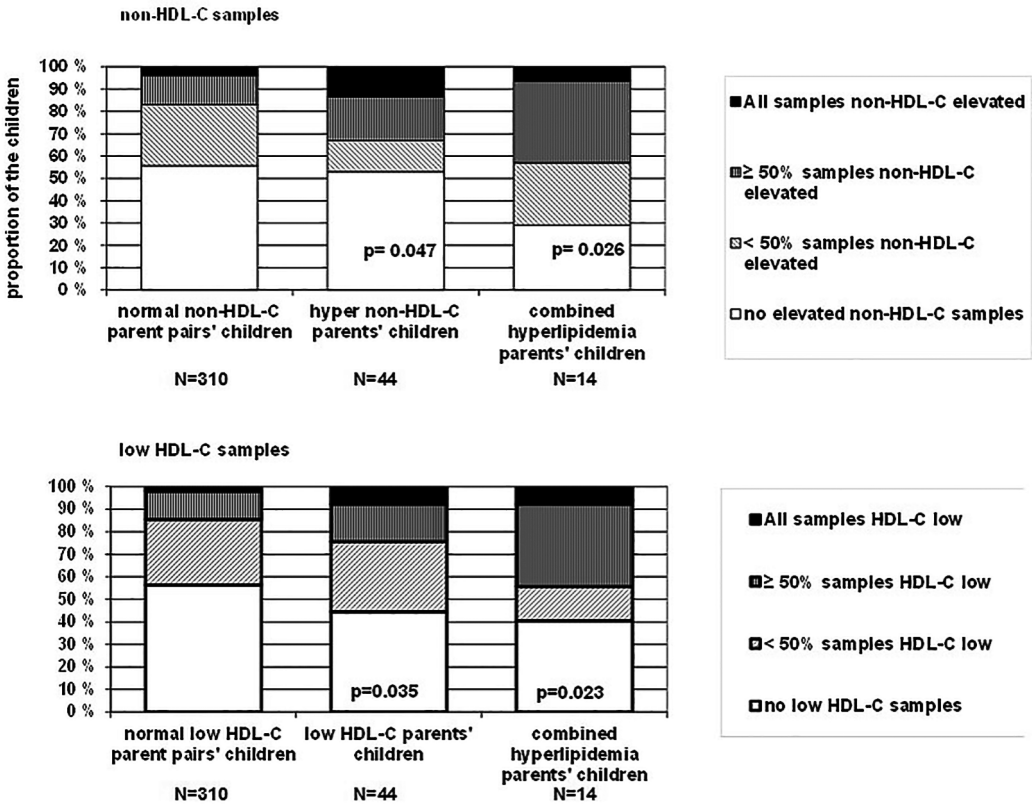

Figure 14. Dyslipidemia frequencies of 354 children by parental dyslipidemia. The parental dyslipidemias were defined as either normal non-HDL-C concentration or elevated non-HDL-C concentration and normal HDL-C concentration or low HDL-C concentration. Combined hyperlipidemia was used as an alternative definition of overlapping parental dyslipidemia. The samples were taken when the children were 1–7 years old, maximum 6 samples. Non-parametric comparisons are against normal non-HDL or HDL-C parent pairs. (unpublished, Study II).

The dyslipidemia frequencies of the control group children were checked against the dyslipidemia of the parents. The frequency distributions of the high non-HDL-C values and low HDL-C values by quintiles are shown for children aged 1–7 years in Figure 14. The frequency distributions of the LDL-C and TG values are shown similarly for children aged 5–7 years in Figure 15. The children to parents with combined hyperlipidemia had the most recurrent and persisting non-HDL-C and HDL-C dyslipidemias. The persistency of LDL-C or TG of these children is not significant, but a higher number of children with high non-HDL-C parents had repeatedly elevated LDL-C or TG values. Thus, childhood hyperlipidemias identified through dyslipidemic parents are characteristically persistent.

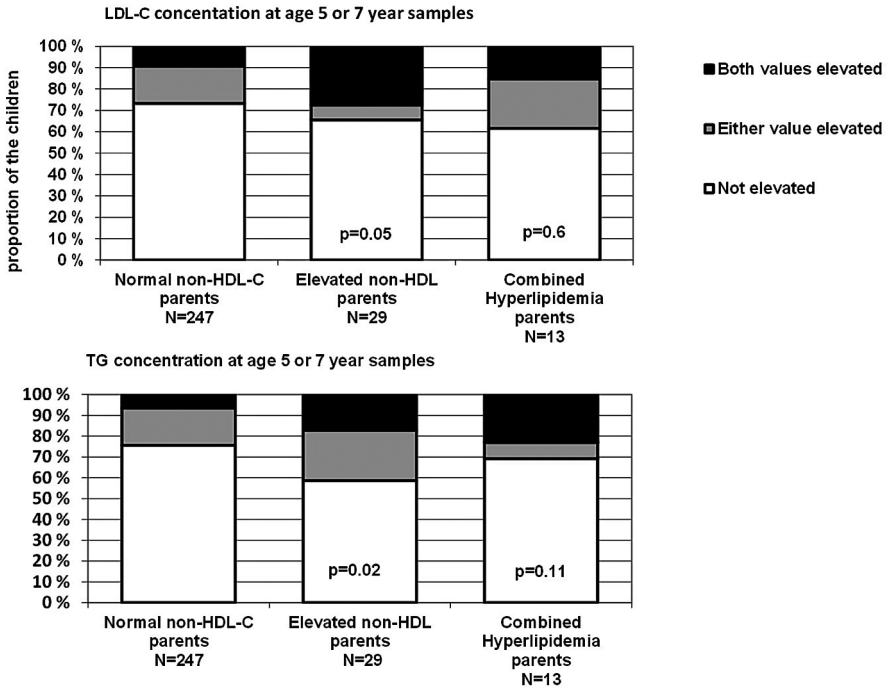

Figure 15. Repeatability of LDL-C and TG concentration measurements in the highest quintile among 276 children by parental dyslipidemia. The parental dyslipidemias were defined as either normal non-HDL-C, elevated non-HDL-C or combined hyperlipidemia, which was used as an alternative definition of overlapping parental dyslipidemia. (unpublished, Study II).

5.4 Lp(a) concentrations by age and gender (unpublished)

Figure 16 shows a combination of longitudinal data covering the STRIP-study children aged 7 months–18 years and the values of later points as based on cross-sectional data of the parents from the beginning of the study. The Lp(a) concentration appears to rise in both sexes at puberty. The shapes of the graphs were similar between the genders, but males seem to have slightly lower Lp(a) values than females.

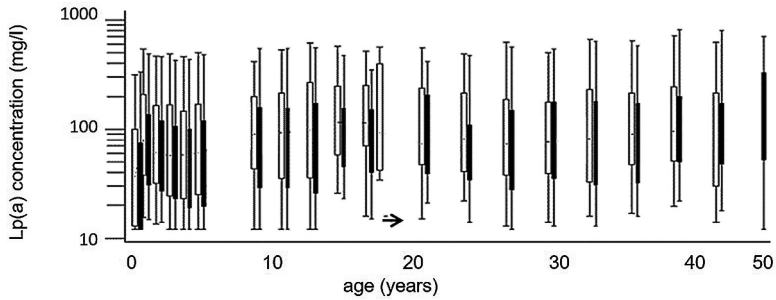

Figure 16. Lp(a) concentration by age and gender. Figure combines two different data shown on a longitudinal axis. The concentrations from 7 months to 18 years are based on the STRIP-study cohort of the children and from 18 years onwards on the cross sectional data of the parents. An arrow indicates the age where the data of the parents from the first study point is shown. white boxes: female, black boxes: male, Boxes show 80% of the values and whiskers the whole range. (unpublished)

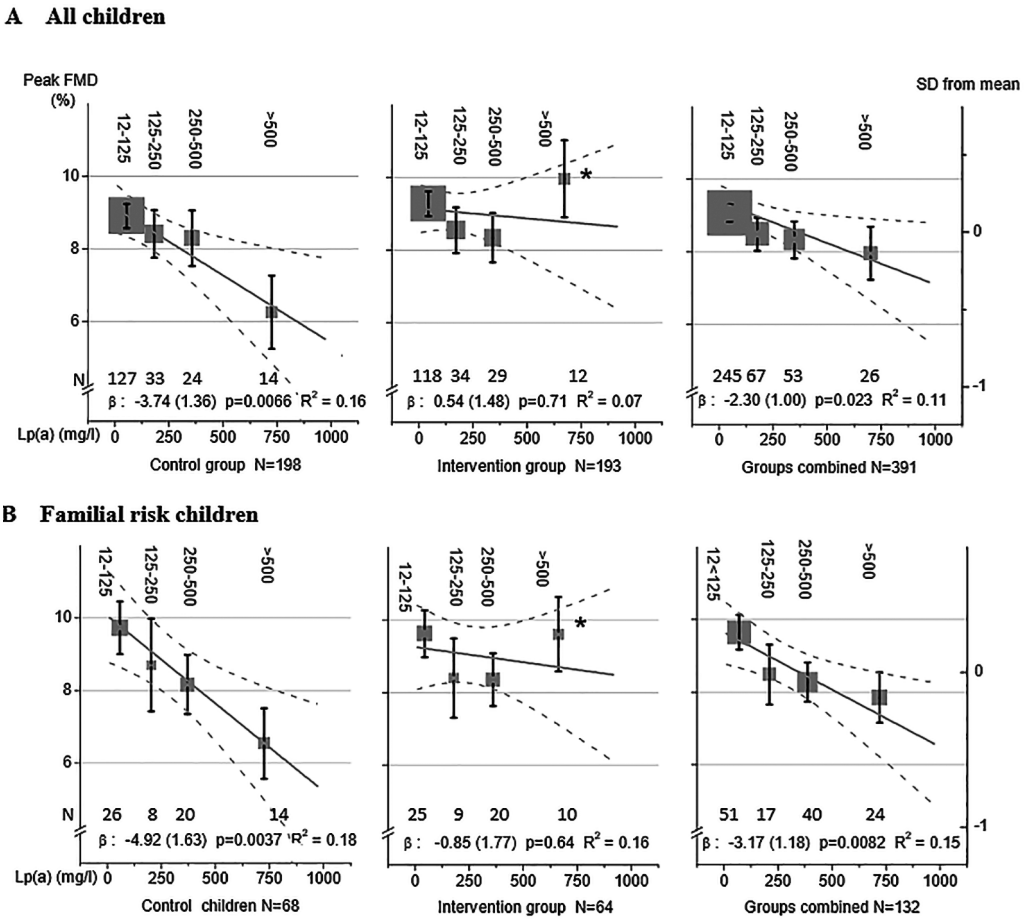

Figure 17. Flow mediated dilatation (FMD) by (Lp(a) concentration slopes among children aged 11 years. All figures express two overlying plots: regression slopes of FMD by Lp(a) concentrations and boxes depicting Lp(a) interval means. The size of the boxes is proportional to the number of children. The upper panel of figures show all children (control, interval and combined groups) and the lower panel of figures the children with parents whose Lp(a) concentration was >250 mg/l. (Modified from the figure in Study III.)

5.5 Effect of Lp(a) concentration on FMD (Study III)

The regression slopes and Lp(a)-intervals by FMD means are seen in Figure 17. The regression slopes suggest a negative relationship between FMD and Lp(a) concentrations in the control group. The negative association is stronger among the familial high-risk control group children than among the intervention group children where FMD and Lp(a) concentrations are not linearly associated. When the control and intervention groups were combined, the negative association reemerged, but a difference between groups appeared with the highest Lp(a) concentrations (>500mg/l), where no attenuation was seen in the intervention group.

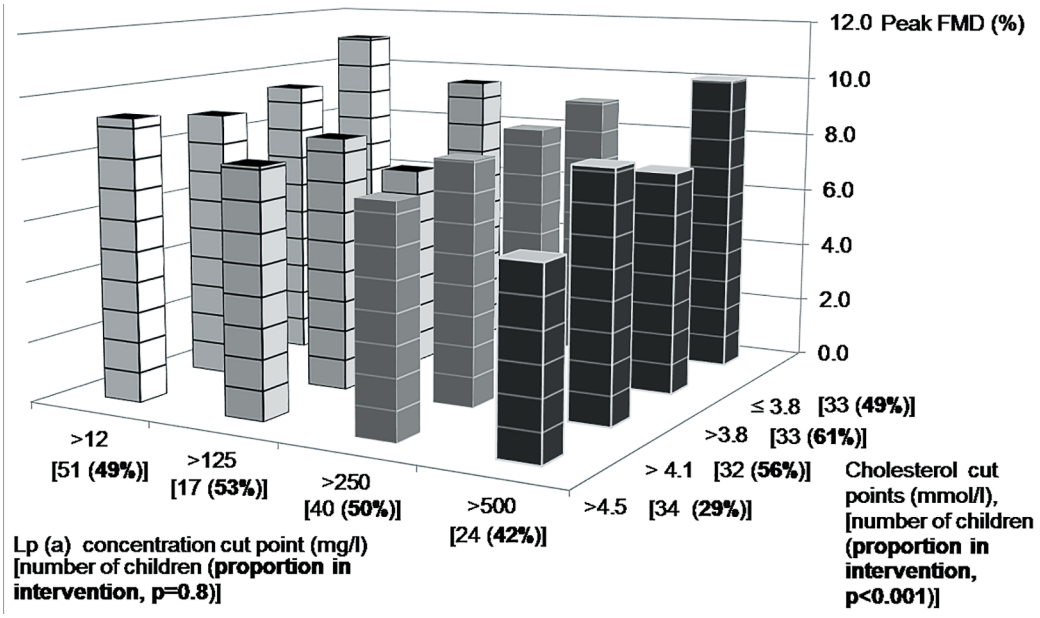

Figure 18. Flow mediated dilatation of the brachial artery at the age of 11 years on the Lp(a) concentration intervals and early age cholesterol concentrations. Children (n=132) of the parents with Lp(a) concentration > 250 mg/l are from the intervention and control groups. Data and Lp(a) intervals are as in substudy III, but Lp(a) concentration adjusted cholesterol of the early age is set in quartiles. P-values are χ^2-estimates for the likelihood of random distribution of the children by their Lp(a) intervals or cholesterol quartiles into the intervention and control groups. (unpublished, Study III)

Figure 18 shows the interplay between early childhood adjusted TC concentrations, Lp(a) concentrations measured at the age of 11 years and FMD. The data in that figure concerning the high parental Lp(a) risk children is derived from the combined control and intervention groups. The TC measured at early age was adjusted for the Lp(a) concentration and divided into quartiles. The proportion of control group children increased in the highest quartile of adjusted TC.

5.6 Effect of dyslipidemia phenotypes and Lp(a) on FMD (unpublished)

The FMD measured of children aged 11 years was studied according to the phenotypes defined at age 5 years. (Figure 19). When all available dyslipidemia phenotypes were grouped, the FMD of the subjects was attenuated compared to the children without dyslipidemia, but the FMD only among type IIA dyslipidemia subjects was alone significantly reduced in comparison with children without dyslipidemia.

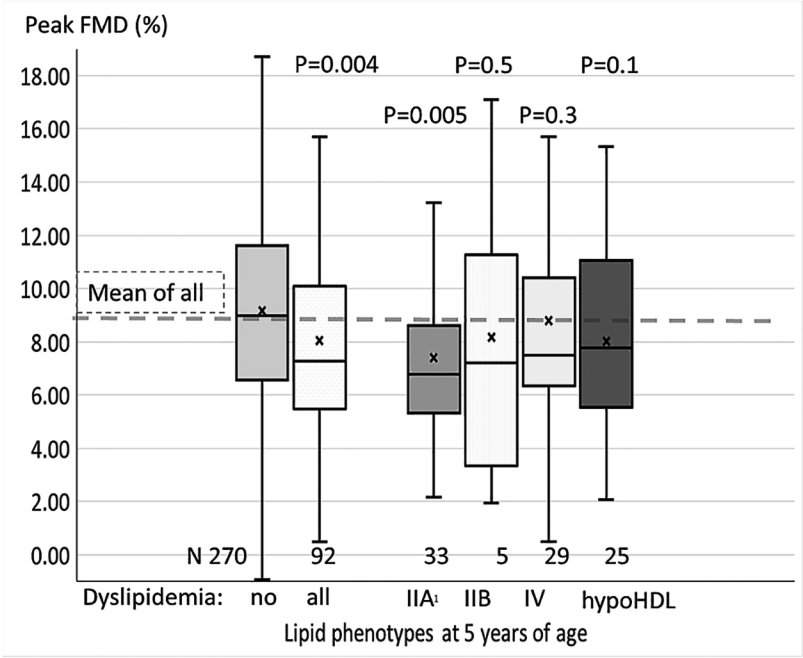

Figure 19. Peak FMD on the dyslipidemias. The peak FMD measured at the age of 11 years on the dyslipidemias defined at 362 children at the age of 5 years. The comparisons indicate lower FMD with all dyslipidemias or IIA phenotype alone compared to the children without a dyslipidemia. Control and intervention group children were combined. Figure shows medians as vertical line, means as crosses, four fifths as box and the ranges with whiskers. No: no dyslipidemia, All: all dyslipidemia phenotypes together. All comparisons are between the dyslipidemia group and no dyslipidemia. (unpublished)

The FMD by time after cuff release was estimated from the mixed model least-square means. The FMD-curves by lipid phenotype at age 5 years were plotted (Figure 20) when the subjects were 11 years old. Only IIA differed significantly from the healthy children with regard to FMD. The Lp(a) concentration measured at age 5 dichotomized by 500 mg/l predicted attenuation of the peak FMD at age 11 (Figure 21). The overall FMD after cuff release was also analyzed with mixed models and plotted. A high Lp(a) concentration was a good predictor of FMD attenuation later in life (Figure 22).

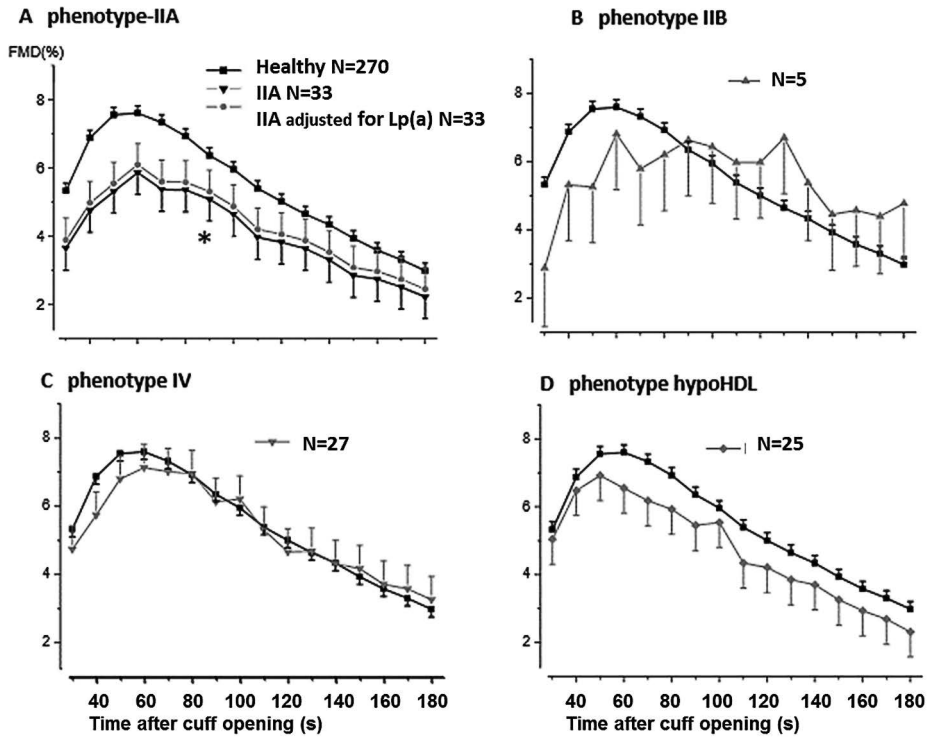

Figure 20. Repeated FMD measures on the dyslipidemia phenotypes. FMD at the age of 11 years according to the lipid phenotypes defined earlier at the age of 5 years. Gender and intervention adjusted FMD percentage relative to baseline are marked with mean and SE. Subfigure A demonstrates the IIA with the covariate effect of the lipoprotein (a) concentration [LP(a)] on the FMD. The graphs are based on mixed models of the FMD on time with autoregressive type-1 covariance model. * p< 0.05 (unpublished)

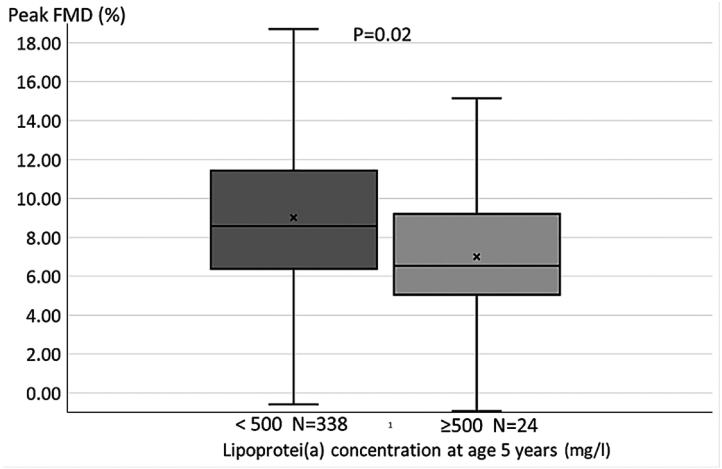

Figure 21. Peak FMD on the Lp(a) concentration. The peak flow mediated dilatation measured at the age of 11 years on the Lp(a) concentration measured in 362 children at the age of 5 years. Control and intervention group children were combined. Figure shows medians as vertical line, means as crosses, four fifths as box and the ranges with whiskers. (unpublished)

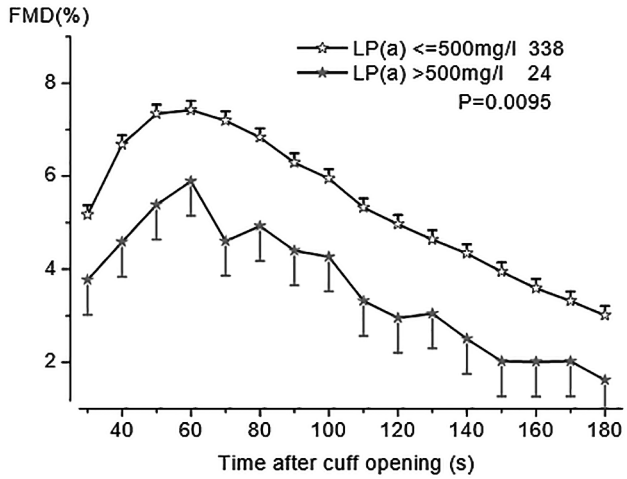

Figure 22. Repeated FMD measures on the Lp(a) concentration. FMD at age 11 years by Lipoprotein(a) (Lp(a) concentration at age 5 years (intervention and control groups combined). Mean FMD adjusted for gender and intervention (percentage of baseline artery diameter) is depicted with standard error bars. The graphs are based on mixed models of FMD over time with an autoregressive type-1 covariance model. (unpublished)

We combined the dyslipidemia phenotypes IIA, IV, IIB and hypoHDL and the Lp(a) concentration above 500 mg/l measured at the age of 5 years as in the two previous analyses. The dyslipidemias were considered as sporadic or familial when they were associated to their parents' respective dyslipidemias. Familial hypoHDL or high Lp(a) concentration were defined only when at least one of the parents had similar expression. High Lp(a)-parents were defined having the Lp(a) concentration > 500 mg/l. It is shown in the Figure 23 that the lower the FMD the higher was the probability of the dyslipidemia and its association in the family.

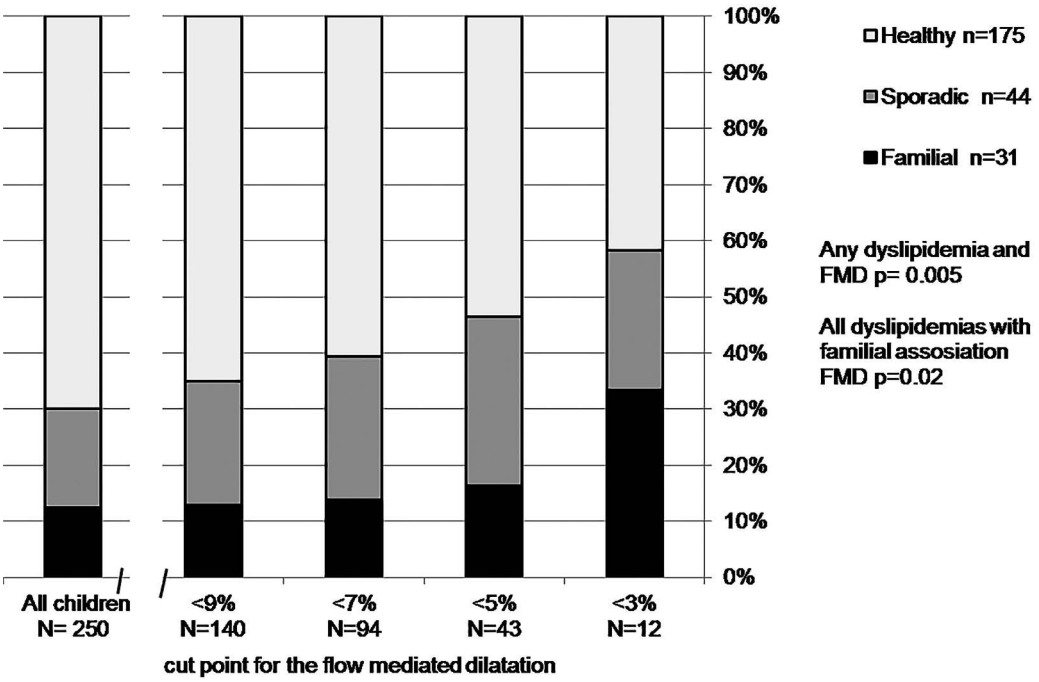

Figure 23. Distributions of childhood dyslipidemias and their familial associations by fractional attenuation of endothelial dysfunction, 250 children (combined intervention and control study groups). Dyslipidemias and their familial associations were assessed earlier, when the children were 5 years (Study II). Flow mediated dilatation (FMD) was measured when they were 11 years. Columns show childhood dyslipidemias: either sporadic or associated with parental dyslipidemia (familial). A high Lipoprotein(a) (Lp(a)) concentration (> 500 mg/l) in the child or the child and parent were alternative criteria for sporadic or familial dyslipidemia.

All dyslipidemias in children are:	
IIA	17
IIB	4
IV	17
hypoHDL	16
high Lp(a) concentration only	13
high Lp(a) concentration and IIA	6
high Lp(a) concentration and IV	2
	75

Familial associations of the a children's dyslipidemias (parent-child):	
IIA-IIA	1
IV-IV	6
FCHL	3
hypoHDL-hypoHDL	4
different hyperlipidemias in parent and child	3
high Lp(a)-high Lp(a)	11
IIA+high Lp(a)-IIA+high Lp(a)	3
	31

If the Lp(a) concentration was above 500 mg/l in a parent or child, high Lp(a) was defined. The family classification is otherwise as in the Table 9. (unpublished)

6. DISCUSSION

6.1 Subjects and study design

The prevalence of CHD has been high in the Finnish population and CHD risk profiles are high, characterized particularly by high TC levels in adults, adolescents and children (Knuiman et al. 1980; Åkerblom et al. 1984; Viikari et al. 1985; Harald et al. 2008). Therefore, this longitudinal atherosclerosis prevention trial with a non-selected cohort of the general population is suitable for studying family risks. However, longitudinal studies suffer from loss of the subjects during follow-up. The motives for non-attendance have been studied and compared to study attenders (Simell et al. 2000). The main reasons for non-attendance were changes in the family situation and change of domicile. In Study II the parents who attended at the 5-year study point were compared to those who did not attend. It turned out that the lipid levels or BMI values did not differ markedly between attenders or non-attenders, but the non-attenders were more likely to be fathers than mothers and tended to be younger than the attenders. The attenders in the main STRIP study and the attenders of the ultrasound studies at age 11 years were compared by Raitakari et al. 2005 who found that there were no significant differences in the lipid, apolipoprotein or anthropometric measures.

The reduced availability of parental lipid data restricted the studies. Attendance of both parents was best in the beginning of the study, but as time passed, attendance especially of the fathers was incomplete. This gave cause to use the values from the beginning of the study in Studies I and III, at a time when the parents were considered to be practically drug naive. Study I used parental longitudinal data only up to the 7th study year and allowed also absent visits and samples for the dyslipidemia definitions for both the children and their parents. In this way, it was possible to sustain as much repeated data of the control group families as possible. The parents' dyslipidemia definitions allowed to exempt some individual measurements taken during pregnancy and still they were valid. This was not the case in the cross sectional of Study II at the 5-year age point. It was the first time samples from the families were generally drawn after an overnight fast and thus it allowed definition of the phenotypes based on fasting blood samples. A substantial number of families was required to establish a significance of the differences, if present, and that is why samples from the control groups as well as the and intervention groups were included. It is important to understand that this study population was a unique childhood cohort tightly followed up from infancy and despite its natural limitations it still represents the normal population as closely as is feasible.

These studies addressed common dyslipidemias within the study population, and rare monogenetic dyslipidemias were not included. The only monogenetic dyslipidemia that was considered was heterozygotic FH, which was excluded from all data analyses.

6.2 Methods

The diagnostic values presented in Study I are for comparison and are not directly transferrable to other pediatric populations or settings. The models converted the mean values of 4 to 6 samples and their variances as exact values to model the true distribution of lipid profiles in the pediatric population. This kind of exact values may not exist in reality, but the diagnostic values are set as examples to show how the selection of children by considering parental dyslipidemia may change markedly the significance of the measured non-HDL-C of the child. The models estimated errors and thus the absolute values of the means are not of crucial importance. The models assume symmetry of the values to the individual means at any age of the child. This kind of symmetry was supported by the selected covariance pattern of the mixed models of non-HDL-C or HDL-C, which was a compounded symmetry pattern. Usually, tracking studies compare initial values with subsequent values. In Study I all early childhood values were assessed *in toto*.

The data is based on nuclear families with one index child and both parents. Only in Study II was information on the siblings additionally used. If larger pedigrees of the nuclear families could have been studied, this would have provided more information and validity about the dyslipidemias on a population level. However, in practice, the requirement of a broader family study is most challenging and studying a parent and child is feasible, practical and yields sufficient data for conclusions.

A family history of premature CHD was not a sensitive tool for screening for childhood dyslipidemias. This is in harmony with other studies (Ritchie et al. 2010), although a family history of CHD has been recognized as a life-time risk of CHD (Bachmann et al. 2012). Also, most parents and even the grandparents were relatively young in the beginning of the STRIP study and thus the coverage was incomplete when the children were 7 years old.

The longitudinal definitions of child dyslipidemia exploiting non-HDL-C and HDL-C quintiles (Study I) were restrictive, since true dyslipidemias were defined based recurrent dyslipidemic blood values over several years. This excluded temporary, short-time factors affecting lipid values. Longitudinal dyslipidemia definitions are seldom used in studies, mainly because of the need of extensive follow-up data (Juonala et al. 2008). The lipid values are always the result of heredity and environment, e.g., dietary factors

shared within families. This study could not distinguish the effect of heredity from the effect of environmental factors.

Fasting as well as non-fasting non-HDL-C values (Study I) are feasible for children and adults as predictors of the metabolic syndrome or CHD and perform at least as well as LDL-C (Cui et al. 2001; Srinivasan et al. 2006), and persistingly high non-HDL-C concentrations in childhood predict advanced atherosclerosis in adulthood (Nuotio J et al. 2015).

The population in Study II exhibited high LDL-C levels based on international reference values (Obisesan et al. 2004), but in the intervention group LDL-C was lower than in the control group, especially among boys. The cut-points were adjusted accordingly. Although intervention may be seen here as a confounding factor, it also widens the study population to model the present heterogeneous populations who have multiple dietary habits. The background of the families must be evaluated, when dyslipidemia among children is evaluated.

Genetic size variation of the Lp(a) was not included in these studies. The Lp(a) concentration was measured in mass units. Because of individual heterogeneity in the size of the Lp(a) molecule, its mass gives only an approximation of the total number of apolipoprotein-a particles in the circulation. However, it is the mass of the Lp(a) that is associated with CVD according to several studies (Erqou et al. 2009; Guan et al. 2015).

6.3 Results

6.3.1 Diagnostic value of dyslipidemia in the family

Childhood measurements of high non-HDL-C or low HDL-C values can be sporadic or recurrent. Dyslipidemias of the parents were associated with recurrent dyslipidemia in their offspring, and this explains the differences in the diagnostic statistics of children to dyslipidemic vs. healthy parents.

One childhood sample above a given non-HDL-C cut-point may select individuals with a mean non-HDL-C above or below the cut-point (Figure 24 A). The sensitivity of this association is directly dependent on the proportion of children who actually have high non-HDL-C, but sensitivity is not determined by the shape of the prior distribution that may as well be unimodal or combination of two or more distributions. A high PPV indicates that previously, a great proportion of the children must have had their actual non-HDL-C level above the cut-point. When data from a number of children who have high non-HDL-C concentrations is added to normally distributed non-HDL-C levels, the result sum distribution would acquire multiple tops (Figure 24 B). Specificity and NPV were less profoundly affected by the shape of the prior distribution. Study I shows

that there is a marked difference in the PPV of the childhood high non-HDL-C value depending on the lipid status of the parent.

Repeated sampling over the childhood years improves specificity, but the difference in the PPV is only slightly affected (positively). An attempt to find low HDL-C children at an early age from children to low HDL-C parents would only reduce the specificity and NPV. In other words, some children, who would have been excluded from the risk group because of a normal HDL-C level in early childhood would later on turn out to have a low HDL-C level.

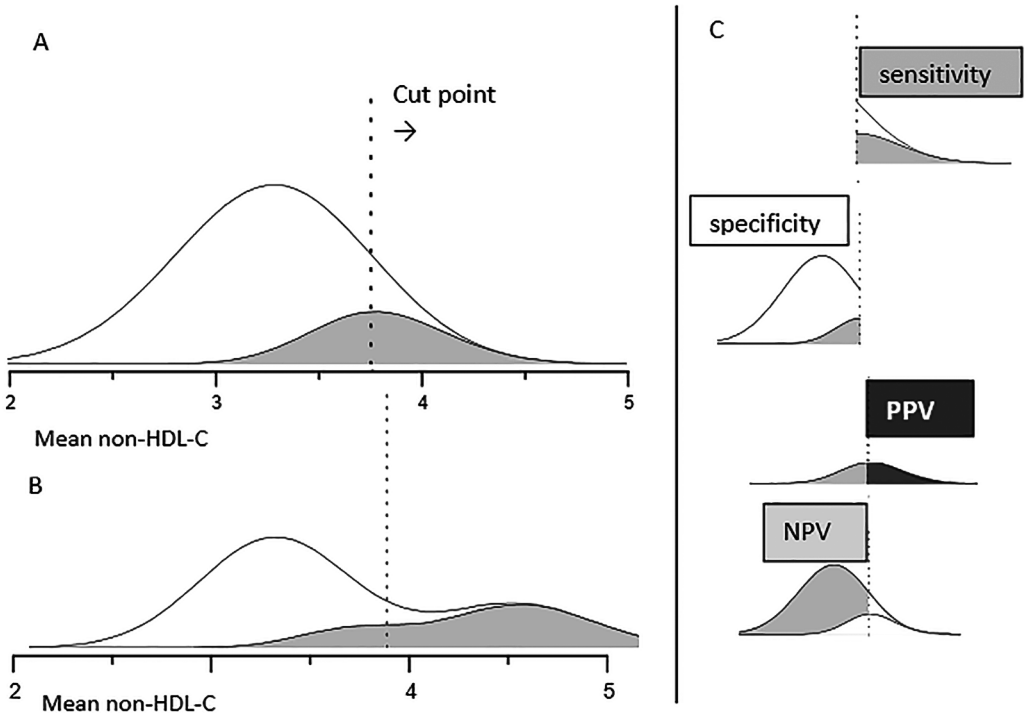

Figure 24. Significance of the prior distribution on the value of one childhood non-HDL-C value. Prior distribution (upper white curves) describe children before any sampling dependent on their parents' dyslipidemias. Gray curves are the children selected from the prior distribution on the basis of one sample that is above the cut-point. Panel A shows a unimodal prior distribution describing the general population and panel B a bimodal prior distribution describing the children to parents with high non-HDL-C. Vertical axis marks the frequency of the children. Horizontal axis is an assumed actual mean non-HDL-C of the child. The areas under the uppermost curves are the children before the study of the lipids. Shadowed areas are the children selected from the prior distribution by one sample with non-HDL-C above the cut-point. C: Diagnostic values illustrated. The illustrations are from panel A. **Sensitivity**: selected actual high mean/ all actual high mean; figure: shadowed / (white + shadowed). **Specificity**: non-selected / all normal mean; figure: white / (white + shadowed). **Positive predictive value (PPV)**: selected high mean/ all selected; figure: dark shadowed / all shadowed. **Negative predictive value (NPV)**: non-selected normal mean / non-selected all; figure: shadowed / (white + shadowed)

The PPV is also an indicator of tracking the extreme dyslipidemia quintile. Therefore, tracking of the non-HDL-C, but not HDL-C concentration, in early childhood is dependent on the parental dyslipidemia burden. The within-child variance of the lipid values is relatively high (Porkka and Viikari 1995), and thus classification of dyslipidemias in pediatrics is not straightforward, not even after multiple measurements. The number of children of dyslipidemic parents was small, but the absolute number of actual childhood dyslipidemias was high also among parents with normal non-HDL-C values. Therefore, the dyslipidemic children on the population level are identified only through general screening and repeated sampling. Nevertheless, the results of substudy I are applicable for an individual child.

6.3.2 Lipid phenotypes and dyslipidemia families

The percentiles of the lipid values are usually based on the study populations' age, gender, genetic, ethnicity and cultural background. Although taking these into account while setting the cut-points, the significance of the deviant value for an individual lies on the absolute value that is not determined by the population mean. Absolute TC and TG levels in the 1990's deviated from the values that of large combined populations, e.g., in the US (Li et al. 2010). The lipid values of the Finnish population are influenced by the selected genetics in the population (Salmela et al. 2008) and the dietary habits. The impact of the diet on the lipid levels can be estimated by the differences between intervention and control groups, but it does not remove the effect of the food culture. Although the percentile cut-points regulate these effects, they do not regulate the coexistences of extreme values limited by the percentile cut-points. The prevalence of phenotype IIB is seldom clearly defined, but Juonala et al. reported a 2% prevalence of phenotype IIB among Young Finns study population aged 12– 18 years (Juonala et al. 2008).

When the common 90th percentile is used for definition, the IIB phenotype is expected to occur at a prevalence of 1%, if LDL-C and TG are individually unrelated, but the theoretical maximum is 10% if the association is complete. Nutrition, exercise, abdominal obesity and insulin resistance influence the occurrence of high TG and LDL-C concentrations. Although IIB can be seen as a coincidence of phenotypes IIA and IV, the occurrence of both dyslipidemias is rare and marks dyslipidemia and overt VLDL and LDL production and thus high propensity to atherosclerosis (Brouwers et al. 2008; Juonala et al. 2008). Expression of FCHL in children has been thought to be incomplete and it is obvious that hyperlipidemias reach their highest values only in adulthood. TC and TG concentrations increase over age and there is no evidence that this would not be the case for the dyslipidemias.

There has been evidence of dyslipidemic expression in young children for decades, as long as the lipid cut-points are properly set for age, gender and the population (Cortner et

al. 1990; Shamir et al. 1996). The lipid phenotypes of children resemble the phenotypes of their parents. The phenotype IIB is often associated with any hyperlipidemia, whereas the other dyslipidemias are associated only with the corresponding ones. The frequencies of the dyslipidemia families are dependent on the parental lipid criteria and number of children examined. The fact that there are often symmetric associations of the hyperlipidemia phenotypes in the nuclear families between young children and their parents suggests that the expression of the dyslipidemia must be in this context (Genest et al. 1992; Perusse et al. 1997). These dyslipidemias have their basis in genetic and environmental factors. For the moment, there are no means for diagnosing FCHL through gene analysis in everyday practice. This is a noteworthy deficiency, because this risk affects about 2% of the healthy population.

The request of exhaustive family data or data on the association with premature CHD in the close relatives is for not always feasible, but it probably leads to a deficiency in the identification of dyslipidemia families and children in these families, who might benefit from recognition of dyslipidemia in time.

6.3.3 Significance of Lp(a)

Age affects the absolute Lp(a) concentration, but does not affect its rank in the population among subjects above 7 months of age. The likelihood of the Lp(a) concentration being in the age-specific top decile is child dependent, but independent of the child's age, gender or intervention vs. no intervention. This suggests that the high rank of the Lp(a) concentration is not dependent on age. It has been also shown that the Lp(a) concentration tracks well from early childhood on (Routi et al. 1997) and therefore age-specific cut-points would segregate children not only with high Lp(a) concentrations but also children who have impaired endothelial function.

The FMD varies markedly intra-individually by time, but for subjects aged 11 years it seems to be very sensitive for several environmental and metabolic factors, such as physical exercise, passive smoking, type 1 diabetes and LDL-C concentration (Järvisalo et al. 2004; Kallio et al. 2007; Pahkala et al. 2011). A reduced FMD at age 11 years does not necessarily predict dysfunction and cannot be converted into a prognosis of clinical atherosclerotic disease in adulthood. FMD is sensitive for effects, but its tracking for life time is not established. Therefore, FMD should not yet be used for clinical evaluation of an individual child.

The numbers of subjects with certain dyslipidemia phenotypes, especially IIB and families with dyslipidemias or high Lp(a) concentration, were small in the present studies and their impact on FMD cannot be ascertained. In the control group, FMD decreased with the increasing Lp(a) concentrations. This relationship was even stronger when children

with different Lp(a) levels were balanced by including the children with parental high Lp(a) concentrations. A linear association was not defined in the intervention group, suggesting that this data was confounded.

The significance of a high Lp(a) value is often ignored studies on the impact of lipids on atherosclerotic vascular disease. High Lp(a) concentrations are relatively seldom encountered among Caucasians (Guan et al. 2015). The Lp(a) concentration is genetically regulated by polymorphism that differs among the major human ethnic groups (Ogorelkova et al. 2001). The FMD of the femoral arteries of 7-year-old FH children was studied by Sørensen et al. who found that a high Lp(a)-concentration associates with impaired endothelial function (Sørensen et al. 1994). Another small study with 30 FH-children and 30 controls with a mean age of 12 years did not, however, show any association between Lp(a) and FMD (Vlahos et al. 2014). Yet, Lp(a) was a significant determinant together with LDL-C for the carotid IMT. Raitakari et al. studied 241 healthy adult subjects and found no correlation between Lp(a) and FMD (Raitakari et al. 1999). Earlier studies have reported an inverse relationship between Lp(a) and FMD in children and adults (Wilmink et al. 2000; Gonzalez-Requejo et al. 2003; Wu et al. 2004), but Kivimäki et al did not find an association between the Lp(a) concentration or its genetic regulation and subsequent FMD or IMT in the Young Finns cohort study (Kivimäki et al. 2011). Due to the bottleneck phenomenon, the Finnish population has been enriched with a splice variant gene that leads to low Lp(a) concentrations, which is protective against CHD among the affected Finns (Lim et al. 2014). So, the evidence of an association between Lp(a) and CVD is concordant, but the contribution of Lp(a) to atherosclerosis and endothelial dysfunction is controversial.

The reason for such discordant results is not known. The populations not exhibiting an association between Lp(a) and early markers of CVD consist mostly of healthy adults, where the effect of Lp(a) on endothelium may be overridden by other factors, such as LDL-C or even by physiologic endothelial regulation. However, the association between Lp(a) and endothelial function does not seem to be age-dependent. Since the association between coronary atherosclerosis and the Lp(a) concentration has been recognized in earlier studies (Sharma et al. 2011; Guan et al. 2015), studies that have not found such an association should be reviewed with special attention and interest. Study III suggested that the association is not inevitable. In that study, attenuated FMD values were expected, but was not seen in the intervention group of children with the highest Lp(a) concentrations. This suggests that the intervention given in this study may eliminate the harmful effects of high Lp(a) concentrations. This tantalizing conclusion is balanced off by the fact that the number of children fulfilling these criteria was too small for any definite conclusions to be drawn and, clearly, further intervention studies with a large cohort of subjects with high Lp(a) concentrations are warranted.

This study does not support the proposition of any natural thresholds but rather that there is a continuous decline in the childhood FMD as Lp(a) concentrations rise. The drop of FMD (%) by 1 mg/l increment in Lp(a) concentration amounted in this study to 0.0037 with covariates in the control group children. It was similar to the figure of 0.004 derived from the report of Wu et al. involving multiethnic subjects (Wu et al. 2004). The Lp(a) cut-point that seemed effective for children was 500 mg/l, i.e., the same value as in the EAS recommendation for adult screening and recently suggested as the cut-point to identify adulthood CVD risk (Guan et al. 2015).

6.3.4 Dyslipidemia phenotypes and FMD

Raitakari et al. have shown among young adults the importance of LDL-C for predicting intima-media thickning (Raitakari et al. 2003). Elevated LDL-C concentration during the first years of life associate with attenuation of the FMD also in the STRIP-study population (Raitakari et al. 2005). Impaired endothelial function and hypercholesterolemia have been restored by lowering LDL-C with statin treatment (Järvisalo et al. 1999; Ferreira et al. 2007). Our study proves that hypercholesterolemia early in life (phenotype IIA) predicts impaired FMD later in life.

These results also suggest that Lp(a) contributes to the effect on FMD in IIA. If the Lp(a) concentration is not measured, an exceptionally high Lp(a) concentration may be missed and the subject may be considered having common hypercholesterolemia. At age 5 years dyslipidemias and dyslipoproteinemias are likely to affect endothelial function relatively more prominently than obesity, blood pressure, hyperinsulinemia and smoking – disadvantageous circumstances of adulthood. Hypercholesterolemia phenotypes and high Lp(a) concentrations at age 5 years predict later endothelial dysfunction. The results support a view that high LDL-C and high Lp(a) concentrations have complementary effects on the atherosclerosis development.

A low HDL-C concentration is a well-established atherosclerosis risk factor and has been associated with endothelial dysfunction in adults and young adults (Toikka et al. 1999; Kuvin et al. 2003). It is not clear if a high TG concentration is associated with impaired endothelial function, as has been suggested by Sezgin et.al. (2006), of the coronary arteries. However, FMD studies have not shown any direct association between FMD and TG (Schnell et al. 1999).

6.3.5 FH, FCHL and FMD

Familial forms of hypercholesterolemia, such as FH, are associated with a reduced FMD value (de Jongh, Lilien, Bakker et al. 2002; Järvisalo et al. 2004). Hyperlipidemic members of the FCHL families have impaired FMD (Engler et al. 2003; Karasek et al. 2006), also children (Mietus-Snyder and Malloy 1998). On the other hand, one study did

not find any effect on FMD when comparing 98 dyslipidemic and 230 non-dyslipidemic FCHL relatives (Ter Avest et al. 2007). Our results show that the lower the FMD was, the higher the likelihood of a familial dyslipidemia or a high Lp(a) concentration.

6.4 Clinical implications

This study shows that lipid screening of children to dyslipidemic parents is feasible and identifies children who could have impaired endothelial function as they grow older. The seven criteria for mass screening of Braveman and Taurimo in Table 10 can be also applied to selective screening. CVD is an increasing worldwide disease (GBD 2013 Mortality and Causes of Death Collaborators 2015) and prevention of CVD is one of the most important challenges of medicine. Dyslipidemias are a surrogate variable for atherosclerotic disease, and atherosclerosis develops on the basis of a lifetime burden of the risk factors, e.g., dyslipidemias. Therefore, the effectiveness of the prevention depends on the age when prevention is initiated. This is especially true for lifestyle factors, like dietary habits, which become established in childhood when it is still possible to affect and amend poor lifestyle habits. The STRIP study has proved that dietary and lifestyle intervention can be a feasible, effective, safe and ethically viable procedure to prevent CHD, at least during childhood and adolescence (Rask-Nissilä et al. 2000; Niinikoski et al. 2007).

Table 10. The seven criteria for deciding whether or not to use health screening (Braveman and Taurino 1994).

	This study
1. Is the condition to be detected of public health importance?	yes
2. Are there effective preventive or curative measures to deal with the condition - when it is detected at an early stage?	yes
3. Is there a safe, ethical, and efficacious procedure for detecting the condition at a sufficiently early stage to permit effective intervention?	yes
4. Are the screening procedures, definitive diagnosis, and the appropriate interventions acceptable to the population?	likely
5. Is it feasible to carry out the relevant screening, diagnostic, and timely intervention practices in a population-based fashion with existing resources or with resources that could be obtained during the planning period, given sufficient political will?	possibly
6. Will the adoption and implementation of the screening, diagnostic, and timely intervention practices strengthen development of the health system and overall societal development, in a manner consistent with primary health care principles?	yes
7. Is the cost of the screening-and-timely-intervention efforts warranted, given all the *considerations in items 1-6 above and in comparison with alternative uses of the resources?*	yes in the selective strategy

Selective screening for FH children is an established current practice (Table 4). Suspicion of FCHL should be an indication for selective screening for dyslipidemia in children. Also, if it is known that a parent has a high Lp(a) concentration screening of the children is indicated. If the parent has either IIB or a high Lp(a) concentration, this clearly warrants a study of the lipids and Lp(a) of the child, especially if the parent has vascular manifestations, as well. This is feasible already from age 5 years.

General screening of the lipids in childhood is currently widely recommended, but not in Finland (Table 4). Screening for dyslipidemias has become increasingly more cost-effective and cost should no longer be a major obstacle in this respect. Screening is available for adults who may undergo numerous laboratory examinations for screening purposes. Extending a study to children is still a challenge and selective screening may be appropriate, depending on the setting. The findings of this thesis clearly support selective screening of the lipids of children.

On the basis of the results of the present strategy of childhood screening for FH, which is highly justified, should be incorporated into a general screening strategy (Table 4). However, on the basis of this thesis we added a selective strategy to identify dyslipidemic children (Figure 25). The main advantage of selective compared to general screening is the smaller number of subject required. Although primarily non-fasting blood samples for non-HDL-C should be taken, fasting samples are needed if hyperlipidemia is present and the lipid phenotypes should be established. If there is hyperlipidemia in the family, and e.g. a type IIB phenotype is encountered in the child, this would suggest FCHL. Although FCHL ideally requires more familial evidence, there is a strong possibility that the child may be subjected to a harmful hereditary structural dyslipidemia. The results of this thesis and literature data suggest that recommendations to measure Lp(a) can be extended from adults to children.

Alternatives for childhood dyslipidemia screening would be screening later in adulthood or no screening based on familial lipid values at all. However, the earlier the reduction in the TC or LDL level takes place, the higher the likelihood of survival without CHD (Juonala, Viikari et al. 2010; Ference et al. 2012; Hartiala et al. 2012).

6.5 Future research needs

The power of a single measurement of serum cholesterol in childhood or adolescence is low with regard to prediction of an increase in the IMT later in life (Magnussen et al. 2009) On the other hand, repeatedly elevated non-HDL-C is a known risk factor for dyslipidemia in adulthood (Nuotio J. et al. 2015).

The progression and evolution of the dyslipidemia of a person in transition from childhood to adulthood should also be evaluated in relation to the need for intervention.

Although active intervention was discontinued, as planned, in the STRIP trial, the trial will continue. It is now in its 26th year study and follow-up will show if the benefit gained during the first 20 years of intervention has yielded sustained benefit in the lipid levels. If the lipid levels in the intervention and non-intervention groups were to converge, the question arises whether the metabolic legacy effect of the markers of atherosclerosis persists, as has been demonstrated in diabetes intervention studies (Lachin et al. 2014).

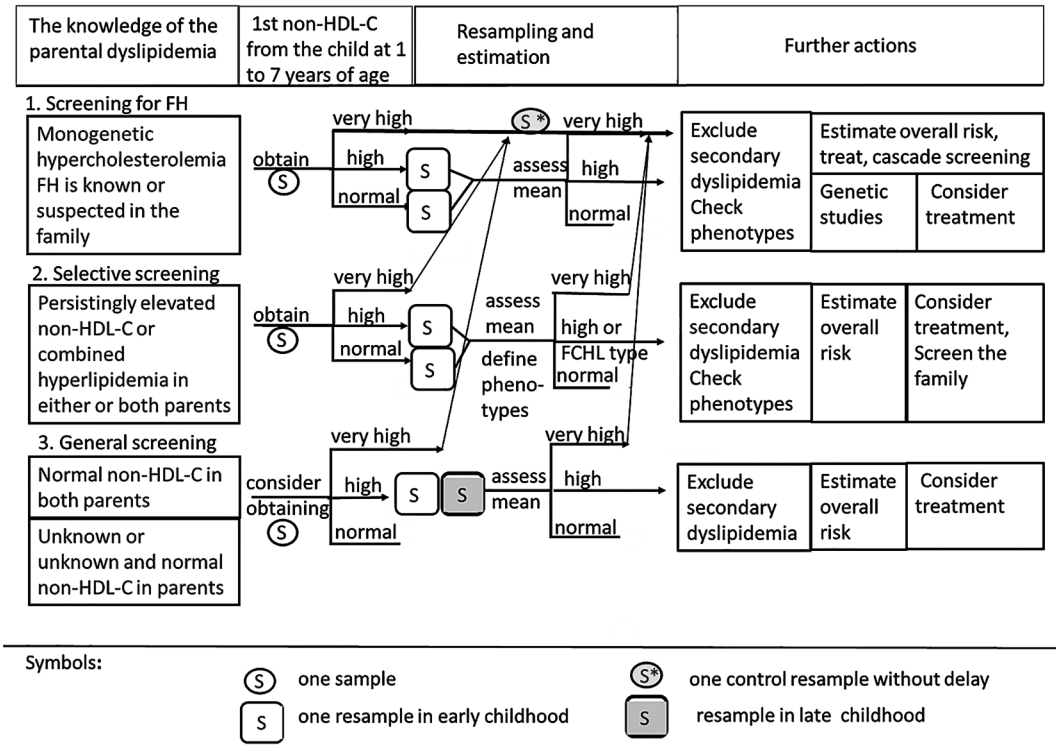

Figure 25. Childhood lipid screening strategies using non-HDL-C concentrations. **High** non-HDL-C concentration for the sample 1 and for the mean is above 3.7 mmol/l, gender and age adjusted if possible. **Very high** non-HDL-C is above 6 mmol/l. All very high childhood values and means should be considered as FH and the uppermost route used. Samples can be either non-fasting fasting. The latter enables the lipid phenotype definitions. **Exclude secondary** hyperlipidemia: Exclude hypothyroidism, diabetes, kidney and liver diseases. **Estimate overall risk**: weight and obesity, blood pressure, physical exercise, diet, smoking passive or active. **Monogenetic hypercholesterolemia**: FH is suspected on the basis of premature CHD and hypercholesterolemia family pedigrees, possibly tendon xanthomas and genetic diagnosis from the family. **Lp(a) concentration** should be studied, when 1. there is an indication to study the lipids of the child and either of the parents have Lp(a) level above 250 mg/l. 2. The treatment response of the hyperlipidemia is poor without an apparent cause. 3. When there is a need to estimate the total atherosclerosis risk as with FH children.

7. CONCLUSIONS

The presence of parental non-HDL-C dyslipidemia increases the likelihood of finding truly elevated non-HDL-C concentrations in the offspring at age 1–7 years. HDL-C is associated only later in childhood with low HDL-C in the parents.

The most common familial dyslipidemias occurring in excess of what is expected from sporadic occurrence are types FCHL, IIA, IV and hypoHDL. The occurrence is dependent on the criteria and the number of children studied. Parents with type IIB dyslipidemia have often hyperlipidemic children with persisting dyslipidemias.

A high Lp(a) concentration is negatively associated with FMD, but this association becomes weaker by intervention.

All dyslipidemic phenotypes, hypercholesterolemia alone and high Lp(a) at age 5 years are associated with a reduced FMD at age 11 years. The likelihood of childhood dyslipidemia increases in relation to the degree of reduction of FMD. The same holds true for a familial coexistence of dyslipidemias and high Lp(a) concentrations.

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