Familial hypercholesterolemia in childhood

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Chapter 1

General introduction:
Familial Hypercholesterolemia
Familial hypercholesterolemia

1. History
2. Epidemiology
3. Lipid metabolism
   3.1 Lipoproteins
   3.2 Lipoprotein transport
      3.2.1 Exogenous lipoprotein transport
      3.2.2 Endogenous lipoprotein transport
   3.3 LDL-receptor
   3.4 LDL-receptor gene mutations
4. Atherosclerosis
5. Clinical characteristics and therapy in FH children
   5.1 Diagnosis
   5.2 Therapy
6. Assessment of sub-clinical atherosclerosis in FH children
   6.1 Endothelial function
   6.2 Intima media thickness (IMT) of the carotid artery
7. Outline of this thesis
Familial hypercholesterolemia

Heterozygous familial hypercholesterolemia (FH) is a common and inherited autosomal dominant disorder of lipoprotein metabolism. Clinically, FH is characterized by elevated levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) from birth onwards, and at older age by xanthomas on the extensor tendons of the hands and feet and on the Achilles tendon. The elevated LDL-C levels strongly predispose for premature atherosclerotic disease.

1. History

FH was first described by Fagge (1873) as a skin disease, in a patient with jaundice and xanthelasmas. Autopsy in this patient showed xanthelasma-like, white-yellow patches in the aorta and its branches, now known as atherosclerotic plaques. Sixty-five years later Müller (1938) associated the xanomas with a high incidence of premature atherosclerosis and revealed the dominant inheritance of disease. Both Wilkinson (1948) and Khachadurian (1964) confirmed Müller's finding that FH was transmitted in a Mendelian dominant mode. The heterozygous and homozygous forms of FH, and their clinical expression, were also recognized at that time. In the sixties and seventies it became evident that increased plasma LDL-C was characteristic for this disorder. This observation lead to the discovery of the receptor for LDL on the cell membrane of different cell types. It was later demonstrated that the underlying molecular defect of FH consisted of mutations in the gene coding for the LDL-receptor protein. Mutations in this gene lead to the absence or impairment of LDL-receptor function, and consequently result in increased levels of LDL-C in plasma. These findings resulted in the Nobel Prize for medicine by Goldstein and Brown in 1985.

2. Epidemiology

The frequency of FH in the populations of Europe and North America averages about one in 500. However, in some regions of the world the prevalence is much higher owing to founder effects consequent to emigration or based on to local
habits that encourage consanguineous marriages. The estimated FH population in the Netherlands is 35,000 persons, but case-finding research in the northern part of the country showed an even higher prevalence of 1:400. The highest frequency of FH occurs in South Africa, where one in 100 white Afrikaners have the disorder. These Afrikaners are descended from settlers who emigrated from Germany and Holland three centuries previously, who presumably carried the two mutations known to be responsible for much of FH in South Africa.

The homozygous form of FH, encountered in one birth per million, is characterized by the absence of functional LDL receptors and plasma LDL can reach levels up to 26 mmol/l. Xanthomata develop in childhood, and massive premature atherosclerosis is frequently encountered. An identical syndrome to FH can occur in individuals with normal LDL receptors as a result of inheritance of a mutation at the apolipoprotein B (apo B) gene locus. Apolipoprotein B is an important protein of LDL and a mutation in this gene results in a functionally defective form of LDL. This disorder, familial defective apoB-100 or FDB, has a prevalence of one in 1000 of people of European descent.

FH patients show symptoms of atherosclerotic cardiovascular disease at a relatively young age. In men with untreated FH, the risk of clinically overt coronary heart disease (CHD) is approximately 5% by the age of 30, 20% by the age of 40, and 50% by the age of 50 years. Without proper treatment, only about 15% of FH males reach an age of 65 without an ischemic coronary event and about 25% die from CHD before the age of 50. Although the cause of FH is monogenic, there is wide variation in the onset and severity of atherosclerotic disease symptoms. Additional atherogenic risk factors in conjunction with the LDL-receptor gene mutation are presumed to influence the clinical phenotype in FH.

The importance of selective screening in families with FH has been recognized internationally and attempts are being made to implement the ‘Make Early Diagnosis Prevent Early Death’ approach (or MEDPED program) at a global level, assisted by the World Health Organisation. The vast majority of FH patients have still not been diagnosed and of those identified, more than half are not receiving proper medical treatment, as confirmed by a recent World Health organization Survey. Therefore, the Dutch foundation for tracing hereditary hypercholesterolemia (StOEH) was founded to identify all new FH patients in the Netherlands.
3. Lipid metabolism

3.1 Lipoproteins
Lipoproteins are macromolecular complexes consisting of lipids (cholesterol, cholesterol esters, triglycerides and phospholipids) and apolipoproteins. Apolipoproteins are specialized proteins that define the different functions and final destination of the lipoprotein particles. They are indispensable for the structure of lipoproteins and their specific functions, such as activation or inhibition of enzymes involved in lipoprotein metabolism, or the interaction with lipoprotein-receptors.

3.2 Lipoprotein transport
Lipoproteins are transported in the body by three different pathways: a) the exogenous pathway that regulates the uptake of dietary lipids. This pathway is transporting lipids from the intestine to the liver. b) the endogenous pathway that transports the lipids from the liver to the peripheral tissue and c) the reverse cholesterol pathway that transports cholesterol from the peripheral tissues back to the liver (figure 1). High-density lipoprotein (HDL) is the main lipoprotein involved in reverse cholesterol transport. It can take up excess cholesterol from tissues and can transfer it to the liver and to other lipoproteins such as very low-density lipoprotein (VLDL) 17. The exogenous and endogenous pathway plays an important role in FH.

3.2.1 Exogenous lipoprotein transport
Daily 0.5 grams of cholesterol and about 100 grams of triglycerides (TG) are ingested and absorbed by the gut (figure 1). The TG uptake takes primarily place in the duodenum and upper jejunum. Cholesterol is only partially absorbed, the whole length of the small intestine takes up 25-75 %, and the remainder is removed by fecal excretion. In the enterocyte, free cholesterol (FC) is converted into cholesterol-esters (CE) by the action of the membrane-bound enzyme, acyl coenzyme A transferase (ACAT). Here triglyceride and cholesterol-esters are converted into chylomicrons and are transported through the mucosa cells to the mesenteric lymph vessel and subsequently via the thoracic duct to the intravascular circulation. In the circulation, the enzyme lipoprotein lipase (LPL) hydrolyses the TG into free fatty acids and glycerol. Free fatty acids are used by muscle tissue as an energy source or are stored in adipose tissue. Chylomicron
and chylomicron-remnants are removed from the circulation by the LDL receptor (LDLR) and LDL-receptor related protein (LRP) on the liver cell surface.

### 3.2.2 Endogenous lipoprotein transport

Hepatocytes synthesize VLDL from TG and cholesterol. VLDL is secreted into the circulation and hydrolyzed by the enzyme lipoprotein lipase (LPL) (figure 1). Loss of lipid from the hydrophobic core of VLDL leads to shrinkage and remodeling of the particle to intermediate-density lipoprotein (IDL) and LDL. Seventy-five percent of the cholesterol transport is accounted for by LDL and of the LDL particles approximately 70% are cleared from the plasma by the LDLR present on the surface of liver cells. The remainder is cleared by the LDLR present on the peripheral tissues of organs that require cholesterol as a substrate. LDL can also be modified (e.g., by oxidation) and the modified LDL is taken up by scavenger receptors (SR) in the macrophage. Intracellular accumulation of cholesterol-esters converts a macrophage into a foam cell, one of the first steps in atherogenesis.

![Figure 1. lipid metabolism](image)

A = exogenous lipoprotein transport, B = endogenous lipoprotein transport, C = reverse cholesterol transport, Ape; apolipoprotein; LPL; lipoprotein lipase, LRP; LDL-receptor related protein, LDLR; LDL-receptor, VLDL: very low-density lipoprotein, IDL: intermediate-density lipoprotein, LDL: low-density lipoprotein, HDL: high-density lipoprotein, HL: hepatic lipase, SR: scavenger receptor.
3.3 LDL-receptor

The main role of the LDL-receptor is to maintain a constantly available source of cholesterol throughout the body for cell membrane synthesis and for supplying certain organs that require cholesterol as a substrate for their metabolic products like bile acids, sex hormones and corticosteroids. Thus the liver, gonads and adrenals are well equipped with LDL-receptors, and the liver, because of its size, is the major site of receptor-mediated LDL catabolism. LDL-receptors also bind VLDL remnants or IDL and a subfraction of HDL, which contains apolipoprotein E.

Figure 2 shows a schematic representation of this receptor in action. Fibroblasts from normal subjects possess specific cell surface receptors that recognize both apolipoprotein B$_{100}$ and apolipoprotein E (B,E receptors) and thereby bind LDL by means of a high affinity, saturable mechanism. The bound LDL is incorporated into the fibroblast within endocytotic vesicles, derived from clathrin-coated pits in which LDL-receptors cluster on the cell surface. After shedding their coats, the endocytotic vesicles fuse to become endosomes within which the LDL dissociates from its receptor. The latter is recycled to the surface and re-enters another coated pit whereas the LDL undergoes lysosomal digestion. This results in degradation of apolipoprotein B$_{100}$ and hydrolysis of cholesterol esters. The free cholesterol that is released serves to control the rate of cholesterol synthesis within the cell by down-regulating the enzyme HMG CoA reductase.

**Figure 2. LDL receptor pathway**

![Diagrammatic representation of a fibroblast showing uptake and partial degradation of LDL via the LDL receptor pathway.](image)

*Proc Natl Acad Sci USA 1979;76: 3330-7*
Excess free cholesterol is re-esterified within the cell by ACAT, which preferentially uses oleate for this purpose. The rate of synthesis of LDL receptors is in turn regulated by a feedback mechanism linked to the cholesterol content of the cell, and mediated by the LDL-receptor gene via sterol regulatory elements of the promoter.

3.4 LDL-receptor gene mutations
The LDL-receptor gene occupies approximately 45 kb on the short arm of chromosome 19 and compromises 18 exons and 17 intervening introns. The gene for the LDL-receptor is a so-called housekeeping gene, which is, in almost all tissues, continuously translated into LDL-receptors. The transcription is regulated by means of negative feedback mechanism by certain sterols.

At present, more than 600 mutations of the LDL receptor gene, responsible for FH are known. LDL receptor mutations can be divided into five classes based on their phenotypic effects on the protein. Class 1 mutations fail to produce receptor protein. The so-called null-allele is the most prevalent and makes up more than 50% of the total number of mutations. Class 2 mutations encode proteins that are blocked, either partially or completely, in transport between the ER and the Golgi complex (transport-defective alleles). Class 2 mutations can be subclassified into a class 2A and a class 2B mutations. Class 2A mutations produce proteins that are transported at a detectable, but reduced rate. Class 2B mutations encode for proteins that are characterized by slow transport to the Golgi.

Class 3 mutations encode proteins that are synthesized and transported to the cell surface, but fail to bind LDL normally (binding-defective alleles). Class 4 mutations have a normal synthesis of the LDL receptor protein and normal binding of LDL, but clustering in coated pits and internalization of the receptor complex does not take place (defective-alleles). These mutated receptors are synthesized normally, folding and transport are normal, but clustering in coated pits is impossible (class 4A) and sometimes the receptors are even secreted after they have reached the cell surface (class 4B). Class 5 mutations are mutations in the domain that mediates the acid-dependent dissociation of receptor and ligand in the endosome, an essential event for receptor recycling (recycling-defective alleles).
4. Atherosclerosis

The process of atherosclerosis starts with early lesions consisting of subendothelial accumulation of cholesterol-engorged macrophages (foam cells). These lesions, called ‘fatty streaks’, are not clinically significant, but they are the precursors of more advanced lesions (fibrous lesions) characterized by the accumulation of lipid-rich debris and smooth muscle cells (SMCs). The ‘fibrous lesions’ have a ‘fibrous cap’ consisting of SMCs and extracellular matrix that encloses the beginning of a lipid-rich ‘necrotic core’. These plaques can become ‘complex lesions’ by calcification and ulceration at the luminal surface, and by small vessels that grow into the lesion from the media of the blood vessel wall. Although advanced lesions can grow so large as to block blood flow, the most important clinical complication is an acute occlusion due to the formation of a thrombus or blood clot, resulting in myocardial infarction or stroke. Usually this process is associated with rupture of the atherosclerotic lesion 23.

5. Clinical characteristics and therapy in FH children

5.1 Diagnosis

The clinical diagnosis of FH is based on family history, physical examination, and laboratory findings. Because of its autosomal dominant inheritance mode each child with FH consequently has one affected parent and often a positive family history for premature atherosclerosis. Clinical symptoms of the disease are the result of cholesterol deposits in the cornea (arcus lipoidus), on the eyelids (xanthelasmas), on the Achilles tendons and extensor tendons of hands and feet (xanthomas) (figure 3 and 4) and tuberous xanthomas on the processus olecrani and tibial tuberosity 24. However, the disease is mostly asymptomatic in children. Less than 10 % of heterozygous FH children have tendon xanthomas and these are primarily found in the second decade of life 25. In children the disease is characterized by plasma levels of TC and LDL-C above the 95th percentile for age and gender (table 1) and HDL-C, TG and VLDL-C in the normal range, although HDL-C plasma levels are usually slightly decreased in children 26. A mutation in the LDL-receptor gene as described in paragraph 3.4 is the only unequivocal diagnosis.
Figure 3. Achilles tendon xanthomas

Figure 4. Extensor tendons xanthomas of the hand

Thus the diagnosis of FH in children is usually based on elevated levels of LDL-C. Therefore the Expert Panel on Blood Cholesterol Levels in Children and Adolescents recommended screening guidelines to identify these children at risk and they recommended that a lipoprotein level should be obtained in children:
1) whose parents and grandparents underwent coronary arteriography below 55 years of age and were found to have coronary atherosclerosis, 2) whose parents, grandparents had a previously documented myocardial infarction, angina pectoris, peripheral vascular disease, cerebral vascular disease or sudden cardiac death below 55 years of age, 3) of a parent who has been found to have high blood cholesterol (TC >6.2 mmol/L), and 4) whose parental or grandparental history is unobtainable and the children have 2 or more other cardiovascular risk factors 27.

Table 1. 95th percentile plasma lipid values of normal subjects in the first two decades of life

<table>
<thead>
<tr>
<th>Age (y)/Sex</th>
<th>TC (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>TG (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>4.91</td>
<td>3.26</td>
<td>1.89</td>
<td>1.14</td>
</tr>
<tr>
<td>F</td>
<td>5.12</td>
<td>3.45</td>
<td>1.87</td>
<td>1.19</td>
</tr>
<tr>
<td>10-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>5.25</td>
<td>3.37</td>
<td>1.89</td>
<td>1.41</td>
</tr>
<tr>
<td>F</td>
<td>5.33</td>
<td>3.47</td>
<td>1.81</td>
<td>1.48</td>
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<tr>
<td>15-19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>5.10</td>
<td>3.86</td>
<td>1.76</td>
<td>1.67</td>
</tr>
<tr>
<td>F</td>
<td>5.38</td>
<td>3.78</td>
<td>1.92</td>
<td>1.40</td>
</tr>
</tbody>
</table>

5.2 Therapy in children
The recommended therapy for FH children consists of dietary intervention, but the long-term efficacy of a lipid-lowering diet in children is very poor 28. The US National Cholesterol Education Program (NCEP) recommends drug therapy for children above 10 years whose LDL-C remains elevated (>4.9 mmol/L) after diet therapy, and for children whose LDL-C remains above 4.1 mmol/L in combination with a positive family history of premature cardiovascular disease (CVD) or the child has 2 or more other risk factors 27. Bile acid sequestrants are considered the drugs of choice, but the lipid-lowering efficacy is modest (10-15%) and long-term compliance remains poor 29; 30. The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) are effective, safe, and well tolerated lipid-altering agents in adults. Statins have proven to reduce the incidence of CHD, stroke, and peripheral vascular disease 31; 32 and are currently widely used in adults.

Thus far, only a few studies have been conducted to evaluate statin therapy in children and adolescents. Stein and colleagues (1989) were the first to show a
40% reduction of LDL-C in FH children treated with lovastatin or simvastatin, but this study was not controlled and only involved a small group of boys. In 1992, another small (n=32) and uncontrolled study with simvastatin showed a 37% LDL-C reduction and excellent tolerability. In addition, three other statin studies in children or adolescents have been reported: two placebo-controlled and one uncontrolled. In the first placebo controlled study, 72 FH children (66% girls), age 10-16 years, were randomized to placebo or pravastatin 5, 10, or 20 mg. After 12 weeks, LDL-C levels were reduced by 23%, 24%, and 33% in the groups receiving pravastatin 5, 10, and 20 mg, respectively. Short-term safety and tolerability were excellent. In the second controlled study, 132 boys, between 10 and 17 years of age were randomized to either lovastatin or placebo. Lovastatin was started at 10mg/day and the dosage was doubled every 8 weeks to a maximum of 40mg/day. Mean LDL-C levels decreased significantly relative to placebo in all active treatment groups. Data on growth and hormonal status indicated no significant differences between lovastatin and placebo in a 48 week time period. Lambert and colleagues reported an uncontrolled study in which boys were randomized to lovastatin 10, 20, 30, or 40 mg/day for 12 weeks. LDL-C levels were reduced by 21 to 36% and lovastatin was again well tolerated with no serious adverse events. Although these studies showed good efficacy of statins in children, they were short term, had a limited sample size, were mostly conducted in boys or did not provide extensive information about growth and development.


In the general population, autopsy studies of young healthy adolescents have shown that the first sign of atherosclerosis are present already in the young. The Bogalusa Heart Study performed autopsies on 204 young persons and correlated the risk factors with the extent of atherosclerosis in the aorta and coronary arteries. They showed a 10 to 20% prevalence of fibrous plaque lesions in children between 2 and 20 years of age in the coronary arteries and the aorta. Another autopsy study demonstrated a prevalence of 3.2% of coronary stenosis in adolescents between the age of 15-19. Combining these data with the aggressive nature of vascular disease in adult FH patients, one can safely assume that atherosclerotic changes in FH begin in early childhood.
Surrogate markers are able to assess sub-clinical atherosclerosis. They are predictive for cardiovascular disease and improvement in the marker correlates with improvement in the atherosclerotic process. Flow-mediated dilatation (FMD) to measure endothelial function and B-mode ultrasound to measure the intima media thickness (IMT) of the carotid artery are useful surrogate markers to assess sub-clinical atherosclerosis in clinical intervention trials.

6.1 Endothelial function

Endothelial dysfunction is an early reversible stage in the development of atherosclerosis, it is detectable before morphological changes are present. Dysfunctional endothelium promotes atherosclerosis through vasoconstriction, platelet activation, leukocyte adhesion, thrombogenesis, inflammation, smooth muscle cell proliferation, and collagen breakdown. Coronary and brachial artery endothelial dysfunction is associated with advanced age, male sex, hypercholesterolemia, cigarette smoking, hypertension, diabetes mellitus, high-homocysteine levels, high-fat diet, physical inactivity and family history of premature coronary heart disease.

An impaired endothelial function has a predictive value for future cardiovascular events. In the last decade, a number of studies have shown that endothelial function measured as flow-mediated dilatation (FMD) is impaired in FH children. This indicates that in these young asymptomatic children the process of atherosclerosis has already begun.

The FMD technique is based on nitric oxide (NO) availability of the endothelium. Increased arterial blood flow (shear) is sensed by the endothelium with a subsequent release of NO and other vasodilators. By inflating a blood pressure cuff upon the forearm and releasing it after a few minutes shear stress is imitated in the brachial artery (figure 5). Using ultrasound sonography one can easily measure the diameter of the vessel before and after the period of ischemia. FMD is expressed as the proportional increase of the diameter after ischemia compared to before ischemia. An abnormal response consists of lesser vasodilatation and thus of endothelial dysfunction.

Hydroxymethylglutaryl coenzyme A reductase inhibitors (statins) have been shown to reverse endothelial dysfunction, however, only in adult dyslipidemic patients. The correlation between FMD improvement and the degree of LDL-C lowering implies a role for cholesterol lowering per se in mediating the beneficial effects of statins. However, statins might also exert lip independent
pleiotropic effects on the vasculature, for instance by up regulating endothelial cell NO synthase (ecNOS) through stabilization of mRNA levels \(^{53}\).

**Figure 5.** Schematic overview of the FMD technique

6.2 Intima media thickness (IMT) of the carotid artery

Early morphological changes in arterial walls are characterized by sub-endothelial accumulations of cholesterol in macrophages and SMC growth and proliferation. This process results in arterial wall thickening \(^{23}\). The arterial wall can be visualized by means of non-invasive B-mode ultrasound imaging of large peripheral arterial walls. The edges of the lumen-intima and media-adventitia ultrasound interfaces of the posterior artery walls represent the boundaries of the intima-media complex. The distance between the interfaces is therefore called intima-media thickness (IMT) (figure 6) \(^{54,55}\).

Several cross-sectional studies have shown that increased carotid artery IMT is associated with cardiovascular risk factors like LDL-C and age \(^{56}\). Increased IMT is also associated with increased risk for myocardial infarction and stroke in adults \(^{57-59}\). Furthermore, in prospective studies carotid IMT has shown to be an independent predictor for cardiovascular disease \(^{60}\). In FH children a number of B-mode ultrasound studies demonstrated increased IMT compared to healthy controls. It could therefore be concluded that already in a young FH population morphological arterial wall changes can be measured \(^{61-65}\). These findings emphasize the importance of inhibiting arterial wall thickness progression at young age in this population at risk.

Recently, our center reported that LDL-C reduction with atorvastatin 80mg over a two year period in adult FH patients suffering from FH was accompanied by carotid IMT regression \(^{66}\). Other intervention studies have also demonstrated
that B-mode ultrasonography is an useful tool to monitor IMT progression changes in time. However, it still remains to be established whether statin therapy effects can be observed in FH children.

**Figure 6.** Schematic overview of the B-mode (IMT) ultrasound technique.

B-mode ultrasound image the distal common carotid artery of a child aged 15.1yrs (left). The distance between the leading ultrasound interfaces of the double-line pattern of the arterial far wall, the intima-media thickness (IMT) are directly related to the thickness of the histological defined intima-media complex.

### 7. Outline of this thesis

The studies described in this thesis were designed to investigate several aspects concerning the diagnosis, treatment and sub-clinical atherosclerosis of FH in childhood.

In chapter 2 the 12-year referral data of our pediatric lipid out-patient clinic were analyzed. The objectives were: first to establish specific LDL-C levels or percentile cut-offs that provide the most specific and sensitive means for the diagnosis of FH children; secondly, to address whether the variations in lipoproteins in these FH children can be explained by physical or lifestyle characteristics, which then could be focus of more hygienic intervention; thirdly, to determine whether the levels of lipid and lipoprotein in these children are associated with the occurrence of premature CVD in their families. In chapter 3 the objective was to investigate, whether children with a positive family history for cardiovascular events are more at risk for future cardiovascular disease than children with a negative family history. The FMD technique was used to estimate the process of sub-clinical atherosclerosis in these children. In chapter 4 the baseline characteristics, of a large randomized double-blind placebo-controlled trial, evaluating the efficacy, safety and tolerability
of simvastatin therapy in FH children, are presented. The results of this large international multi-center trial are described in chapter 5. The impact of this statin therapy on quality of life, anxiety and concerns in FH children and their parents was studied (chapter 6). Extensive self-report questionnaires were evaluated and discussed.

The next two chapters describe two placebo-controlled studies, evaluating the effect of statin therapy on sub-clinical atherosclerosis. Endothelial function was assessed by using the non-invasive technique of flow-mediated dilatation of the brachial artery (FMD) and arterial wall thickening was assessed by measuring the intima media thickness of the carotid artery (IMT), using B-mode ultrasound. In chapter 7 the objectives were to evaluate whether young asymptomatic FH children are already characterized by the presence of endothelial dysfunction and whether statin therapy improves endothelial function. Chapter 8 evaluates the effect of statin therapy on the IMT of the carotid artery in FH children. In the last chapter of this thesis (chapter 9) the objectives were, to investigate whether pre-pubertal FH children are already characterized by an impaired FMD and whether the use of plant sterol spread improves the endothelial function. We used a double-blind placebo controlled crossover design to answer these study questions.

This thesis ends with a summary and general discussion in which the main results are placed in a wider context. Furthermore, the implication of our findings on the approach of FH in childhood and recommendations for future research are discussed (chapter 10).
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Chapter 1


