Pharmacokinetics, efficacy, and safety of pravastatin in children

Studies in children with heterozygous familial hypercholesterolemia and in pediatric cardiac transplant recipients

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Academic Dissertation

To be publicly discussed, with the permission of the Faculty of Medicine of the University of Helsinki, in the Niilo Hallman-Lecture Hall, Hospital for Children and Adolescents, on November 24th at 12 noon.

Helsinki
2006

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ISBN 952-92-1293-3 (paperback)
ISBN 952-10-3527-7 (pdf)

Helsinki University Printing House
2006

To my Grandma Toini

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LIST OF ORIGINAL PUBLICATIONS

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

I. Hedman M, Neuvonen PJ, Neuvonen M, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in children with familial hypercholesterolemia. Clin Pharmacol Ther 2003;74:178-85.

II. Hedman M, Neuvonen PJ, Neuvonen M, Holmberg C, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in pediatric and adolescent cardiac transplant recipients on triple immunosuppression. Clin Pharmacol Ther 2004;75:101-9.

III. Hedman M, Matikainen T, Föhr A, Lappi M, Piippo S, Nuutinen M, Antikainen M. Efficacy and safety of pravastatin in children and adolescents with heterozygous familial hypercholesterolemia: a prospective clinical follow-up study. J Clin Endocrinol Metab 2005;90:1942-52.

IV. Hedman M, Miettinen T, Gylling H, Ketomäki A, Antikainen M. Serum noncholesterol sterols in children with familial hypercholesterolemia undergoing pravastatin therapy. J Pediatr 2005; in press.

V. Hedman M, Pahlman R, Sundvall J, Ehnholm C, Syvänne M, Jokinen E, Jauhiainen M, Holmberg C, Antikainen M. Low HDL cholesterol predicts the onset of transplant vasculopathy in pediatric cardiac recipients on pravastatin therapy. Pediatr Transplant 2006; submitted.

ABBREVIATIONS

ACTH, adrenocorticotropic hormone

ALT, alanine aminotransferase

ApoA-I, apolipoprotein A-I

ApoA-II, apolipoprotein A-II

ApoB, apolipoprotein B

ApoC, apolipoprotein C

AUC, area under the concentration-time curve

BA, bone age

CA, chronologic age

CHD, coronary heart disease

CK, creatine kinase

C_{max}, peak concentration

CoA, coenzyme A

CYP, cytochrome P450

EDTA, ethylenediaminetetraacetic acid

ER, endoplasmic reticulum

FDA, Food and Drug Administation

FH, familial hypercholesterolemia

FSH, follicle-stimulating hormone

GLC, gas-liquid chromatography

GnRH, gonadotropin-releasing hormone

HDL, high-density lipoprotein

HeFH, heterozygous familial hypercholesterolemia

HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A

IDL, intermediate-density lipoprotein

ISHLT, International Society of Heart and Lung Transplantation

K_e, elimination rate constant

KELA, Social Insurance Institution of Finland

LDL, low-density lipoprotein

LFA-1, leukocyte function antigen-1

LH, luteinizing hormone

LPL, lipoprotein lipase

MHC II, major histocompatibility complex class II

MMF, mycophenolate mofetil

NCEP, National Cholesterol Education Program

PEG, polyethylene glycol

$T_{1/2}$, half-life

Tmax, time to peak concentration

TxCAD, accelerated transplant vasculopathy

VLDL, very-low-density lipoprotein

W/H, weight for weight index

ABSTRACT

Background. Hyperlipidemia is a common concern in patients with heterozygous familial hypercholesterolemia (HeFH) and in cardiac transplant recipients. In both groups, an elevated serum LDL cholesterol level accelerates the development of atherosclerotic vascular disease and increases the rates of cardiovascular morbidity and mortality. The purpose of this study is to assess the pharmacokinetics, efficacy, and safety of cholesterol-lowering pravastatin in children with HeFH and in pediatric cardiac transplant recipients receiving immunosuppressive medication.

Patients and Methods. The pharmacokinetics of pravastatin was studied in 20 HeFH children and in 19 pediatric cardiac transplant recipients receiving triple immunosuppression. The patients ingested a single 10-mg dose of pravastatin, and plasma pravastatin concentrations were measured up to 10/24 hours. The efficacy and safety of pravastatin (maximum dose 10 to 60 mg/day and 10 mg/day) up to one to two years were studied in 30 patients with HeFH and in 19 cardiac transplant recipients, respectively. In a subgroup of 16 HeFH children, serum non-cholesterol sterol ratios (10^2 x mmol/mol of cholesterol), surrogate estimates of cholesterol absorption (cholestanol, campesterol, sitosterol), and synthesis (desmosterol and lathosterol) were studied at study baseline (on plant stanol esters) and during combination with pravastatin and plant stanol esters. In the transplant recipients, the lipoprotein levels and their mass compositions were analyzed before and after one year of pravastatin use, and then compared to values measured from 21 healthy pediatric controls. The transplant recipients were grouped into patients with transplant coronary artery disease (TxCAD) and patients without TxCAD, based on annual angiography evaluations before pravastatin.

Results. In the cardiac transplant recipients, the mean area under the plasma concentration-time curve of pravastatin [AUC(0-10)], 264.1 ± 192.4 ng·h/mL, was nearly ten-fold higher than in the HeFH children (26.6 ± 17.0 ng·h/mL). By 2, 4, 6, 12 and 24 months of treatment, the LDL cholesterol levels in the HeFH children had respectively decreased by 25%, 26%, 29%, 33%, and 32%. In the HeFH group, pravastatin treatment increased the markers of cholesterol absorption and decreased those of synthesis. High ratios of cholestanol to cholesterol were associated with the poor cholesterol-lowering efficacy of pravastatin. In cardiac transplant recipients, pravastatin 10 mg/day lowered the

LDL cholesterol by approximately 19%. Compared with the patients without TxCAD, patients with TxCAD had significantly lower HDL cholesterol concentrations and higher apoB-100/apoA-I ratios at baseline (1.0 ± 0.3 mmol/L *vs.* 1.4 ± 0.3 mmol/L, P = 0.031; and 0.7 ± 0.2 *vs.* 0.5 ± 0.1, P = 0.034) and after one year of pravastatin use (1.0 ± 0.3 mmol/L *vs.* 1.4 ± 0.3 mmol/L, P = 0.013; and 0.6 ± 0.2 *vs.* 0.4 ± 0.1, P = 0.005). Compared with healthy controls, the transplant recipients exhibited elevated serum triglycerides at baseline (median 1.3 [range 0.6-3.2] mmol/L vs. 0.7 [0.3-2.4] mmol/L, P=0.0002), which negatively correlated with their HDL cholesterol concentration (r = -0.523, P = 0.022). Recipients also exhibited higher apoB-100/apoA1 ratios (0.6 ± 0.2 vs. 0.4 ± 0.1, P = 0.005). In addition, elevated triglyceride levels were still observed after one year of pravastatin use (1.3 [0.5-3.5] mmol/L vs. 0.7 [0.3-2.4] mmol/L, P = 0.0004). Clinically significant elevations in alanine aminotransferase, creatine kinase, or creatinine ocurred in neither group.

Conclusions. Immunosuppressive medication considerably increased the plasma pravastatin concentrations. In both patient groups, pravastatin treatment was moderately effective, safe, and well tolerated. In the HeFH group, high baseline cholesterol absorption seemed to predispose patients to insufficient cholesterol-lowering efficacy of pravastatin. In the cardiac transplant recipients, low HDL cholesterol and a high apoB-100/apoA-I ratio were associated with development of TxCAD. Even though pravastatin in the transplant recipients effectively lowered serum total and LDL cholesterol concentrations, it failed to normalize their elevated triglyceride levels and, in some patients, to prevent the progression of TxCAD.

1. INTRODUCTION

Familial hypercholesterolemia (FH) is a common genetic disease resulting from functionally significant mutations of the LDL receptor gene.[1, 2] Consequently, the total and LDL cholesterol concentrations of the affected subjects are significantly elevated. High serum cholesterol, observable already at birth,[3] in a life-long manner, predisposes the subjects with HeFH to premature coronary heart disease (CHD). In untreated men, the mean age of diagnosed CHD is 48 years,[4] whereas untreated women usually develop CHD approximately 10 years later.[4] In both FH-subjects and non-FH-subjects, numerous trials suggest that effective cholesterol-lowering therapy can prevent, delay, and even regress the development of CHD.[5-15]

Accelerated coronary heart disease (TxCAD) is a common and a serious complication in both pediatric[16] and adult[17] cardiac transplant recipients following heart transplantation. Various immunological (e.g. rejections), inflammatory (chronic inflammatory response from the recipient to the donor endothelial cells), and infectious factors (i.e. cytomegalovirus)[18-21] as well as metabolic factors (i.e. hypercholesterolemia, hypertriglyceridemia and insulin resistance)[22-30] are thought to predispose transplant recipients to TxCAD.

In adults with HeFH[11, 31] and in adult cardiac transplant recipients,[32-40] statins, potent inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis, have markedly decreased the mortality and morbidity of cardiovascular disease. Despite the potential beneficial effects, the lack of safety and efficacy data has prevented the use of statins in children until recently. Currently, a few statin trials in children with HeFH[41-54] and in pediatric cardiac transplant recipients[55-58] exist, although many of them are limited by the short follow-up time or the small number of participants. The purpose of this series of studies is to investigate the pharmacokinetics, safety, and efficacy of pravastatin in children with heterozygous FH and in pediatric cardiac transplant recipients.

2. REVIEW OF LITERATURE

2.1. Cholesterol and cholesterol metabolism

2.1.1. Cholesterol

Cholesterol is an insoluble lipid, which has one hydroxyl group and one double bond in its sterol nucleus, and a single side chain consisting of eight carbon atoms. Cholesterol has several vital roles; it is, for example, an essential component of cell membranes and lipoproteins, and a precursor for bile acids, adrenal steroids (hydrocortisone and aldosterone) and sex hormones (estrogens and androgens), and vitamin D metabolites, and is an important factor in neural myelinization and brain growth.[59]

Humans obtain cholesterol from diet (exogenous pathway) and from *de novo* synthesis within the body (endogenous pathway) (Figure 1).

2.1.2. Cholesterol transport

Due to their lipophilic nature, cholesterol and triglycerides are transported in blood by lipoproteins classified by increasing density as chylomicrons: chylomicron remnants, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), intermediate-density lipoproteins (IDL), and high-density lipoproteins (HDL) (Figure 1).[60] The basic composition of the lipoproteins is similar, consisting of a core of cholesteryl esters and triglycerides surrounded by a surface coat of phospholipids, unesterified cholesterol, and apoproteins. The quantities and qualities of apoproteins vary in the particles: Apolipoprotein B (apoB) is the chief apolipoprotein of LDL, VLDL, and IDL, whereas apolipoprotein As, which can be subdivided into apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II), are the main protein constituents of HDL. HDL particles also contain and secrete apolipoprotein C (apo C).

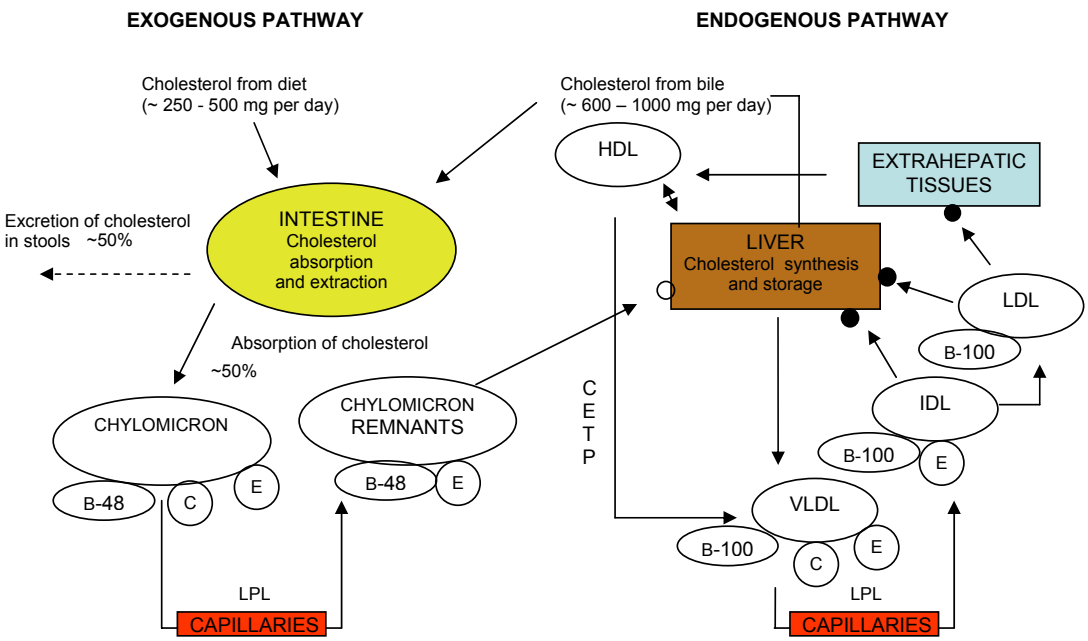

Figure 1. Exogenous and endogenous pathways of cholesterol metabolism and mechanisms of cholesterol transport. B-100, apolipoprotein B100; B-48, apolipoprotein B48; C, apolipoprotein C; E, apolipoprotein E; CETP, cholesteryl ester protein; LPL, lipoprotein lipase. Small solid and open circles represent the LDL receptors and the chylomicron remnant receptors, respective.

2.1.3. Exogenous pathway

The exogenous pathway of lipids originates from the intestine.[61] In adults, the daily amounts of dietary and biliary cholesterol vary between 250 - 500 mg and 600 - 1000 mg, respectively. Approximately half of this cholesterol is absorbed in the small intestine and half is excreted in stools. Exogenous fats are packed into chylomicrons, which are transported via lymphatic vessels into the circulation.[60] Lipoprotein lipase (LPL), located at the surface of the vascular endothelium of the adipose and muscle tissue, hydrolyzes most of the triglycerides into free fatty acids.[60] The resulting particles, referred as to chylomicron remnants, are then cleared from the circulation by the liver.[62]

2.1.4. Endogenous pathway

The endogenous lipid pathway originates from the liver.[63-65] The liver secretes cholesterol into the circulation in triglyceride-rich VLDL particles, which can then be transformed into IDL in a process where by LPL most core triglycerides are removed, and some surface molecules are lost to HDL.[60] The IDL particles can be either removed from the circulation by the hepatic LDL receptors,[63] or further converted into LDL particles by lipolysis of the core triglycerides.[60, 64] LDL is an essential lipoprotein particle, which carries cholesterol to the peripheral cells.

Cells are capable of taking up cholesterol from lipoproteins that contain apolipoprotein B100 (apoB-100) (LDL, partially catabolised VLDL, IDL). ApoB-100 is a surface protein that binds to the LDL-receptor.[60] The majority of LDL-receptors are located at the parenchyma of hepatocytes. The lipoprotein particles bound the receptor are taken into the cell by endocytosis,[66, 67] after which the receptor and its ligand dissociate and the receptor is recycled back to the cell surface. In most cells, the cholesterol released from LDL particles down-regulates the synthesis of new LDL receptors, and thus inhibits the cellular accumulation of cholesterol.

Even though the liver is generally considered the primary organ for newly synthesized cholesterol, all dividing human cells are capable of synthesizing cholesterol.[68] Cholesterol is synthesized within the cells in peroxisomes and in the endoplasmic reticulum (ER). Figure 2 shows a simplified presentation of cholesterol synthesis: an early stage in cholesterol synthesis is the conversion of acetyl-CoA via two enzymatic steps to HMG-CoA, which is then converted to mevalonate.[69-71] The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme of this irreversible step, and the activity of the enzyme is down-regulated by cholesterol. Newly synthesized cholesterol can be utilized by the liver, stored in the liver in an esterified form, excreted into bile, or used for the synthesis of lipoproteins.

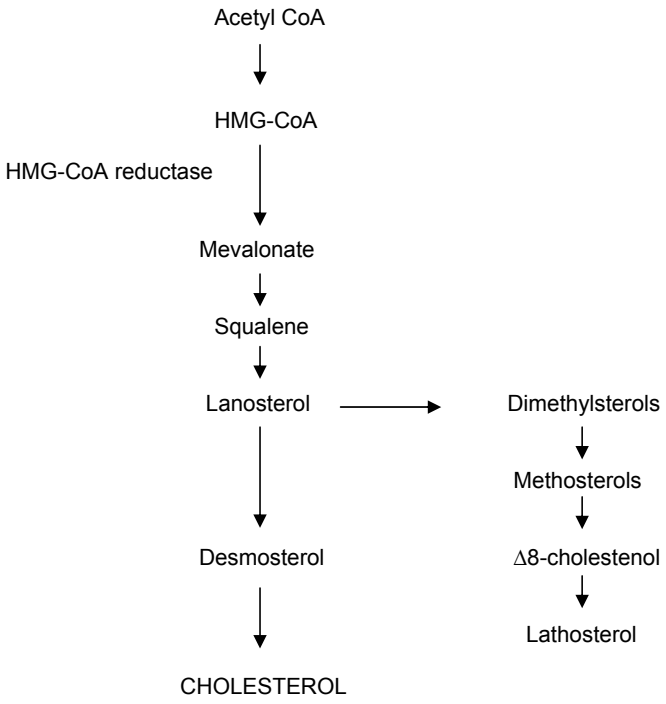

Figure 2. Cholesterol synthesis. Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; CoA, coenzyme A.

2.1.5. Elimination of cholesterol

Except for the small portion of cholesterol that is lost from the body as steroid hormones or through the renewal of skin and hair, elimination of excess cholesterol occurs in the liver through excretion via bile as an unaltered cholesterol molecule or as cholesterol incorporated into bile acids.[68, 72, 73] Cholesterol and bile acids continuously cycle between the intestine and the liver in a process known as enterohepatic circulation. Approximately 50% of the cholesterol and most of the bile acids entering the enterohepatic circulation are reabsorbed.

2.1.6. Regulation of cholesterol metabolism

The serum LDL cholesterol concentration is modified by the degree of hepatic cholesterol synthesis, cholesterol uptake from the blood by LDL-receptors, cholesterol elimination via bile, and cholesterol (both biliary and dietary cholesterol) absorption in the intestine. Overall, the absorption and synthesis of cholesterol are tightly linked to maintain cholesterol homeostasis; and, in general, as absorption increases, synthesis decreases, and *vice versa*.[73-75]

2.1.7. HDL particles and reverse cholesterol transport

Approximately one third of serum cholesterol is carried in the HDL particles. HDL particles participate in the reverse cholesterol transport process: they remove free cholesterol from peripheral tissues and transport it to the liver.[60, 76, 77] HDL particles also have antioxidant and anti-inflammatory properties[78, 79] that prevent the oxidation of LDL particles.[60] The anti-inflammatory effects of HDL have been shown to occur at endothelial level, where HDL particles decrease the action of cytokine-mediated adhesion molecules.[80-82] HDL also seems to protect the endothelial cells from cytotoxic damage caused by remnants of triglyceride-enriched lipoproteins,[83] and to trigger the proliferation of endothelial cells in the repair process.[78]

2.2. Heterozygous familial hypercholesterolemia

2.2.1. Pathogenesis

Familial hypercholesterolemia (FH) is an autosomal co-dominantly inherited disease resulting from mutations of the LDL receptor.[1, 2, 84] The LDL receptor gene is located on the short arm of chromosome 19, and its mutations can lead to abnormalities in receptor synthesis or transport, the LDL-binding capacity of the receptor, the internalization of LDL, or the recycling of the receptor back to the cell surface.[85] In heterozygous FH (HeFH), the affected individual inherits an LDL receptor gene with a functionally significant mutation from one parent, resulting in an approximately 50% reduction in the number of functional LDL receptors.[86] In its homozygous form, both genes are defective, leaving all of the LDL

receptors defective. Altogether over 900 different mutations of the LDL-encoding gene have been identified word-wide (www.ucl.ac.uk/fh/). In Finland, seven different mutations of the LDL receptor gene (FH-Helsinki [large deletion], FH-North Karelia [small deletion], FH-Turku [G823D], FH-Pori [L380H], FH-Pogosta [R574Q], FH-Fin11 [D558N], and FH-Fin12 [C331W]) are responsible for approximately 93% of all FH cases (www.ucl.ac.uk/fh/).[87-90] In the Caucasian population, HeFH affects approximately one in 500 individuals,[86] and is thus one of the most commonly known single-gene-determined disorders in man. Homozygous FH (not further discussed in this review) is rare, affecting only approximately one in a million individuals.

The impaired LDL receptor function leads to an accumulation of LDL cholesterol in the blood due to the defective hepatic uptake. An additional elevation in serum LDL cholesterol levels results as the cellular uptake of other lipoproteins containing the apo B100 (i.e. VLDL, IDL) (Figure 1) is also delayed, and these precursor lipoproteins are consequently catabolized into LDLs.[1]

2.2.2. Symptoms and signs

High serum cholesterol in subjects with HeFH can already be detected at birth,[3] and hypercholesterolemia is often the first sign of the disease.[91] A distinctive difference in cholesterol levels between children with HeFH and healthy subjects develops during the first year of life,[3] and this difference remains unchanged until adulthood. In children, HeFH is the most common cause for marked hypercholesterolemia,[92] but the distribution of cholesterol concentrations may overlap with those of the general population. Over the course of a lifetime, high cholesterol strongly predisposes the untreated subjects with HeFH to premature CHD.[93-95] Men with untreated HeFH may develop symptomatic CHD as early as in their thirties, and the cumulative probability of CHD increases markedly with age: 20% by 40 years of age, 45% by 50, and 75% by 60.[96, 97] Mortality among untreated men is 23% by 50 years. [96, 97] Untreated women with HeFH usually develop CHD approximately 10 to 15 years later than men do, and by ages 50 and 60, the cumulative incidence of CHD is approximately 20% and 32%, respectively.[95-97]

Early signs of atherosclerosis, such as fatty streaks, carotid artery plaques,[98] increased carotid artery intima-media thickness,[98-102] endothelial dysfunction[103] and decreased

carotid-artery elasticity,[101, 104, 105] can already be detected in some patients by ages 3 to19 years. Tendon xanthomas, which usually occur in the Achilles, patellar, and extensor tendons of the hand, are also pathognomonic to FH, and often appear in the second decade of life.[106, 107] Most individuals affected by HeFH develop xanthomas at some point: Kwiterovich et al. reported xanthomas based on clinical examination by an experienced clinician in 3% of patients aged 1-9 years, in 13% of patients aged 10-19 years, in 70% of patients aged 20-29 years, and in 90% of patients aged 30-39 years.[106] Based on ultrasonography examinations, researches have reported an even higher incidence of xanthomas.[108] The detection of tendon xanthomas is clinically relevant, since a recent study by Civeira et al. showed that tendon xanthomas are associated with higher CHD risk.[109] The authors suggested that patients with xanthomas may require more aggressive lipid-lowering therapy. Other clinical manifestations of hypercholesterolemia include corneal arcus and xanthelasma, of which the former occurs in approximately 50% of HeFH patients over 30 years of age.[86] Despite these early clear clinical signs, the diagnosis of HeFH is unfortunately often missed until symptomatic vascular disease develops, which will likely worsen the prognosis.

2.2.3. Diagnosis

In adults, HeFH constitutes only a small portion of hypercholesterolemia, whereby it is difficult to differentiate between HeFH and other forms of hypercholesterolemia based solely on increased cholesterol levels. A clinical diagnosis is more reliable if other factors, such as the presence of tendon xanthomas and personal or family history of premature CHD, are also considered. For eligibility of the lower special reimbursement for statins, the Social Insurance Institution of Finland (KELA) defines FH in children by the following criteria (www.kela.fi/in/internet/suomi.nsf/alias/laake211):

1. **Main criteria** (the patient must meet both criteria):
   A) Serum total cholesterol concentration is > 8 mmol/L, despite dietary intervention.
   B) Secondary causes of hypercholesterolemia and hypercholesterolemia caused by hypertriglyceridemia have been excluded.
2. **Additional criteria** (the patient must meet ≥ 1 criteria):
   A) At least one first degree relative has verified FH.
   B) The patient has tendon xanthomas

C) At least one first degree relative has developed CHD at an exceptionally young age (men under 45 years of age and women under 55 years of age).

HeFH can also be verified by DNA analysis of the underlying molecular defect in the LDL receptor gene. [110] DNA analysis is convenient in regions such as Finland, where only a few mutations are responsible for the majority of the HeFH cases. [87-90] In some other regions, however, the notable mutational heterogeneity in the LDL receptor gene may limit its use in routine diagnosis. Another option is to conduct an *in vitro* functional assay to measure the binding, internalization, and degradation of ^{125}I-labeled LDL by cultured skin fibroblasts,[111] or to measure the ability of freshly-isolated lymphocytes to proliferate when cultured in a lipoprotein-deficient medium in the presence of mevinolin, an inhibitor of endogenous cholesterol synthesis.[112]

2.2.4. Psycosocial aspects of early diagnosed HeFH

A study by de Jongh et al. showed that 44% of HeFH children emotionally suffered from the disease, and that 38% of the parents considered HeFH to be a burden on their family.[113] However, 62% of the HeFH children said that pharmaceutical treatment of hypercholesterolemia made them feel safer. [113] Several studies have shown that the psychosocial cabability of children with HeFH is similar to that of the general age-matched population. [113-115] The emotional impact of the premature death of an affected parent is, on the other hand, very significant,[116] and is associated with poor school performance and increased expression of anger.[113]

2.3. Accelerated coronary artery disease of the heart transplant (TxCAD)

2.3.1. Patient survival and accelerated coronary artery disease

Since 1967, when the first human heart transplantation was carried out, both technical procedures and immunosuppressive medication have developed considerably, leading to substantial improvement in the graft and patient survival. However, as reported by the International Society of Heart and Lung Transplantation (ISHLT) which maintains a registry of annual transplantation data from over 80 centers in the USA and Europe, long-term survival after cardiac transplantation in both adult[17] and pediatric[16] cardiac transplant

recipients remains rather poor (Figure 3 A). As Figure 3 B shows, accelerated coronary artery disease of the heart transplant (TxCAD) is an important cause of poor long-term graft survival. According to a recent ISHLT report, the incidence of angiographic detectable TxCAD seven years after transplantation in adult cardiac transplant recipients is 45.7%, and in pediatric recipients, 15.2% (Table 1).[16, 17] Because qualitative coronary angiography underestimates the severity of the coronary disease, even more patients would exhibit intimal thickening if examined by the intravascular ultrasound (IVUS) method.[117-119]

Various immunological (rejections),[20] inflammatory (chronic inflammatory response from the recipient to the donor endothelial cells),[21] and infectious factors (cytomegalovirus, *Chlamydia pneumonie*, enterovirus infection etc)[18, 19] are thought to predispose transplant recipients to TxCAD. Also, metabolic abnormalities, such as hypercholesterolemia,[27] hypertriglyceridemia,[23, 26, 28, 30, 120] increased body mass index,[22, 25] and glucose intolerance,[24, 28, 29] are frequently observed in transplant recipients, and are considered significant risk factors for TxCAD. However, TxCAD can also appear in the absence of any known risk factors. Hypercholesterolemia frequently manifests after cardiac transplantation,[58, 121, 122] and is often aggravated by the use of cyclosporine.[123, 124] In both adult[28] and pediatric[125] cardiac transplant recipients, the use of corticosteroids can lead to a metabolic abnormality characterized by a combination of high triglycerides together with insulin resistance and low HDL cholesterol.[24, 28, 29] In Finland, the main immunosuppressive therapy in pediatric recipients consists of cyclosporine, azathioprine, and methyl prednisone, while tacrolimus and mycophenolate mofetil (MMF) serve as secondary options if acute rejections occur.

Figure A

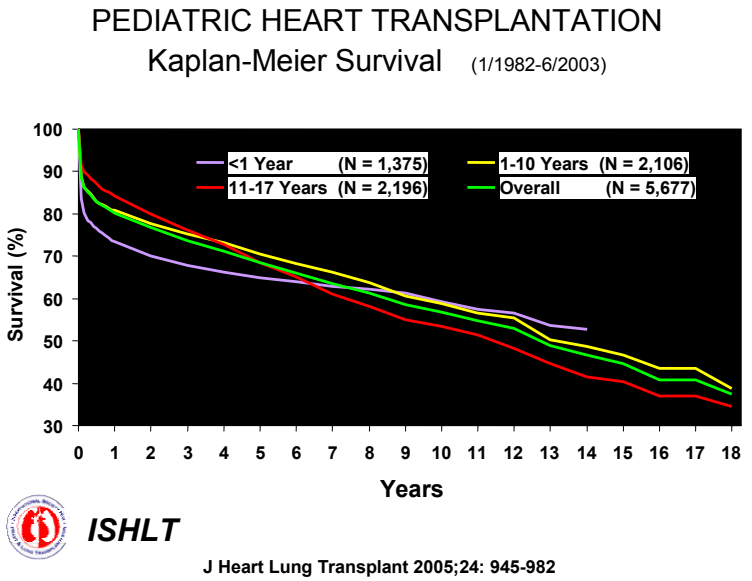

Figure B

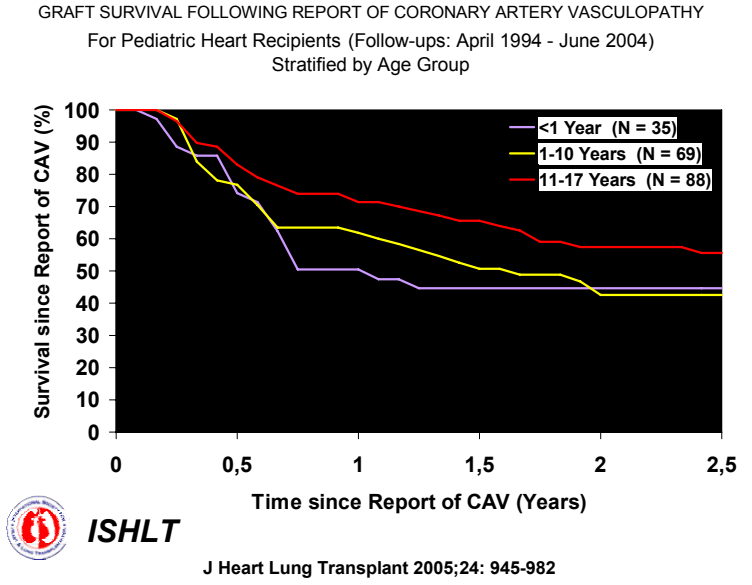

Figure 3. **Panel A** shows the patient survival of pediatric patients following heart transplantation. **Panel B** shows the graft survival after diagnosis of coronary artery vasculopathy in pediatric patients. The data by the International Society of Heart and Lung Transplantation (ISHLT) are available at www.ishlt.org/registries/slides.

Table 1. The prevalence of TxCAD and some known cardiovascular risk factors in adult and pediatric cardiac transplant recipients following heart transplantation.

The ISHLT registry data.

	1 y after Tx	3 y after Tx	7 y after Tx
Adults	n = 9659	n = 6221	n = 2103
Pediatric	n =2184	n =696	n = 357
TxCAD			
Adults	8.7%	32.3%	45.7%
Pediatric	2.5%	11.0%	15.2%
Hypertension			
Adult	72.6%	94.0%	97.7%
Pediatric	46.7%	61.4%	65.8%
Renal dysfunction			
Adult	25.7%	32.4%	35.6%
Pediatric	5.8%	9.4%	11.4%
Hyperlipidemia			
Adults	49.8%	85.1%	91.2%
Pediatric	10.1%	21.4%	26.3%
Diabetes			
Adults	23.7%	33.2%	36.5%
Pediatric	3.2%	4.6%	3.9%

Data in percentages
Reference: Taylor et al. 2005;[17] Boucek et al. 2005[16]
Tx, heart transplantation

2.4. Atherosclerosis

2.4.1. Typical atherosclerosis vs. transplant vasculopathy

Figure 4 illustrates the variant characteristics of typical atherosclerosis and allograft vasculopathy. Typical atherosclerosis shows characteristic focal, eccentric, proximal thickenings of the inner portion of the artery wall in association with fatty deposits (see also chapter *Pathophysiology of atherosclerosis* below).[126] It usually affects the large and

medium-sized arteries such as the aorta, and the iliac, femoral, coronary, and cerebral arteries.[127] The internal elastic lamina is typically disrupted, and calcium deposits are frequently present.[126] The development of typical atherosclerotic lesions may take years. The early signs of atherosclerosis appear in the arterial intima during childhood and adolescence. [103, 104, 128-135] In fact, some have been found in stillborn and newborn babies.[136, 137] The early development of atherosclerosis can be greatly enhanced by maternal hypercholesterolemia.[136] In the presence of risk factors, this process then continues throughout life. High serum total and LDL cholesterol, low HDL cholesterol, high blood pressure, poor glucose intolerance, cigarette smoking, and obesity, are known risk factors for atherosclerosis in adults, but have also been shown to correlate with the development of atherosclerotic changes in children.[130, 133-135, 138] Without lipid-lowering therapy, serum cholesterol measured at 22 years of age seems to predict the risk of coronary heart disease (CHD) over the next 30 to 40 years among the general population.[139] Autopsy studies, such as Pathobiological Determinants of Atherosclerosis in Youth and the Bogalusa Heart Study, indicate that early signs of atherosclerosis in adolescents are common.[128, 135] In autopsy studies, the extent of both fatty streaks and fibrous plaque have been shown to correlate with non-HDL cholesterol levels (determined post-mortem).[140] Furthermore, atherosclerotic plaques seem to develop later in the same regions in which the early atherosclerotic changes occur. [127, 133]

The pathological processes in transplant vasculopathy, in turn, favor concentric, occlusive distal, diffuse coronary changes rather than conventional atheroma-like lesions (Figure 4).[141] In typical transplant vasculopathy, the internal elastic lamina is usually intact, calcium deposits are absent, and signs of vasculitis occur infrequently. The development of transplant vasculopathy is expected to take months, rather than years, as in typical atherosclerosis.[126] Histological studies suggest, however, that transplant vasculopathy has a spectrum of pathologic features, and that lipid deposits and calcification may also appear later on in the older cardiac allografts.[142] This is not surprising, since both adult and pediatric cardiac transplant recipients also commonly have a high incidence of the traditional risk factors for cardiovascular diseases, such as hypertension, hyperlipidemia, renal dysfunction, and altered glucose metabolism (Table 1) (ISHLT registry data).[16, 17] Thus, in addition to changes in the endothelium and smooth muscle cells, the accumulation of lipids within the artery wall evidently contributes to the development of TxCAD.

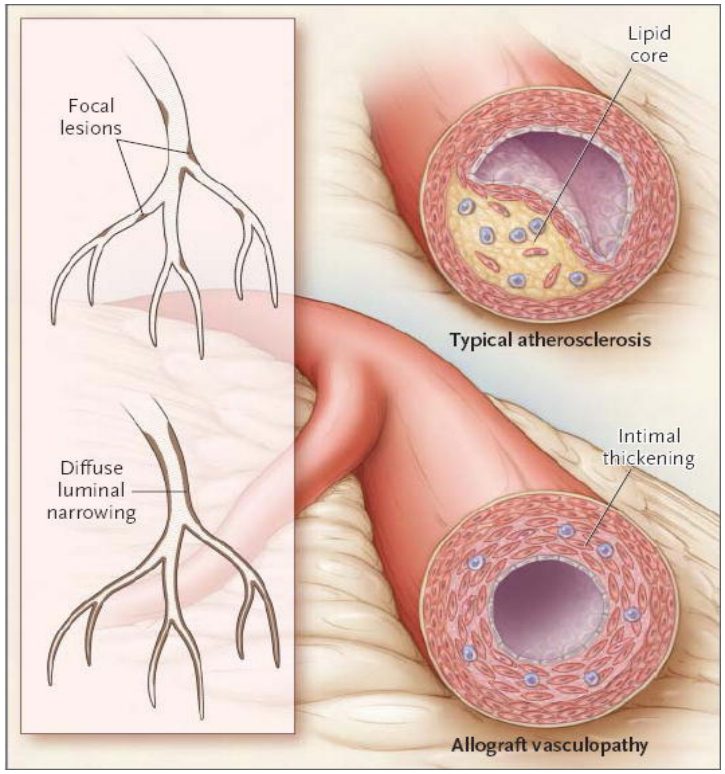

Figure 4. Characteristics of typical atherosclerosis and transplant vasculopathy. Adapted with permission 2006 from Avery.[141] . Copyright © 2003 Massachusetts Medical Society. All rights reserved.

2.4.2. Pathophysiology of atherosclerosis

The development of atherosclerosis is a complex process in which several factors and processes play a role.[143] Local injury and the increased permeability of the intima enhance the entrapment of LDL particles and VLDL remnants in the arterial wall, which is usually considered the first step in atherosclerosis. Macrophages engulf modified LDL from the arterial walls through scavenger receptors. In contrast to the LDL receptor-mediated pathway, in which the cholesterol released from LDL particles down-regulates the synthesis of new LDL receptors, the scavenger receptors are not similarly down-regulated. Hence, macrophages continue to take up cholesterol regardless of its intracellular

accumulation. This leads to the formation of foam cells, which contain large droplets of cholesterol ester. Foam cells are distinctive to atherosclerosis: the accumulation of foam cells and intercellular lipid in the intima of arteries leads to the formation of fatty streaks, which may then be further converted into fibrous plaques and finally into complicated atherosclerotic lesions. [144] Even though a plaque itself restricts the blood flow by narrowing the vessel lumen, it does not typically cause clinical symptoms (e.g. chest pain) until the degree of stenosis reaches approximately 70% or greater.[145] However, even smaller plaques can cause myocardial infarction by rupturing, because the resulting thrombus may completely occlude the vessel lumen and cease the blood flow from downstream.

Factors contributing to increased infiltration of lipoproteins into the arterial wall, decreased cholesterol transport away from arterial wall, increased endothelial permeability or vulnerability, or enhanced thrombosis can promote the development of atherosclerosis.[146, 147] Large experimental, epidemiological, and clinical studies have demonstrated that high LDL cholesterol and low HDL cholesterol are independent risk factors for atherosclerosis, both of which enhance the accumulation of cholesterol in the arterial wall.[148-152] In the context of metabolic syndrome, hypertriglyceridemia and altered glucose metabolism lead to the enrichment of HDL particles with triglycerides, and consequently, these triglyceride-enriched HDL particles function as a good substrate for hepatic lipase. Hepatic lipase hydrolyzes HDL triglycerides (also phospholipids), causing a formation of small apoA-I enriched particles that are prone to catabolism via the kidney function.[153, 154] This cascade leads to an overall reduction in levels of circulating HDL cholesterol. Besides the traditional risk-factors, infectious, inflammatory and immunological factors are also known to play a role in the pathogenesis of atherosclerosis; and inflammatory cells (i.e. T-cells), macrophages and mast cells, have been found in atherosclerotic lesions.[146, 155-161] In fact, both the metabolic risk-factors (i.e. high triglyceride levels, persistent glucose intolerance, and other abnormalities associated with metabolic syndrome), as well as a variety of immunological and inflammatory factors, have been shown to predict the development of TxCAD in adult recipients.[20, 22, 25, 28-30, 120, 162]

2.5. Treatment of hypercholesterolemia

2.5.1. Non-pharmaceutical therapy of hypercholesterolemia

The U.S. National Cholesterol Education Program (NCEP) guidelines[163, 164] and the Finnish national Current Care guidelines[165] (Käypä hoito, published in August 2004; www.kaypahoito.fi) primarily recommend dietary intervention and physical exercise for the treatment of hypercholesterolemia. Table 2 summarizes the effects of non-pharmaceutical interventions on serum lipids and lipoproteins as well as on cardiovascular mortality. The U.S. NCEP guidelines propose that if a diet (American Heart Association Step 1 diet) that restricts the daily amount of saturated fat to < 10% of total calories, total fat to < 30%, and cholesterol to < 300 mg, is insufficient, a diet (Step 2 diet) that restricts total fat to 15% of total calories is recommendable. In general, dietary therapy lowers the serum total cholesterol by 3 to 6%.[165-167] Stricter interventions that reduce the daily fat content from 35 - 40% of energy to 15 - 20% of energy further reduce serum total cholesterol, with a total reduction of approximately 10 - 20%.[168, 169] Besides cholesterol-lowering therapy, the elimination of other risk factors for cardiovascular disease, such as obesity, smoking, high blood pressure, and physical inactivity, is also considered important.[163-165, 170]

Plant sterols and stanols are structurally related to cholesterol, but cannot be synthesized in humans, and therefore originate from the diet (i.e. nuts, vegetable oils, seeds, cereals, and beans). Plant sterols and stanols are nowadays incorporated into some food products because of their cholesterol-lowering effects. The daily intake of 2.0 – 2.5 g of plant sterols or plant stanols reduces the serum levels of total and LDL cholesterol by approximately 10%.[171-174] Although the underlining mechanism is not fully understood, it seems that plant sterols and stanols decrease cholesterol absorption by displacing cholesterol from mixed mishelles.[175-177] Plant sterols and stanols do not affect the serum HDL cholesterol or triglycerides.

Table 2. Effects of non-pharmaceutical interventions on the levels of serum lipids and lipoproteins, and on overall cardiovascular morbidity.

Intervention	Lipids	Morbidity
Reducing dietary fat intake	Total cholesterol decreases by 3 – 6%	Decreases
Suplementation of plant sitosterols or sitostanols	Total cholesterol decreases by 6 – 10%	Effects unknown
Increasing dietary fiber intake	Total cholesterol decreases by 5 – 6%	Decreases
Physical exercise	HDL cholesterol increases by 5% Triglycerides decrease by 4% LDL cholesterol decreases by 5%	Effects unknown

Table modified from the Finnish national Current Care guidelines (Käypä hoito) for the treatment of dyslipidemias.[165]

2.5.2. Pharmaceutical treatment of hypercholesterolemia

Aggressive drug therapy is often required to normalize the serum cholesterol levels in HeFH. According to the Finnish national Current Care guidelines (Käypä hoito), the target total and LDL cholesterol concentrations among the general population are defined as < 5.0 mmol/l and < 3.0 mmol/l, but for high risk patients (i.e. subjects with HeFH), the targets are set even lower (4.5 mmol/ and 2.5 mmol/l, respectively).[165] Several types of drugs are available for the treatment of HeFH: Bile acids binding agents (or ion exchange resins) (i.e. colestipol and colestyramine) bind to bile acids in the small intestine, and prevent their reabsorption in the enterohepatic circulation, and thus decrease the endogenous cholesterol stores as new bile acids form in the liver with cholesterol as their precursor.

Bile acid binding agents lower serum cholesterol 10 to 15%.[178-181] Fibric acid derivatives (i.e. gemfibrozil, clofibrate, bezafibrate and fenofibrate) substantially decrease serum triglycerides and moderately increase HDL cholesterol concentrations, but only modestly (approximately 5 to 15%) lower total and LDL concentrations.[182, 183] Until recently, the underlining mechanisms for fibric acid derivatives remained unclear, but recent studies have shown, that they increase lipoprotein lipase-mediated lipolysis and alter the transcription of proteins that control lipoprotein metabolism.[184] Statins (e.g. simvastatin, lovastatin, pravastatin, fluvastatin, atrovastatin and rosuvastatin) inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol synthesis, and decrease serum LDL cholesterol by 30-70%.[185] Ezetimibe is a selective inhibitor of cholesterol absorption[186] that decreases serum total cholesterol by 20%.[187] In clinical practice in adults, ezetimibe is also often combined with a small statin dose, yielding a total reduction of serum cholesterol of approximately 60%. [187]

2.5.3. Treatment of hypercholesterolemia in childhood and adolescence

The Finnish national Current Care guidelines (Käypä hoito) and the U.S. NCEP guidelines recommend dietary therapy as the primary cholesterol-lowering treatment for children with HeFH.[163-165] The benefits of dietary therapy in healthy children have been documented as early as during the first year of life.[188-190] Large, randomized controlled trials up to seven years of duration assessing the efficacy and safety of cholesterol-lowering diets in hypercholesterolemic children have been promising, suggesting no adverse effects from moderate fat restriction in young children, assuming that caloric intake is adequate.[191, 192] In children, plant stanols or sterols also safely lower serum total and LDL cholesterol levels by approximately 6-10% and 9-19%, respectively.[193-196] Due to high baseline levels in HeFH, dietary interventions are, however, seldom sufficient to normalize serum cholesterol concentrations, and drug therapy is often required.

Due to the many crucial roles of cholesterol in growth, brain myelinization, and pubertal development, cholesterol-lowering drug therapy in children has raised concerns.[197] At present, the Finnish national Current Care guidelines (Käypä hoito) recommend that if total and LDL cholesterol concentrations remain significantly elevated (> 7 mmol/l and > 5.5 mmol/l), the child or adolescent should be remitted to a pediatrician specialized in the treatment of dyslipidemias.[165] The guidelines also emphasize that target cholesterol levels

should be determined individually taking into account the patient's family history of CHD. Bile acid binding agents such as colestipol and colestyramine are recommended as the primary choice of drug therapy for hypercholesterolemic children; the guidelines state that the use of statins in children requires special consideration due to limited long-term safety data. The U.S. NCEP similarly recommends bile acid sequestrants for children > 10 years of age with insufficient dietary response.[163, 164] However, bile acid sequestrants are generally insufficient in children with HeFH due to the sequestrants' modest efficacy, unpleasant side-effects, and poor compliance.[180, 181, 198] While the long-term safety of resins is generally accepted, niacin has been avoided in the treatment of children because of serious side-effects, such as hepatotoxicity. The European Commission and the U.S. Food and Drug Administration (FDA) have currently approved pravastatin for the treatment of such HeFH-children over eight years of age, whose LDL cholesterol levels remain elevated after adequate dietary therapy. In addition, the U.S. FDA has approved atorvastatin for pediatric use by in children over ten years of age.

Several studies in children with HeFH and a few retrospective studies in pediatric cardiac transplant recipients addressing the short-term safety and tolerability of statins are available nowadays (Table 3).[41, 43-58, 199] Until recently, the long-term safety of statins in children remained poorly understood. However, recent studies of the growth and development of adolescents with HeFH receiving statins have been promising .[43, 45, 47, 54]

Table 3. Studies with statins in children and adolescents with heterozygous familial hypercholesterolemia (HeFH) and in pediatric cardiac transplant recipients

Trial (publication year)	Design	Placebo group (patients)	Statin group (patients)	Dose of statin (mg)	Age range (y)	Follow-up time (weeks)	LDL-C reduction (%)
HeFH patients							
Pravastatin							
Knipscheer et al.[41] (1996)	a randomized, double-blind, placebo-controlled study	18	54	5-10-20	8-16	12	23-33
Mc Crindle et al.[199] (2002)	a randomized, crossover open-label study*		36	10 + colestipol 5 g	9-18**	18	17
Wiegman et al.[43] (2004)	a randomized, placebo-controlled study	108	106	20-40	8-18	104	24
Simvastatin							
Couture et al.[46] (1998)	a randomized, double-blind, placebo-controlled study	16	47	20	8-17	6	31-38
Dirisamer et al.[48] (2003)	uncontrolled		20	5-10-20	10-17	52	25-36
Ducobu et al.[49] (1992)	uncontrolled		32 (male)	10-20-40	<17	104	37
de Jongh et al.[47] (2002)	a randomized, double-blind, placebo-controlled study	69	106	10-20-40	10-17	48	31-41
Stefanutti et al.[53] (1999)	uncontrolled	8	8	10	4-12	52	29
Lovastatin							
Lambert et al.[50] (1996)	a randomized, double-blind study with a four-week placebo period		69 (male)	10-20-30-40	13†	8	21-36
Sinzinger et al.[52] (1992)	–		9	20	6-13	208	28

Stein et al.[54] (1999)	a randomized, placebo-controlled study	65 (male)	67 (male)	10-20-40	10-17	48	17-27
Clauss et al.[45] (2005)	a randomized, double-blind, placebo-controlled study	54 (female)	35 (female)	20-40	11-18	24	23-27
Atorvastatin							
Athyros et al.[44] (2000)	uncontrolled		16 (male)	10-20-40	10-17	156	45
Mc Crindle et al.[51] (2003)	a randomized, placebo-controlled study	47	140	10-20	10-17	26	40
Pediatric cardiac transplant recipients							
Pravastatin							
Penson MG et al.[57] (2001)	uncontrolled**		22	10 to 20	10-21	56	29
Mahle et al.[56] (2005)	uncontrolled**		22	0.1-0.3 mg/kg	NA	52	15***
Seipelt et al.[58] (2004)	uncontrolled**		20	5-20	0.1-16	26	34
Atorvastatin							
Chin et al.[55] (2002)	uncontrolled**		23-38	0.2 ± 0.1 mg/kg	12†	19	24-39

* Combination therapy of pravastatin and colestipol in patients with familial hypercholesterolemia or with familial combined hyperlipidemia. ** Retrospective studies. *** Reduction in total cholesterol. NA, data unavailable for the cohort. † mean age

2.6. Statins

2.6.1. Background

Statins reduce hepatic cholesterol synthesis by inhibiting the rate-limiting enzyme of cholesterol synthesis, HMG-CoA reductase (Figure 2).[69, 200] As a consequence of reduced cholesterol synthesis, hepatocytes increase the number of LDL receptors on their cell surface in order to meet cholesterol demands.[200] This leads to a reduction in serum total and LDL cholesterol concentrations. The first drug of this class, mevastatin (also known as ML-236B and compactin), was isolated as a fungal metabolite from cultures of *penicillium citrinum*.[201, 202] Other early statins (lovastatin, pravastatin, and simvastatin) that, unlike mevastatin, are still in clinical use, are also modified fungal extracts, whereas the newer statins, fluvastatin, atorvastatin, pitavastatin (currently in clinical use in Japan), and rosuvastatin, are synthetic compounds.[185, 203-205] Pravastatin and rosuvastatin are the most hydrophilic statins, whereas simvastatin and lovastatin are the most lipophilic. [185, 203-205]

The metabolism of various statins differ considerably (Table 4).[185, 203-207] The active compounds are acids derived from the hydrolysis of precursor drugs. Statins have several metabolic pathways. Rosuvastatin is glucuronidated for excretion, while simvastatin, lovastatin, and atrovastatin are metabolized by cytochrome P450 (CYP) 3A4.[205] Fluvastatin is metabolized by CYP 2C9.[203] Pravastatin is cleared via both renal and non-renal routes, and its systemic elimination does not use CYP3A4 oxidation to any great extent, but rather uses multiple other oxidative and conjugative pathways.[208]

Pharmacokinetic interactions of statins, with for example cyclosporine, itraconazole, and macrolide antibiotics, have been reported[206, 209-212] to lead to markedly increased plasma concentrations of statins[213, 214] and to severe side-effects, such as muscle toxicity.[215-217] Because cyclosporine inhibits CYP 3A4,[218] drug transporters such as P-glycoprotein,[219] intestinal efflux transporter MRP2,[220] and liver-specific uptake transporters OATP1B1,[221, 222] interactions with statins have been hypothesized to result from the competitive inhibitory effects of cyclosporine on drug catabolism or from the inhibition of statin transport. Unlike many other statins, pravastatin is unsusceptible to CYP-mediated drug interactions and is only a weak substrate of P-glycoprotein.[208, 211, 223, 224] However, cyclosporine has been suspected to interact with pravastatin by inhibition of OATP1B1 or MRP2.[210, 221, 222, 225, 226]

Table 4. Characteristics and metabolism of various statins in adults

		Lovastatin	Simvastatin	Pravastatin	Fluvastatin	Atorvastatin	Rosuvastatin *
Origin		Microbial	Semi-synthetic	Semi-synthetic	Synthetic	Synthetic	Synthetic
Lipophilic/hydrophilic		lipophilic	lipophilic	hydrophilic	hydrophilic	lipophilic	hydrophilic
Absorption	(%)	31	60-85	35	98	30	50
Bioavailability	(%)	< 5	< 5	17	10-35	12	20
T_{max}	(h)	2.8	1.3-2.4	0.9-1.6	0.5-1.5	2.0-4.0	3-5
Half-life ($T_{1/2}$)	(h)	2.5-15	1.9-15.6	0.8-3.0	0.5-2.3	11-30	19
Protein binding	(%)	95	95	48	> 99	> 98	88
CYP substrate		Yes	Yes	Clinically irrelevant	Yes	Yes	Weak
CYP2C9		(+)	(+)	(+)	+		+
CYP2D6		(+)	(+)		(+)		Clinically irrelevant
CYP3A4		+	+	(+)	(+)	+	Clinically irrelevant

Modified from Igel M et al.[203] (2001); * References[204, 205]

2.6.2. Adverse effects of statin monotherapy

Statins are generally safe and well-tolerated:[227-230] The most common clinical side-effects include gastrointestinal symptoms (i.e. nausea, flatulence, diarrhea, and constipation), headache, and muscle pain. Elevated liver transaminases or muscle enzymes occur in 0.5-2.0% and 0.08-0.09% of users, respectively.[230] Rhabdomyolysis is a rare, but potentially dangerous, side-effect.[216, 217, 231, 232] Statins must enter cells to exert their action, and the uptake of hydrophilic pravastatin, for example, requires an active transportation system. Therefore, differences in uptake may in theory explain some of the tissue-specific side-effects of both drugs and individuals. However, the overall tolerability of statin monotherapy is generally good with no clinically significant differences in the safety profiles of different statins.[233]

2.6.3. Lipid- and lipoprotein-modifying effects of statins

The cholesterol-lowering efficacy of statins varies considerably (Figure 5). Pravastatin and fluvastatin are the weakest statins, yielding a maximum LDL reduction of ~ 30% at top dosage (40 and 80 mg, respectively).[185, 234] Lovastatin, simvastatin, atorvastatin, and rosuvastatin reduce LDL cholesterol levels dose-dependently by a maximum of 30-70%.[185, 187, 233-236] Doubling the statin dose generally results in an additional 5 – 7% reduction in LDL cholesterol concentrations.[229, 234] Because triglyceride-carrying lipoproteins (IDL, VLDL) are also transported into hepatocytes via the LDL receptor, statins lower the level of serum triglycerides to some degree.[185] The effects of statins on HDL cholesterol are poorly understood and are somewhat drug specific: pravastatin, lovastatin, fluvastatin, simvastatin and rosuvastatin increase HDL cholesterol by a maximum of ~ 4 – 10%, whereas a low dosage of atorvastatin increases HDL cholesterol, but a high dosage decreases it.[185, 234]

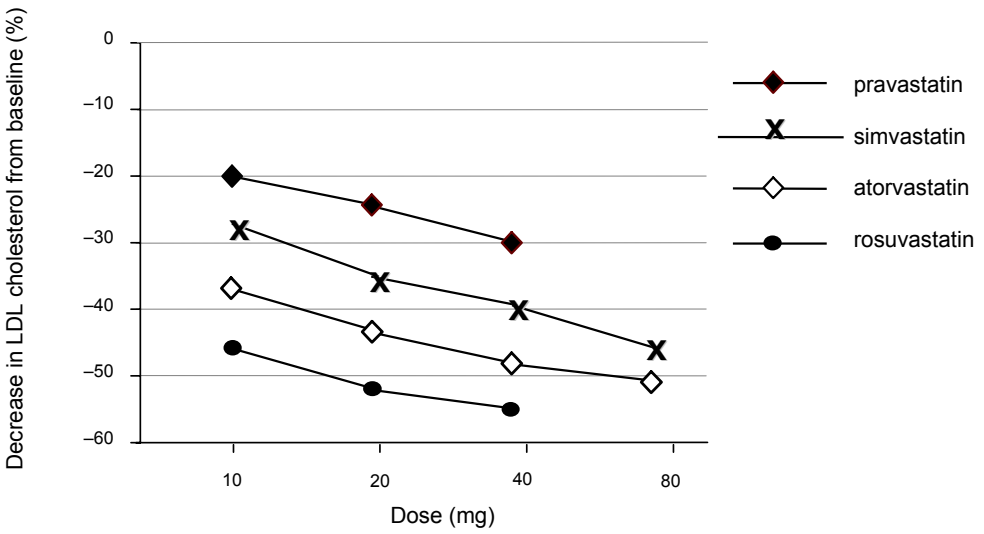

Figure 5. The LDL cholesterol-lowering efficacy of pravastatin, simvastatin, atorvastatin and rosuvastatin in increasing doses. Figure modified from Schuster.[234]

2.6.4. Effects of statins on atherosclerosis and CHD

Numerous studies have proven that, in the primary and secondary prevention of CHD in high-risk patients with and without hypercholesterolemia, effective cholesterol-lowering therapy with statins decreases the risk of acute coronary events.[6, 7, 9, 10, 12, 237-239] A recent meta-analyses by Law et al. indicated that the lower the serum cholesterol levels with statins, the lower the risk of cardiovascular events.[240] Statin therapy has been shown to stabilize endothelial inflammation and atherosclerotic plaques and to decrease serum C reactive protein levels.[241-245] In a study by Smilde et al., the thickness of the intima-media in the carotid arteries of adults with HeFH was reduced by 0.031 mm over a two-year period with 80 mg atorvastatin, but was increased by 0.036 mm with 40 mg simvastatin.[11] Nolting et al., however, reported a reduction of 0.081 mm in the thickness of the intima-media in the carotid arteries with two years of 80 mg simvastatin.[8] Furthermore, Wiegman et al. and de Jong et al. have recently demonstrated that, respectively, pravastatin can reduce intima-media thickness and restore the endothelial function in children with HeFH.[99, 246]

2.6.5. Statins in the treatment of cardiac transplant recipients

In adult cardiac transplant recipients, pravastatin and simvastatin therapy has markedly decreased blood cholesterol concentrations, rejection rates, and overall cardiovascular morbidity and mortality, thereby improving patient and graft survival.[32-40] Additional studies are, however, still warranted, since other trials on kidney transplant recipients have reported no effects of statins on the rejection rates.[247, 248] Besides their cholesterol-lowering effects, some researchers believe that statins also benefit transplant recipients by exerting a variety of immunosuppressive and immunomodulatory effects.[21, 249-251] Cholesterol-reductions can also benefit cardiac transplant recipients in several additional ways. The reduction of lipoproteins may increase in the biological activity of cyclosporine by increasing the fraction of unbound cyclosporine. Furthermore, as hypercholesterolemia seems to provoke lipid peroxidation, cause oxidative stress, increase the activation of resting T-cells and endothelial cells, and increase the secretion of various cytokines, chemokines and growth factors by macrophages, reduction of cholesterol may hinder these inflammatory pathways.[21] Recent studies suggest, however, that statins would also exhibit cholesterol-independent effects. Kwak et al. reported in an *in vitro* study that statins also act as repressors of major histocompatibility complex class II (MHC-II) expression.[252] Weitz-Schmidt et al. discovered that statins prevent the costimulation of T-cells by selectively blocking the leukocyte function antigen-1 (LFA-1).[253] Katznelson et al. showed that pravastatin acts synergistically with cyclosporine to inhibit vascular smooth muscle mitogenesis and to reduce cytotoxic T-lymphocyte activity.[254, 255] In many centers, statins are nowadays used in routine post-transplantation therapy. Several major trials have yielded promising results concerning the safety and cholesterol-lowering efficacy of statin therapy in cardiac transplant recipients.[37, 38, 55, 57, 121, 232, 256-260] In pediatric and adolescent transplant recipients, however, the safety data on statins are limited, and only a few retrospective studies have been published (Table 3).[55-58] Careful follow-up of transplant recipients receiving statins and cyclosporine is important, since their co-administration is known to lead to increased plasma concentrations of statins[213, 214] as well as to serious side-effects, such as rhabdomyolysis.[216, 217, 231, 232]

2.6.6. Pravastatin

In adults, about 34% of the oral dose of pravastatin is absorbed.[203, 261] The overall bioavailability of pravastatin averages 18%, since about 50% of the absorbed drug is subject to pre-systemic hepatic metabolism.[261] Pan et al. have shown, that the bioavailability of pravastatin dropped markedly when the drug was taken with a meal. [262] Hence, in pharmacokinetic studies, it is important to evaluate the fasting concentrations of pravastatin. However, because reductions in total and LDL cholesterol were equal in the fasting and non-fasting groups, in clinical practice pravastatin can be administered regardless of meal time.[262]

The major metabolites of pravastatin, 3-α-iso-pravastatin, 6-epi-pravastatin, 3α,5β-dihydroxy-pravastatin and 3-hydroxypravastatin,[263] are almost inactive.[203] Unlike with many other statins, CYP enzymes do not significantly biotransform pravastatin,[208] and it thus is not susceptible to CYP3A4-,[211] CYP2C9-[224] or CYP2C19-mediated[224] drug interactions. Pravastatin is cleared via both renal and nonrenal routes, (47% and 53%, respectively), and the half-life in adults is 1 to 3 hours.[261, 263, 264]

Pravastatin is a substrate of at least canalicular multispecific organic anion transporter (cMOAT or MRP2)[225, 226] but not that of P-glycoprotein.[265] Pravastatin is also transported by members of the OATP-family.[221, 222] OATP1B1 is responsible for the hepatic uptake of pravastatin.[266, 267] OATP2B1 transports pravastatin from the gut lumen to the cytosol of intestinal epithelial cells and may facilitate its absorption there,[268] while MRP2 transports absorbed pravastatin back to the gut lumen and also mediates the biliary excretion of pravastatin from hepatocytes.[265] Organic anion transporter 3 (OAT3), a member of the SLC22 superfamily, may affect the urinary excretion of pravastatin.[269]

3. AIMS OF THE STUDY

The aim of this dissertation was to study pharmacokinetics, efficacy, and safety of pravastatin therapy in children.

The specific subjects included were (Table 5a):

The pharmacokinetics of pravastatin in children with HeFH and in pediatric cardiac transplant recipients.

- To assess the pharmacokinetic profile of pravastatin in children with HeFH and in pediatric cardiac transplant recipients receiving triple immunosuppressive medication
- To determine, whether pravastatin pharmacokinetics differ between children and adults by comparing the results of this study to those of previous studies in adults.
- To assess the effects of immunosuppressive medication on pravastatin pharmacokinetics by comparing the HeFH children (receiving no concomitant drug therapy) to the cardiac transplant recipients

The efficacy of pravastatin in children with HeFH and in pediatric cardiac transplant recipients

- To determine the lipid- and lipoprotein-modifying efficacy of pravastatin in children with HeFH and in pediatric cardiac transplant recipients
- To investigate the effects of pravastatin therapy on non-cholesterol sterols in children with HeFH, and to investigate whether differences in cholesterol absorption and synthesis, assayed with serum non-cholesterol sterols, could explain differences in responsiveness to pravastatin.
- To investigate whether lipid and lipoprotein levels or lipoprotein compositions differ between pediatric cardiac transplant recipients and healthy pediatric controls
- To investigate, whether lipid or lipoprotein levels or lipoprotein composition differ between the transplant recipients who show signs of TxCAD and those who do not.

The safety of pravastatin in children with HeFH and in pediatric cardiac transplant recipients

- To evaluate the potential clinical side-effects of pravastatin, and to assess adverse effects of pravastatin on liver, kidney, and muscle cells in children with HeFH and in pediatric cardiac transplant recipients
- To assess the effects of pravastatin on growth, pubertal development, and hormone levels in children with HeFH.

4. PATIENTS

4.1. Children with HeFH

Consecutive patients admitted to the Hospital for Children and Adolescents, Helsinki University Central Hospital, due to hypercholesterolemia were considered candidates for an open, clinical follow-up study of pravastatin treatment. According to the normal clinical protocol of the Hospital for Children and Adolescents, during the first visit, the hypercholesterolemic children were clinically examined, their family history was reviewed, and their cholesterol levels were evaluated. In addition, a dietician reviewed their diets. With the consent of the patients and their families, FH was diagnosed by LDL receptor mutation analysis[90] or by the lymphocyte test.[112] If patients tested negative for the four most common LDL receptor mutations in Finland, detection of familial hypercholesterolemia was carried out by assaying low-density-lipoprotein receptors on lymphocytes. The patients then visited the hospital again after two months of dietary intervention consisting of a low-fat (energy from fat < 30% of total energy), reduced saturated fat (< 10% of total energy), and low cholesterol (< 100 mg/day) diet, with supplementation of plant stanol or sterol esters (2.0 g/d). Those patients with genetically-verified HeFH who were at least four years of age, who had serum total cholesterol ≥ 6 mmol/L regardless of the dietary intervention, and who, together with their families, volunteered for the study and accepted the study protocol, were enrolled without selection. Figure 6 illustrates the study protocol and the examinations performed at each visit. Besides HeFH, all our patients were healthy, and prior to our study, none received daily medication.

Table 5 a. Goals of the studies

Study I	To assess the pharmacokinetic profile of pravastatin in children with HeFH, and to determine whether pravastatin pharmacokinetics differ between children and adults by comparing the results to known adult data.
Study II	To assess the pharmacokinetic profile of pravastatin in pediatric cardiac transplant recipients receiving immunosuppressive medication, and to assess whether it differes from that of HeFH children in the absence of concomitant drug therapy.
Study III	To study the safety and cholesterol-lowering efficacy of pravastatin in children with HeFH.
Study IV	To determine the effects of pravastatin on noncholesterol sterols in children with HeFH, and to evaluate whether differences in cholesterol metabolism (assayed with noncholesterol sterols) could explain differences in responsiveness to pravastatin therapy.
Study V	To study the cholesterol-lowering efficacy of pravastatin in pediatric cardiac transplant recipients, to investigate whether lipid and lipoprotein levels differ between transplant recipients and healthy controls, and to determine whether lipid or lipoprotein levels or composition differ between those patients who develop transplant vasculopathy and those who do not.

Table 5 b. Characteristics of children with heterozygous familial hypercholesterolemia (HeFH) and pediatric cardiac transplant recipients in Studies I to V.

	Patients	Girls/Boys	Age (years)		Weight (kg)		BMI (kg/m^2)		Time from Tx (years)	
Study I	20 patients with HeFH	13/7	10.3 ± 2.9	(4.9 – 15.6)	38.1 ± 13.8	(16.4 – 63.0)	18.2 ± 3.4	13.8 – 24.0	—	—
Study II	19 transplant recipients	11/8	12.1 ± 5.0	(4.4 – 18.9)	41.6 ± 22.4	(13.4 – 98.0)	19.4 ± 5.3	12.4 – 33.5	3.7 ± 3.2	(0.1 – 11.2)
Study III	30 patients with HeFH	19/11	10.1 ± 3.4	(4.9 – 18.5)	38.6 ± 17.4	(16.4 – 89.9)	18.5 ± 4.2	13.8 – 30.1	—	—
Study IV	16 patients with HeFH	10/6	9.8 ± 2.7	(4.9 – 14.0)	35.8 ± 13.0	(16.4 – 63.0)	17.5 ± 2.9	13.8 – 21.8	—	—
Study V	19 transplant recipients	12/7	11.8 ± 4.7	(4.4 – 18.5)	41.0 ± 21.4	(13.4 – 94.5)	19.4 ± 5.0	12.4 – 32.3	3.6 ± 3.2	(0.1 – 11.0)

Data are mean ± SD (range).

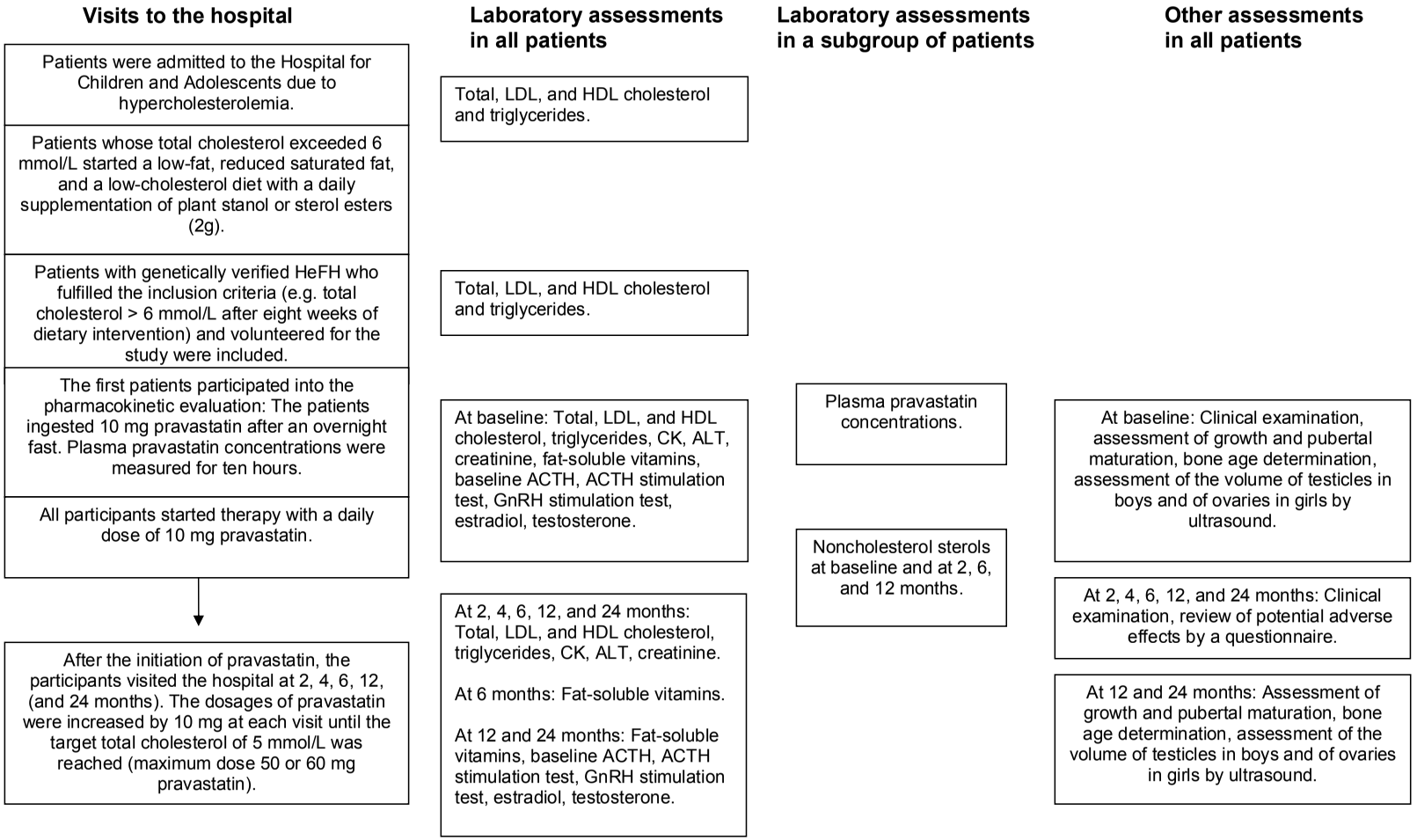

Figure 6. Visits to the Hospital for Children and Adolescents in patients with heterozygous familial hypercholesterolemia. CK, creatine kinase. ALT, aminotransferase. Fat soluble vitamins include vitamins A, D, and E. ACTH, adrenocorticotropic hormone.

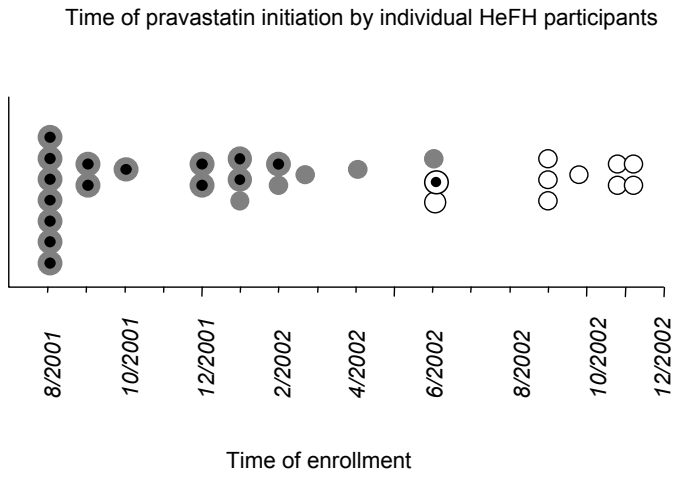

Figure 7. The figure shows the patients with heterozygous familial hypercholesterolemia enrolled in the series of studies. The gray dots represent the 20 patients who participated in the pharmacokinetic study (Study I); the dots with a black center represent the 16 patients selected for the non-cholesterol sterol study (Study IV); all 30 patients participated in the safety and efficacy study (Study III).

The goals of this series of studies are described in Table 5a. Figure 7 illustrates the HeFH patients enrolled in the series of studies at different points in time. The first 20 children and adolescents with HeFH who fulfilled the inclusion criteria (above) and volunteered for the study were included in the pharmacokinetic evaluation (**Study I**) with 10 mg pravastatin (Figures 6 and 7). The clinical characteristics of the patients are described in Table 5. Of these 20 patients, 10 tested positive for the FH-Helsinki LDL receptor mutation, 6 patients for the FH-North Karelia LDL receptor mutation, and 4 patients exhibited defective cholesterol intake in the lymphocyte test.

Altogether 35 children and adolescents (including the 20 patients in Study I) with HeFH were recruited for the long-term safety and efficacy study (**Study III**). The final number of patients included in the analyses was 30 since 5 patients failed to complete the first year of

follow-up and were thus excluded: one patient discontinued after six months of therapy due to persistent abdominal pain (subsequently diagnosed as lactose intolerance), one patient discontinued at baseline due to lack of motivation, and three patients violated the study protocol. Aside from having HeFH, all our patients were healthy and, before the study, none was taking daily medication. Of the 30 patients, 16 tested positive for the FH-Helsinki LDL receptor mutation, 6 patients for the FH-North Karelia LDL receptor mutation, 2 patients for the FH-Turku LDL receptor mutation, and 6 patients exhibited defective cholesterol intake in the lymphocyte test. The characteristics of the patients appear in Table 5. Of note is that the patients were seldom over weight, as the mean W/H, which represents the ratio of weight for height (W/H) to the mean W/H in the normal population of the same age and gender, in girls was 2.3 ± 14.7%(-20 – 27%), and in boys, 13.3 ± 24.1% (-11-58.0%). One patient had been smoking 20 cigarettes/day for three years.

Pravastatin was administered to the HeFH patients at 10 mg per day, with a dose escalation by 10 mg at 2, 4, 6, and 12 months of follow-up (maximum dose 50 mg), until cholesterol level reached the target (≤ 5mmol/L) (Figure 6). The maximum dose was increased to 60 mg in the patients with an exceptionally high total cholesterol level (> 7 mmol/L) after 50 mg pravastatin.

All HeFH patients were encouraged to use plant stanol or sterol ester products regularly with pravastatin. Of the 30 participants **of Study III**, a subgroup of 16 patients, who reported regular use of the non-absorbable stanol ester products throughout the study, were included in **Study IV**. Of these 16 participants, 7, who had reached the target total cholesterol level of 5 mmol/L with one year of pravastatin therapy, were characterized as Group A patients (patients with a sufficient response to pravastatin), and 9 patients, who had not, as Group B patients (patients with an insufficient resonse to pravastatin). The characteristics of the patients appear in Table 5. In Group B patients, the maximum dose of pravastatin at one year was 40 mg in all nine patients, and in Group A patients, 40 mg in two patients, 20 mg in four, and 10 mg in one.

4.2. Cardiac transplant recipients and healthy pediatric controls

The pharmacokinetic study (**Study II**) comprised 19 pediatric and adolescent cardiac recipients receiving triple immunosuppressive medication. The patient characteristics appear in Table 5. One patient was included in the pharmacokinetic study, but was excluded from the efficacy and safety studies because he was transferred from the children's hospital to an adult unit; this patient was replaced by another cardiac transplant recipient in **Study V** (Table 5). Since August 2001, according to the general treatment protocol of the Hospital for Children and Adolescents, 10 mg of pravastatin per day has been administered to all cardiac transplant recipients during either their annual control visits (patients who had undergone cardiac transplantation before the initiation of the study) or four to six weeks after transplantation (patients who underwent cardiac transplantation after the study initiation). The dose of pravastatin was maintained at 10 mg/day because of potential safety concerns (e.g. cyclosporine is known to increase the plasma concentrations of pravastatin in adults, and transplant recipients receive a variety of different drugs that can affect their renal or hepatic function).

In **Study V**, the cardiac transplant recipients were divided into two groups based on the angiography findings before pravastatin: those who had coronary abnormalities were classified as patients with TxCAD, and those who had normal coronary arterieswere classified as patients without TxCAD. In the recipients who received their graft before the initiation of our study (September 2001), this division was based on earlier annual angiographies (up to 2001). In the new recipients who received their graft after the initiation of the study, the patients were grouped according to the findings of an angiography performed three months after transplantation. The condition of the coronary arteries was also documented in 2005, after approximately four years of pravastatin therapy.

The same cardiologist, unaware of the patients' clinical conditions, evaluated the coronary angiographies in the transplant recipients. The findings were graded according to International Society for Heart and Lung Transplantation Registry guidelines.[270]

The patients with TxCAD did not differ from the patients without TxCAD by age (11.0 ± 5.4 y *vs.* 12.1 ± 4.6 y, P = 0.630), time from transplantation to the initiation time of pravastatin

therapy (2.4 ± 3.0 y *vs.* 4.0 ± 3.3 y, P = 0.416), weight (39.3 ± 21.2 kg *vs.* 41.7 ± 22.4 kg, P = 0.823), height (135.6 ± 22.9 cm *vs.* 141.2 ± 24.0 cm, P = 0.634), or body mass index (BMI) (19.7 ± 4.9 kg/m^2 *vs.* 19.3 ± 5.3 kg/m^2, P = 0.896).

In both studies, the immunosuppressive protocol included triple therapy with cyclosporine in microemulsion formulation (17 patients) or tacrolimus (2 patients), methylprednisolone (19 patients), and azathioprine (18 patients), or mycophenylate mofetil (1 patient). The target cyclosporine whole-blood trough level (measured by RIA method, Cyclo-Trac, Diasorin, Minnesota, USA) was 300-500 µg/l at 1-4 weeks, 200-400 µg/l at 1-3 months, 150-300 µg/l at 3-6 months, 150-250 µg/l at 6-12 months, and 100-200 µl/L after the first year, respectively. The cyclosporine dose was adjusted individually, according to through levels, to maintain sufficient immunosuppression and to avoid toxic side-effects, such as nephrotoxicity. Cyclosporine was given in two or three daily doses. The target tacrolimus whole-blood trough level (measured by fluoroimmunoassay, IMX-system, Abbott Diagnostics, Abbott Park, Illinois, USA) was 8 – 12 µg/l at 0 – 12 months, and 5 – 7 µg/l after the first year. Recommendations for daily methylprednisone dosage were 0.30 mg/kg/d for one to four weeks, 0.25 mg/kg/d for 1 to 6 months, and 0.37 mg/kg/d given on every other day thereafter. For practical reasons, the older recipients (time from transplantation > 6 months) in Study II were randomly divided into two groups: those who received methylprednisone on the pravastatin pharmacokinetic study day, and those who received methylprednisone on the previous day. Azathioprine was recommended at 1 mg/kg/d during the first six months and 1.4 mg/kg/d months thereafter.

Antihypertensive therapy was used if blood pressure was repeatedly higher than the age-specific reference values. Additional medication on the day of the pravastatin pharmacokinetic study was as follows (the number in parenthesis refers to the number of patients receiving the drug): diltiazem (3), felodipine (5), nifedipine (1), furosemide (5), propranolol (3), atenolol (1), bisoprolol (1), valganciclovir (2), aciclovir (1), pivmesillinam (1), cephalexin (1), oxycarbazepine (1), and omeprazole (1).

Study V included 21 healthy pediatric controls (10 girls). The healthy controls were 7 to 18 years of age, and participated only in the baseline (without pravastatin) lipid, lipoprotein, and apolipoprotein assessments.

5. METHODS

5.1. Pharmacokinetics of pravastatin

In the pharmacokinetic studies, the patients, who had not previously received pravastatin, ingested a 10 mg oral dose of pravastatin (a half of Pravachol 20 mg tablet, Bristol-Myers Squibb, Epernon, France) with 150 mL water after an overnight fast. The cardiac transplant recipients ingested their morning medication with pravastatin. The patients were not allowed to eat less than 1.5 hours after administration of pravastatin in order to prevent possible food-drug interactions.[262] Timed blood samples (1 ml each) for the determination of plasma pravastatin concentration were drawn from a cannulated vein into chilled tubes containing EDTA. In the children with HeFH, the samples were drawn before pravastatin administration and 0.5, 1, 1.5, 2, 3, 4, 8, and 10 hours later, and in the cardiac transplant recipients, before pravastatin and 0.5, 1, 1.5, 2, 3, 4, 8, 12, and 24 hours later. Two children with HeFH were sampled only for four hours due to their young age (4.9 and 6.0 years). Plasma was separated within 120 minutes and stored at –20 °C until analysis. The maximum storage time before analysis was 12 months. The concentrations of pravastatin in plasma were measured by liquid chromatography-ionspray tandem mass spectrometry with use of the PE SCIEX API 3000 LC/MS/MS system (Sciex Division of MDS Inc, Toronto, Canada).[271] The ion transition monitored was *m/z* 442 to *m/z* 269, and the limit of quantification for pravastatin was 0.25 ng/mL. The day-to-day coefficient of variation was 7.8% at 1 ng/mL (n = 6). The limit of quantification was 0.25 ng ml-1.

The pharmacokinetics of pravastatin were characterized by peak concentration in plasma (C_{max}), time to peak concentration (t_{max}), elimination half-life ($t_{1/2}$), and area under the plasma concentration-time curve from 0 to 24 hours [AUC(0-24 h)] in cardiac transplant recipients, and from 0 to 10 hours [AUC (0-10)] in HeFH patients. The C_{max} and t_{max} were determined visually and taken directly from the original data. The terminal log-linear phase of the plasma concentration-time curve was identified visually for each curve. The elimination rate constant (ke) was determined by log-linear regression analysis of the log-linear phase of the plasma drug concentration curve. The criteria were the following: regression coefficient > 0.900, three to six time points at the log-linear phase, and the time

interval was visually chosen to represent the elimination phase, rather than the terminal elimination phase. The $t_{1/2}$ was calculated by equation $t_{1/2} = \ln 2/k_e$. The AUC values were calculated with the linear trapezoidal rule with extrapolation to infinity, when appropriate, by division of the last measured concentration by k*e*. In order to compare the groups, AUC (0-10) values of the cardiac transplant recipients were calculated by estimating the concentrations at ten hours with the help of the elimination curve. The two FH-patients who were sampled for only four hours owing to young age, and one FH-patient whose terminal log-linear phase of the plasma drug concentration curve had only two time points, were excluded from the $t_{1/2}$ and AUC analyses.

5.2. Efficacy of pravastatin

5.2.1. Lipid and lipoprotein determinations in HeFH patients

The fasting (at least 10 hours) concentrations of serum total, LDL, and HDL cholesterol and of serum triglycerides were determined before and at 1, 2, 4, 6, 12, and 24 months of pravastatin treatment (**Study III**). The concentrations of serum total and HDL cholesterol and of serum triglycerides were analyzed enzymatically by use of HITACHI 917 or MODULATOR automatic analyzers with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The inter-assay coefficients of variation for low and high serum cholesterol concentrations (3.3 mmol/L and 7.1 mmol/L), HDL cholesterol concentrations (0.9 mmol/L and 1.6 mmol/L), and triglyceride concentrations (1.0 mmol/L and 2.0 mmol/L) were 2.1% and 1.8%, 2.2% and 2.7%, and 3.0% and 2.3%, respectively. The LDL-cholesterol concentration was calculated from the formula of Friedewald et al.[272] In the 30 HeFH patients, the highest fasting concentrations, prior to dietary or drug interventions, of serum total, LDL, and HDL cholesterol and of serum triglycerides were recorded from the patient chart. The response to dietary therapy was determined by the difference of the former and the baseline cholesterol concentration.

5.2.2. Noncholesterol sterols in HeFH patients

In **Study IV**, the 16 participants were grouped as Group A patients (those who reached the target total cholesterol 5 mmol/L after one year of pravastatin) and Group B patients (those who did not reach the target total cholesterol 5 mmol/L after one year of pravastatin) according to the total cholesterol concentrations obtained in **Study III**. The serum total cholesterol and non-cholesterol sterols in **Study IV** were measured by gas-liquid chromatography (GLC) on a 50-m-long capillary column (Ultra 2, 5890, Hewlett Packard, Littlefalls (Wilmington), Delaware, USA) from frozen (-20°C) serum samples drawn at baseline and at 2, 6, and 12 months of pravastatin treatment. Because serum non-cholesterol sterols are transported mainly in cholesterol-containing particles, the absolute concentrations were adjusted for serum cholesterol analyzed by the same GLC run, and are expressed as ratios (10^2 x mmol/mol cholesterol). The ratios of cholestanol, campesterol, and sitosterol are referred to as absorption markers of cholesterol, and those of desmosterol and lathosterol, as cholesterol synthesis markers.

5.2.3. Lipid, lipoprotein and apolipoprotein determinations in cardiac transplant recipients and in healthy pediatric controls

In **Study V**, venous blood samples for the lipoprotein analysis were collected in the morning after a fasting period of at least eight hours in the pediatric cardiac transplant recipients before and after one year of pravastatin, and in the healthy controls without pravastatin at baseline. Serum was separated by low-speed centrifugation (2000 rpm/10 minutes at + 5 ˚C), divided into aliquots, and stored at -70˚ C until lipid and lipoprotein analysis. Parts of the aliquots without freezing were immediately used for lipoprotein separation and some were frozen at -20 ˚C for a maximum of 12 weeks until lipoprotein separation.

Serum lipoproteins were separated by sequential ultracentrifugation using Optima TL Ultracentrifuge and TI 100.3 rotor (Beckman Instruments, Palo Alto, CA, U.S.A.) at the densities adjusted with KBr: VLDL (< 1.006 g/ml, 100000 rpm/ h), IDL (1.006 - 1.019 g/ml, 100000 rpm/2 h), LDL (1.019 - 1.063 g/ ml,100000 rpm/2 h), HDL_2 (1.063 – 1.125

g/ml,100000 rpm/3 h). A bottom fraction of d >1.125 g/ml represented HDL3 fraction. Isolated lipoprotein subfractions were frozen at -20 ˚ C (2-12 weeks) until the completion of lipid and total protein determinations.

Serum and lipoprotein subfractions were analyzed for total cholesterol and triglycerides with an automated Optima analyzer (Thermo Electron Corporation, Vantaa, Finland) by fully enzymatic methods (Thermo Electron Corporation kits 981812 and 981301). Serum HDL cholesterol was quantified with a homogenous enzymatic test with polyethylene glycol (PEG) modified enzymes (Thermo Electron Corporation kit 981655). Serum LDL cholesterol was calculated with the formula of Friedewald et al.[272]

Serum concentrations of apoA-I, apoA-II, and apoB-100 were measured by immunoturbidometric methods using an Optima analyzer (for apoA-I and apoB-100, Thermo Electron Corporation kits 981662 and 981663; for apoA-II, Thermo Electron Corporation and polyclonal antibodies produced in rabbits against apoA-II). Phospholipids in lipoprotein fractions were measured using a colorimetric enzyme method[273] (Wako Chemicals GmbH, Germany kit 990-54009).

It is important to note that in the transplant recipients, the serum lipoproteins were thus analyzed by two separate methods: i) In order to determine and calculate the specific mass composition of the major lipoproteins, the lipoprotein fraction was separated from the others by ultracentrifugation, and its content of triglycerides, cholesterol, phospholipids, and proteins was determined. ii) In order to determine the serum levels of lipids or lipoproteins, serum samples were analyzed by the same enzymatic lipid assay methods in order to derive serum total amounts of triglycerides, cholesterol, and HDL cholesterol. LDL cholesterol was then calculated by using the Friedewald equation.[272] These results differ slightly due to the differences in the source of the material (serum vs. lipoprotein fractions).

5.3. Safety

5.3.1. Adverse events

The potential side-effects of pravastatin were monitored using a systematic questionnaire that the patients or the parents or both (depending on the age of the patient) completed at home before each control visit: in children with HeFH at 2, 4, 6, 12, and 24 months of pravastatin administration, and in the cardiac transplant recipients, at 2 months. The presence of similar symptoms prior to statin therapy was also recorded.

5.3.2. Biochemical safety

Clinical examinations and biochemical measurements of safety, including serum alanine aminotransferase (ALT), creatine kinase (CK), and creatinine concentrations were performed in the children with HeFH before treatment and at 2, 4, 6, 12, and 24 months, and in the cardiac transplant recipients (participants of **Study IV**), before treatment and at 12 months. In the HeFH patients, vitamins A, D, and E were determined before treatment and at 6, 12, and 24 months. The vitamins A and E were analyzed by high-performance liquid chromatography (Hewlett Packard Waldbronn, Germany), 1.25-hydroxyvitamin D by radioimmunoassay (Wallac, Turku, Finland), and 25-hydroxyvitamin D by radioimmunoassay (DiaSorin, Stillwater, USA) or by the HPLC-method.[274] In the cardiac transplant recipients, the glomerular filtration rate was determined by ^{51}Cr EDTA clearance before and at one year of pravastatin: a ^{51}Cr EDTA injection (2 uCi/kg, maximum 100 uCi) was given, after which blood samples were collected at 90, 150, 210 and 270 min. The urinary clearance of ^{51}Cr EDTA was calculated from the rate of ^{51}Cr EDTA disappearance from serum. The clearances obtained were corrected for a standard body surface area of 1.73 m^2. The results were considered reliable if the distribution volume of the injection was between 15 and 35%; all observed results fit within these ranges.

5.3.3. Growth in HeFH patients

The height and weight of the 30 patients with HeFH in **Study III** were measured with a wall-mounted stadiometer and an electric scale during each visit. The hSDS was calculated according to the following equation: hSDS = (observed height – mean height for age)/SD, where SD represents the standard deviation for the normal population of the same chronologic age (CA) and gender.[275] The weight for height index (W/H), expressed as a percentage, was determined from the ratio of weight (kg) for height (cm) to the mean W/H in the normal population of the same CA and gender. Bone age (BA) was determined at each visit according to the Greulich-Pyle method[276] by the same pediatric endocrinologist. Height for bone age (hBA) was also calculated and expressed as a standard deviation score.[275] The growth, in relation to gender and pubertal stage, of each patient was individually evaluated by a pediatric endocrinologist using growth charts, clinical data (e.g. changes in ΔhSDS and W/H, pubertal development, time of menarche), hormonal results (estradiol, testosterone), and bone age measurements.

5.3.4. Development in HeFH patients

The pubertal maturation of the 30 patients with HeFH was evaluated clinically by using Tanner's pubertal staging, by the same pediatrician during each visit.[277-280] Early maturation was defined as the development of sexual characteristics before the age of eight years in girls and nine years in boys. Delayed puberty was defined as exhibiting no signs of puberty at the age of 13 years in girls and at 14 years in boys (2 SD above the mean of chronological age for the onset of puberty in Caucasians).[277, 278, 281-283] The menstrual history in girls was recorded. Gonadal maturation in girls was assessed with ultrasonography by a pediatric gynecologist at baseline (in 13 girls), at 12 months (in nine girls), and at 24 months (in two girls). The ultrasonography was performed transabdominally through a distended bladder with an Aloka SSD-1100 ultrasound scanner equipped with a 13 MHz probe. The length of the uterus was recorded. The ovarian structure was evaluated, and the ovarian volume was calculated by: thickness x length x width x 0.52. Testis volume in boys was assessed by high-resolution B-mode ultrasonography (using an ATL HD 5000 ultrasound scanner equipped with a linear 5-12

MHz transducer) before treatment and at 12 and 24 months. The testis volume was calculated by: thickness x length x width x 0.52.[284]

Baseline and stimulated hormonal status was recorded at 0, 12, and 24 months, comprising baseline adrenocorticotropic hormone (ACTH), testosterone, and estradiol levels and ACTH stimulated cortisol secretion, gonadotropin releasing hormone (GnRH) stimulated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. In the GnRH stimulation test, the patient received a dose of 3.5 µg/kg of GnRH (maximum dose 100 µg), and blood samples for LH and FSH measurements were collected at 0, 20, 30, and 60 minutes, and at 0, 30, 60, and 90 minutes, respectively.[285] In the ACTH stimulation test, the patient received a dose of 0.25 mg/1.73 m^2 of ACTH (maximum dose 0.25 mg), and blood samples for cortisol measurements were collected at 0, 60, and 120 minutes. All laboratory measurements were carried out according to the standards of the Helsinki University Central Hospital. Serum FSH and LH were quantitated with time-resolved ultrasensitive immunofluorometric assays (AutoDELFIA™, Wallac, Turku, Finland). Serum estradiol, testosterone, and ACTH were measured by radioimmunoassay (Wallac, Turku, Finland). Serum cortisol was quantitated with an enzyme immunoassay and with reagents on the immunoanalyzer Immunol® (Bayer, Tarrytown, USA).

The boys and girls were divided into subgroups (I A, prepubertal at baseline and at follow-up; I B, prepubertal at baseline and pubertal at follow-up; II, pubertal at baseline; III, postpubertal at baseline) according to the results of the GnRH stimulation test and clinical examination: prepubertal was defined in clinical examination as Tanner stage 1, and peak FSH dominant over peak LH in the GnRH stimulation test; pubertal was defined as peak LH dominant over FSH, and peak LH concentration > 6 IU/L in the GnRH stimulation test; and postpubertal was defined in clinical examination as Tanner stage 5, and in girls, menarche before the onset of this study. The GnRH stimulation test was used because of its sensitivity in detecting central puberty before clinical signs. The development, relative to age and gender, of each patient was individually evaluated by the use of clinical data (e.g. progression in puberty, time of menarche), hormonal results (GnRH stimulation test, estradiol, and testosterone) and changes in testis/ovarian and uterine volumes.

5.4. Statistical analysis

Data are mean ± SD in text and tables (except from triglycerides, median and range). The normality of the distribution was tested with the Shapiro-Wilk W test. Repeated measures analysis of variance (ANOVA) was used to assess changes in the concentrations at different time points. Friedman's non-parametric test was used to analyze differences between time points when the assumptions of the repeated measures analysis of variance were not met. In HeFH patients, all 30 patients participated to the 0 to 12 months follow-up, whereas 14 patients (out of 30) were followed for 24 months. Therefore, both analyzes from 0 to 12 months (in 30 patients) and from 0 to 24 months (14 patients) was performed. When only 2 measurement points were available, statistical comparison of the normally distributed variables between the pretreatment and treatment phases was carried out with the paired *t* test. If the distribution was skewed, the Wilcoxon signed rank test was used. Statistical comparison of the normally distributed variables between different groups was carried out with the student *t*-test for unpaired values, and with the Mann-Whitney test if the distribution was skewed. Pearson´s correlation coefficients were calculated to evaluate degrees of linear association between normally distributed variables and Spearman´s rank correlation coefficients for nonparametric variables. The statistics software used was from StatsDirect Ltd, Cheshire, UK. P values below 0.05 were considered statistically significant.

5.5. Ethical considerations

The study protocol was approved by the Ethics Committee of the Helsinki University Hospital for Children and Adolescents and by the National Agency for Medicines in Finland. Each patient participated on a voluntary basis. Written informed consent was obtained from the parents or children over 18 years of age or both.

6. RESULTS

6.1. Pharmacokinetics of pravastatin in children with HeFH and in cardiac transplant recipients

The mean pravastatin plasma concentration curves in the children with HeFH and in the cardiac transplant recipients appear in Figure 8, and the pharmacokinetic parameters, in Table 6.

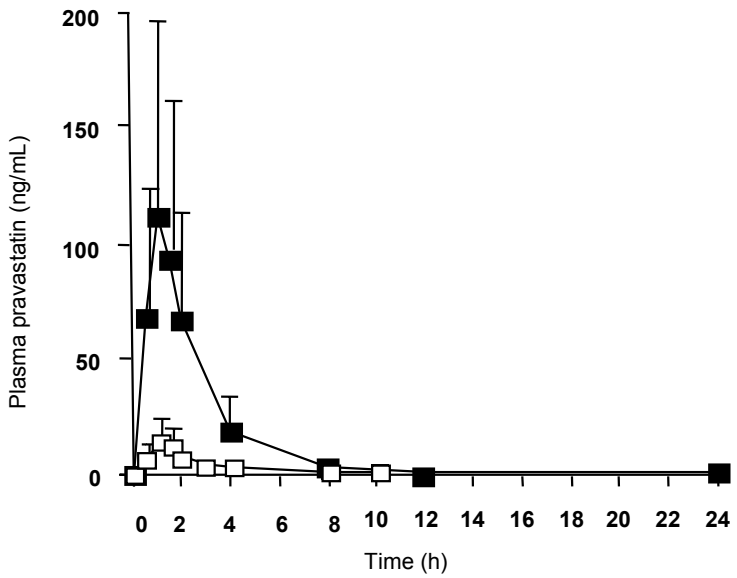

Figure 8. Mean plasma pravastatin concentrations (± SD) after a single oral dose of 10 mg pravastatin in 19 pediatric cardiac transplant recipients on triple immunosuppression (black squares), and in 20 children with familial hypercholesterolemia receiving no concomitant drug therapy (open squares).

Compared to the children with HeFH, the pediatric cardiac transplant recipients exhibited a nearly ten-fold higher mean C_{max} and AUC (0-10) of pravastatin. In both groups, the inter-individual variability in the C_{max} and AUC(0-10) values was considerable. A significant inverse correlation in the HeFH patients emerged between the C_{max} and age (r = -0.52; P = 0.020), weight (r = -0.50; P = 0.026), and body surface area (r = -0.51; P = 0.022). Similar correlations were absent in the cardiac transplant recipients (data not shown).

Table 6. Pharmacokinetic parameters with 10 mg pravastatin in 19 pediatric cardiac transplant recipients on triple immunosuppression and in 20 children with heterozygous familial hypercholesterolemia.

		C_{max}	T_{max}	AUC(0-10)	$t_{½}$
Children with HeFH	mean ±SD	15.7 ± 14.4	1.25	27.8 ± 16.7	1.7 ± 0.7
	range	1.6 – 55.0	0.5 – 4	6.5 – 58.9	1.2 – 4.2
Transplant recipients	mean ± SD	122.2 ± 88.2	1	264.1 ± 192.4	1.2 ± 0.3
	range	11.4 - 305.0	0.5 – 2	30.8 – 701.6	0.9 – 2.2
P-value		< 0.0001	0.065	< 0.0001	0.001

T_{max} is given as median and range

6.2. Efficacy

6.2.1. Efficacy of dietary and pravastatin interventions in HeFH children

The highest total and LDL cholesterol concentrations prior to dietary or drug interventions in the children with HeFH were 9.1 ± 1.4 mmol/L and 7.2 ± 1.4 mmol/L, respectively. The dietary intervention (consisting of a low-fat, reduced-saturated-fat, and low-cholesterol diet with supplementation of plant stanol or sterol esters) lowered the total and LDL cholesterol concentration significantly (-10.7%, P <0.0001 and -12.6% P =0.0001, respectively). The HDL cholesterol concentrations and triglycerides prior to dietary or drug interventions were 1.4 ± 0.3 mmol/L and 1.0 ± 0.5 mmol/L , respectively; an insignificant increase during dietary intervention occurred in the serum triglyceride levels (19.2%, P =0.728), whereas the HDL cholesterol concentrations remained unaffected (0.4%, P =0.841).

Figure 9 shows the serum total, LDL and HDL cholesterol concentrations, and triglycerides in the 30 children and adolescents with HeFH before pravastatin (during diet) and at 2, 4, 6, and 12 months of pravastatin. When compared to the pre-treatment values, at 2, 4, 6, 12, and 24 months of treatment, the total cholesterol levels had decreased by 19%, 20%, 23%, 27%, and 26%, and the LDL cholesterol levels by 25%, 27%, 29%, 33%, and 32%. The respective decreases in the triglyceride concentrations were 5%, 3%, 3%, 11%, and 34%, and the increases in HDL cholesterol concentrations 5%, 8%, 2%, 4%, and 11%. The reductions in total and LDL cholesterol concentrations and triglyceride levels (both in the whole patient group from 0 to 12 months and in the subgroup of 14 patients from 0 to 24 months) were statistically significant (P-values not shown). The increase in HDL cholesterol concentration from 0 to 12 months was also significant. Approximately 70 to 80% of the maximum total and LDL cholesterol-lowering efficacy was achieved with 10 to 20 mg pravastatin. The mean maximum reduction in total cholesterol was 32.4 ± 7.9%, but the cholesterol-lowering efficacy did vary considerably: e.g. in one girl, 10 mg pravastatin reduced the total cholesterol concentration by 46.3%, whereas in another patient, 40 mg pravastatin yielded only a 7.0% reduction in cholesterol. The total cholesterol concentrations reached the target level (≤ 5 mmol/L) in nine of the 30 patients after one year of pravastatin treatment, and in five of the 14 patients after two years.

6.2.2. Noncholesterol sterols in patients with HeFH

To determine whether differences in cholesterol absorption and synthesis could explain the considerable differences in the responsiveness to pravastatin observed in **Study III**, we divided the participants of **Study IV** into those with sufficient response to pravastatin (Group A: patients who reached the target total cholesterol of 5 mmol/L by one year of pravastatin) and those with insufficient response to pravastatin (Group B; patients who failed to reach the target). Besides pravastatin, the patients in both groups used similar amounts of plant stanol ester products (2 g daily). The Group B patients with an insufficient response to pravastatin had higher study baseline (on plant stanol esters) serum cholesterol concentrations (7.7 ± 1.0 *vs.* 6.5 ± 0.9 mmol/L, $P < 0.001$) and higher respective ratios of campesterol (371 ± 99 *vs.* 277 ± 67 10^2 x mmol/mol of cholesterol, $P = 0.049$) and sitosterol (176 ± 37 *vs.* 126 ± 24 10^2 x mmol/mol of cholesterol, $P = 0.008$) than did Group B patients (Figure 10). In general, the higher the ratio of cholestanol to cholesterol at study baseline, the smaller the one-year reduction in cholesterol (Figure 11). Overall, pravastatin decreased the serum levels of cholesterol, and cholesterol synthesis markers, and increased the ratios of cholesterol absorption markers. These effects were similar among the 7 Group A and 9 Group B patients (Figure 10).

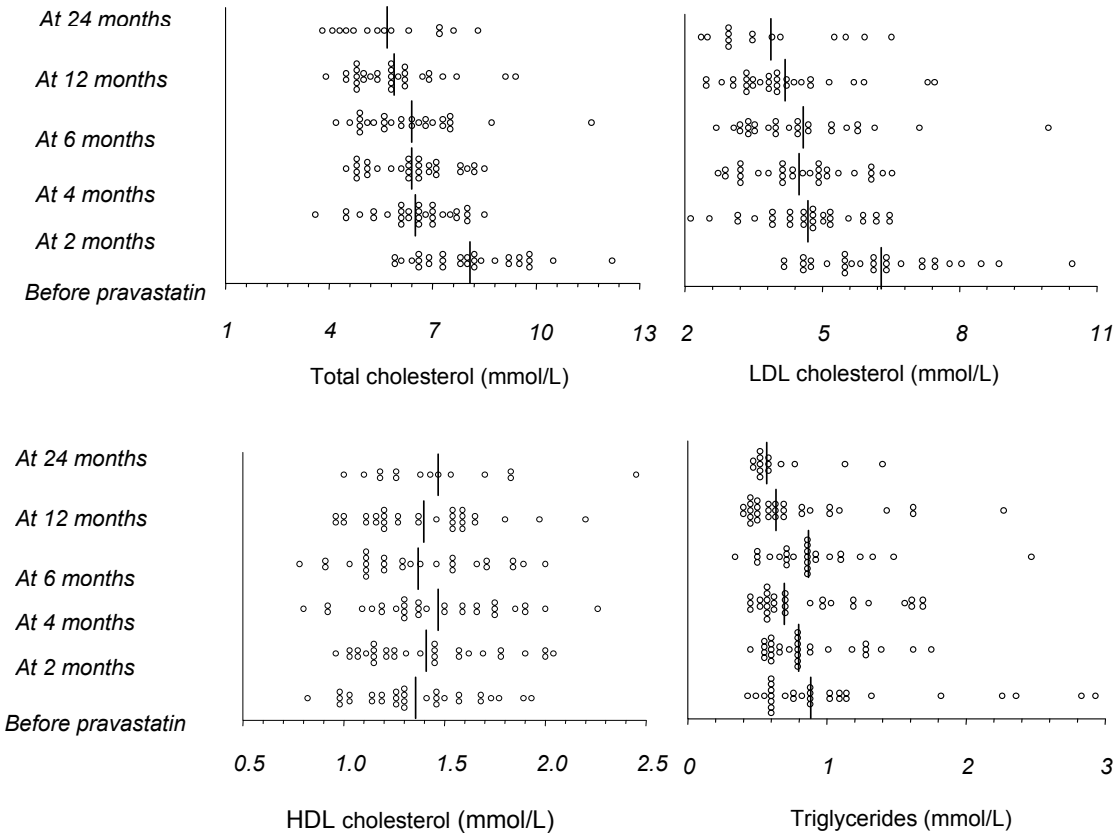

Figure 9. Total, LDL, and HDL cholesterol concentrations and triglycerides in children with HeFH before and during pravastatin treatment with increasing doses (maximum dose 10 to 60 mg). The mean concentrations are indicated by vertical lines (except for triglycerides, indicated as median concentrations).

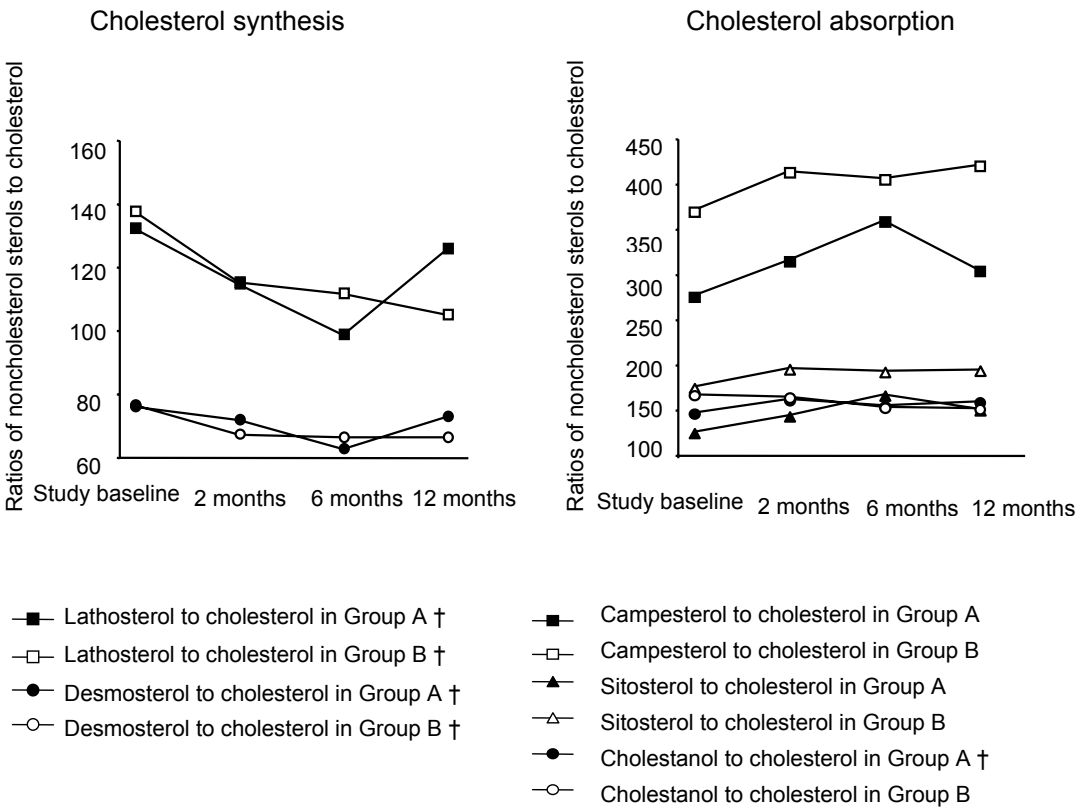

Figure 10. Mean ratios of serum cholestanol, campesterol and sitosterol (absorption markers), and desmosterol and lathosterol (synthesis markers) to cholesterol in 7 Group A patients (those with a sufficient response to pravastatin after one year) and in 9 Group B patients (those patients with an insufficient response to pravastatin after one year) during pravastatin therapy in increasing doses. † Indicates statistical significance in ANOVA.

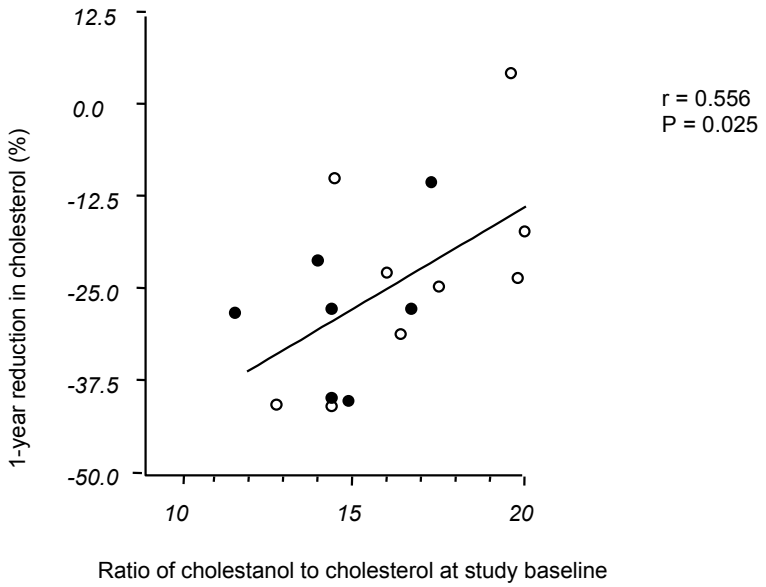

Figure 11. The correlation between the baseline ratio of cholestanol to cholesterol and the one-year reduction (%) in cholesterol in 7 Group A patients (those with a sufficient response to pravastatin after one year; solid circles) and 9 Group B patients (those with an insufficient response to pravastatin after one year ; open circles).

6.2.3. Lipids, lipoproteins, and apolipoproteins in cardiac transplant recipients

Figure 12 shows the serum total, LDL, and HDL cholesterol concentrations and triglyceride levels of the cardiac transplant recipients before pravastatin and at one year, and of healthy pediatric controls without pravastatin at baseline. Of note is that, compared to the healthy controls, the serum total and LDL concentrations of the transplant recipients were not elevated. However, both before pravastatin and at one-year, the serum HDL cholesterol concentrations of the recipients were significantly lower ($P = 0.003$ and $P = 0.009$, respectively), and the serum triglyceride levels were significantly higher ($P = 0.0004$ and $P = 0.0002$, respectively). A negative correlation between serum triglycerides and

HDL cholesterol levels emerged in cardiac transplant recipients prior to pravastatin therapy (r = -0.523, P = 0.022), and a similar tendency was also observed after one year of pravastatin (r = -0.439, P = 0.060).

Table 7 demonstrates the mean concentrations of cholesterol and triglycerides in lipoproteins in transplant recipients (at baseline and at one year) and in controls (at baseline). The quantity of triglycerides in LDL, VLDL and IDL particles was persistently high in the transplant recipients than in the controls (Table 7). Furthermore, the baseline apoB-100/apo-A1 ratios in the recipients were higher, and the HDL_2 cholesterol concentration, lower (Table 7).

After one year of treatment, pravastatin (10 mg per day) had statistically significantly lowered the serum total cholesterol by 10.0 ± 14.6% (P = 0.006) and serum LDL cholesterol by 19.1 ± 24.2% (P = 0.002). HDL cholesterol increased by 5.7 ± 26.4% (P = 0.542). Of note, serum triglycerides slightly, yet non-significantly (10.2 ± 48.7%; P = 0.836) increased, rather than decreased, during pravastatin intervention. Furthermore, the reductions in apo-B level (0.8 ± 0.2 to 0.7 ± 0.2; P = 0.005) and apo-B/apo-A1 ratio (0.6 ± 0.2 to 0.5 ± 0.1; P = 0.004) were also statistically significant.

6.2.4. Lipids, lipoproteins and apolipoproteins in association to TxCAD

The patients with TxCAD had significantly lower serum HDL cholesterol concentrations at baseline (1.0 ± 0.3 mmol/L vs. 1.4 ± 0.3 mmol/L, P = 0.031) and at one year of pravastatin (1.0 ± 0.3 mmol/L vs. 1.4 ± 0.3 mmol/L, P = 0.013), and a significantly higher percentage of triglycerides in the LDL particles at baseline (12.4 ± 2.6% vs. 8.2 ± 1.7%) than did the patients without accelerated coronary artery disease (TxCAD). Furthermore, the patients with TxCAD also exhibited a significantly lower concentration of cholesterol in the HDL_2 particle at one year, and a significantly higher ratio of apoB/apoA1 at baseline and at one year (Table 7).

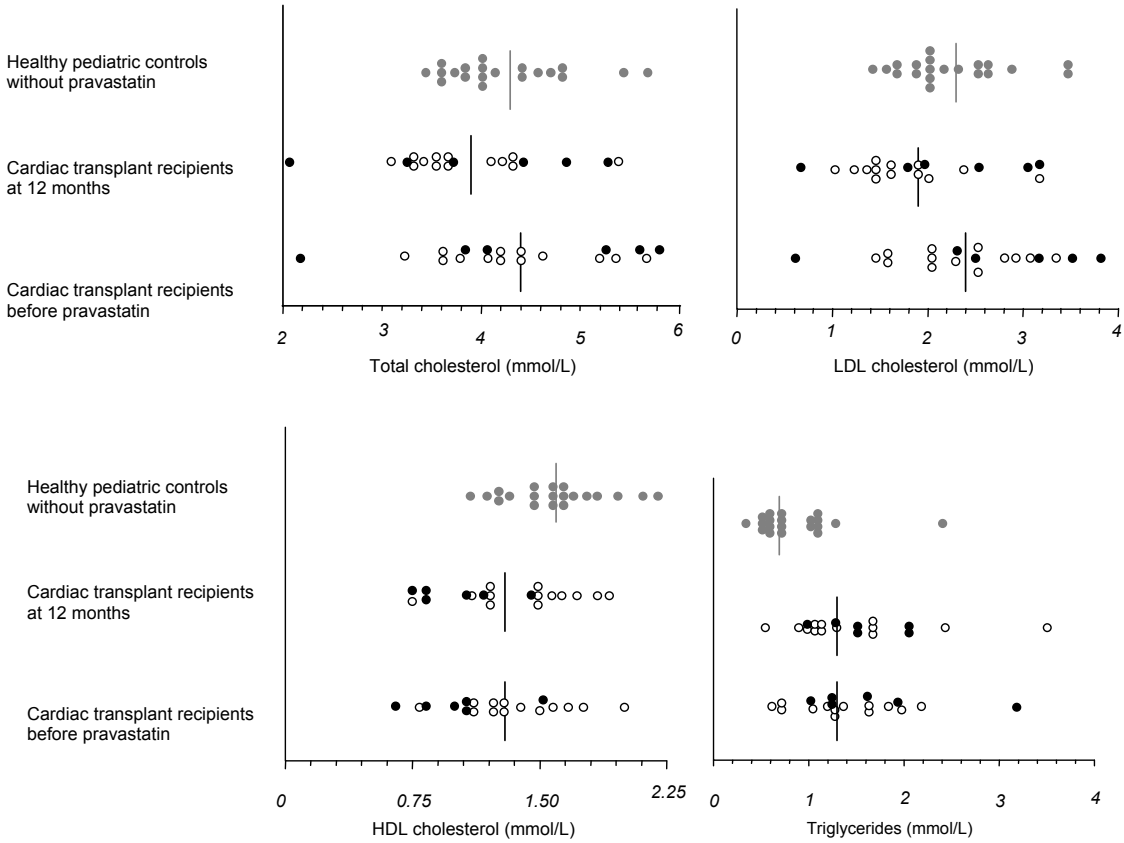

Figure 12. Lipid and lipoprotein levels of 19 pediatric and adolescent cardiac transplant recipients before and at one year of pravastatin treatment (10 mg/day), and of 21 healthy pediatric controls at baseline (without pravastatin). Open circles represent the cardiac transplant recipients without TxCAD. Closed black circles represent the transplant recipients with TxCAD. Vertical lines mark the mean concentrations (except for triglycerides, which mark the median concentrations).

Table 7. Concentrations of cholesterol and triglycerides in lipoproteins in cardiac transplant recipients at baseline and at one year of pravastatin, and in healthy pediatric controls at baseline.

		Patients with TxCAD (n=6)	Patients without TxCAD (n=13)	P-value *	All transplant recipients (n=19)	Healthy controls (n=21)	P-value †
At baseline without pravastatin							
LDL	C (mmol/L)	2.26 ± 0.86	2.29 ± 0.53	0.938	2.28 ± 0.63	1.84 ± 0.44	0.014
	TG (mmol/L)	0.30 (0.13-0.51)	0.22 (0.14-0.30)	0.204	0.22 (0.13-0.51)	0.13 (0.09-0.22)	< 0.0001
VLDL	C (mmol/L)	0.45 ± 0.28	0.29 ± 0.19	0.166	0.34 ± 0.23	0.21 ± 0.14	0.038
	TG (mmol/L)	0.66 (0.46-1.99)	0.51 (0.17-1.28)	0.312	0.53 (0.17-1.99)	0.28 (0.07-1.14)	0.007
IDL	C (mmol/L)	0.32 ± 0.17	0.22 ± 0.12	0.176	0.26 ± 0.14	0.31 ± 0.12	0.162
	TG (mmol/L)	0.22 (0.17-0.48)	0.17 (0.08-0.36)	0.067	0.19 (0.08-0.48)	0.15 (0.03-0.26)	0.014
HDL_2	C (mmol/L)	0.56 ± 0.16	0.70 ± 0.35	0.351	0.66 ± 0.30	0.86 ± 0.24	0.021
	TG (mmol/L)	0.09 (0.04-0.12)	0.07 (0.02-0.21)	0.743	0.08 (0.02-0.21)	0.07 (0.04-0.17)	0.453
ApoA-I ‡	(g/L)	1.2 ± 0.3	1.4 ± 0.2	0.072	1.3 ± 0.3	1.5 ± 0.2	0.059
ApoB-100‡	(g/L)	0.9 ± 0.3	0.7 ± 0.2	0.220	0.8 ± 0.2	0.6 ± 0.1	0.032
ApoB-100‡ / ApoA1	—	0.7 ± 0.2	0.5 ± 0.1	0.034	0.6 ± 0.2	0.4 ± 0.1	0.005
At one year of pravastatin							
LDL	C (mmol/L)	1.82 ± 0.64	1.55 ± 0.46	0.309	1.64 ± 0.52	—	0.184
	TG (mmol/L)	0.16 (0.13-0.27)	0.18 (0.13-0.28)	0.747	0.17 (0.13-0.28)	—	0.002
VLDL	C (mmol/L)	0.44 ± 0.16	0.30 ± 0.18	0.113	0.34 ± 0.18	—	0.014
	TG (mmol/L)	0.91 (0.31-1.20)	0.58 (0.20-2.0)	0.101	0.64 (0.20-2.0)	—	0.0004
IDL	C (mmol/L)	0.38 ± 0.17	0.40 ± 0.24	0.885	0.39 ± 0.22	—	0.394
	TG (mmol/L)	0.21 (0.15-0.27)	0.22 (0.11-0.43)	0.684	0.22 (0.11-0.43)	—	0.0001
HDL_2	C (mmol/L)	0.60 ± 0.12	0.93 ± 0.24	0.006	0.83 ± 0.26	—	0.660
	TG (mmol/L)	0.08 (0.06-0.10)	0.13 (0.06-0.16)	0.107	0.08 (0.06-0.16)	—	0.011
ApoA-I ‡	(g/L)	1.2 ± 0.2	1.4 ± 0.2	0.085	1.3 ± 0.2	—	0.045
ApoB-100‡	(g/L)	0.7 ± 0.2	0.6 ± 0.1	0.156	0.7 ± 0.2	—	0.826
ApoB-100‡ / ApoA1	—	0.6 ± 0.2	0.4 ± 0.1	0.005	0.5 ± 0.1	—	0.143

Data are mean ± SD, except for triglycerides median (range). TxCAD, transplant coronary artery disease; C, cholesterol; TG, triclycerides.
* Patients with TxCAD are compared to patients without TxCAD.
† All cardiac transplant recipients before or at one year of pravastatin are compared to the healthy controls at baseline.
‡ ApoA-I and apoB-100 display values determined from serum.

Table 8. The coronary changes detected by angiography in 19 pediatric cardiac transplant recipients before and after four years of pravastatin intervention (10 mg/day).

Patients	Coronary status prior to pravastatin	Coronary status after four years of pravastatin	Clinical observations during 4 years of pravastatin
1	15% stenosis in RCA	30% stenosis in RCA	grade I rejection
2	20% stenosis in RCA	20%stenosis in LAD 35% stenosis in RCA	–
3	50% stenosis in LCX	progressed up to 95% stenosis in LCX	underwent a stent operation
4	40% stenosis in RCA	35% stenosis in RCA	–
5	15% stenosis in LAD 15% stenosis in RCA	25% stenosis in LAD 20% stenosis in RCA	two grade I rejections nonadherence to therapy
6	severe vasculopathy	severe vasculopathy	died of TxCAD
7	normal	30% stenosis in LAD	two grade I rejections
8	normal	30% stenosis in LCX	grade I rejection metabolic syndrome*
9	normal	normal	rejection
10	normal	normal	grade II rejection
11	normal	normal	–
12	normal	normal	–
13	normal	normal	–
14	normal	normal	–
15	normal	normal	–
16	normal	normal	–
17	normal	normal	–
18	normal	normal	–
19	normal	normal	–

TxCAD, transplant vasculopathy; RCA, right coronary artery; LAD, left anterior descending coronary artery; LCX, left circumflex artery.

* Required treatment with metformin

6.2.5. Changes in coronary status during pravastatin intervention

Table 8 represents the coronary changes detected by angiography in cardiac transplant recipients before and after approximately four years of pravastatin therapy, and the observed rejections after the initiation of pravastatin. Of the six patients with TxCAD at baseline (prior to pravastatin), one had died of severe coronary vasculopathy, and one had undergone stent operation. In the remaining four patients, the coronary changes remained unchanged or only minor progression occurred. Of the 13 patients with healthy coronaries at baseline (patients without TxCAD), 2 had developed 30% stenotic coronary changes during pravastatin intervention. A preceding grade I rejection occurred in both patients. Coronary angiographies of 11 patients without TxCAD remained normal during the four-year pravastatin intervention.

6.3. Safety

6.3.1. Adverse events in HeFH patients

The adverse events reported by the 30 children with HeFH with one to two years of pravastatin therapy appear in Table 9. The most common adverse experiences were headache and gastrointestinal symptoms, affecting at two months 13% and 37% of the patients, respectively. These were, however, common complaints before treatment as well, affecting 10% and 17% of the patients. Most symptoms were mild and disappeared during the first months of therapy. During the up to two year follow-up of the FH-patients in **Study III**, no serious side-effects occurred that would have required discontinuation of pravastatin therapy.

Table 9. Adverse events during pravastatin therapy in children and adolescents with familial hypercholesterolemia.

	Before treatment (n=30)	At 2 months (n=30)	At 4 months (n=30)	At 6 months (n=30)	At 12 months (n=30)	At 24 months (n=14)
Abdominal pain	3	4	0	0	2	0
Loose stools	0	2	1	0	1	1
Flatulence	2	5	1	1	1	0
Headache	3	4	3	1	2	0
Sleep disorder	1	2	0	0	1	0
Muscle tenderness or pain at rest	0	2	1	0	1	0
Muscle tenderness or pain in physical training	0	0	0	0	0	0

Data are the number of subjects.

6.3.2. Adverse events in cardiac transplant recipients

During the two-month follow-up, 11% of the cardiac transplant recipients reported muscle tenderness or pain associated with physical training, while no abdominal pain, loose stools, or sleeping disturbances took place. Headaches occurred in 42% of the cardiac transplant recipients, but its frequency remained unchanged, since eight patients reported headache before and eight patients did so during pravastatin therapy. During the four-year follow-up of the cardiac transplant recipients in **Study V**, no serious side-effects occurred that would have required discontinuation of pravastatin therapy.

6.3.3. Biochemical safety in HeFH children

The ALT, CK and creatinine values of the children with HeFH before pravastatin and at 2, 4, 6, 12 and 24 months appear in Figure 13A. Although some elevations in these

concentrations were statistically significant, they were clinically insignificant. The vitamin levels of the 30 children with HeFH that were followed for one to two years of pravastatin therapy appear in Figure 13B: although the changes in vitamin E concentrations were statistically significant, all concentrations remained within the reference (12 – 40 umol/L). During follow-up, no statistically significant changes in the vitamin A concentrations took place; however two individual patients exhibited vitamin A concentrations (0.8 -0.9 umol/L) slightly lower than the reference (1 – 3 umol/L). A statistically significant increase in the 1.25-hydroxyvitamin D levels also occurred.

6.3.4. Biochemical safety in cardiac transplant recipients

The CK, creatinine and ALT values in the 19 pediatric cardiac transplant recipients before pravastatin and at 2, 4, 6, and 12 months appear in Figure 14 (unpublished data). No clinically or statistically significant elevations in these parameters occurred during pravastatin. The mean GFR before pravastatin was 78.8 ± 25.4, and at one year, was 75.5 ± 28.2 (P = 0.579).

6.3.5. Growth in HeFH children

The parameters regarding growth and development appear in Table 10. The boys and girls were divided into subgroups (I A, prepubertal at baseline and at follow-up; I B, prepubertal at baseline and pubertal at follow-up; II, pubertal at baseline; III, postpubertal at baseline) according to results from the GnRH stimulation test and clinical examination (please see *Methods*). Growth, as individually evaluated, and related to age, gender, and pubertal stage, was normal in all patients. At baseline, the two boys (9.8 and 10.7 years of age) with HeFH in Group II were pubertal according to the GnRH stimulation test (LH dominant over FSH, peak LH 6.7 IU/L in both), but had prepubertal testicular volumes (< 4 ml). Their mean height velocity at one year was at > 97^{th} percentile. However, at two years, the height velocity of one of the boys had normalized to ~ 97^{th} percentile. The growth of these two boys, as individually analyzed by using growth charts, was normal, and no accelerated bone maturation occurred.

Figure A

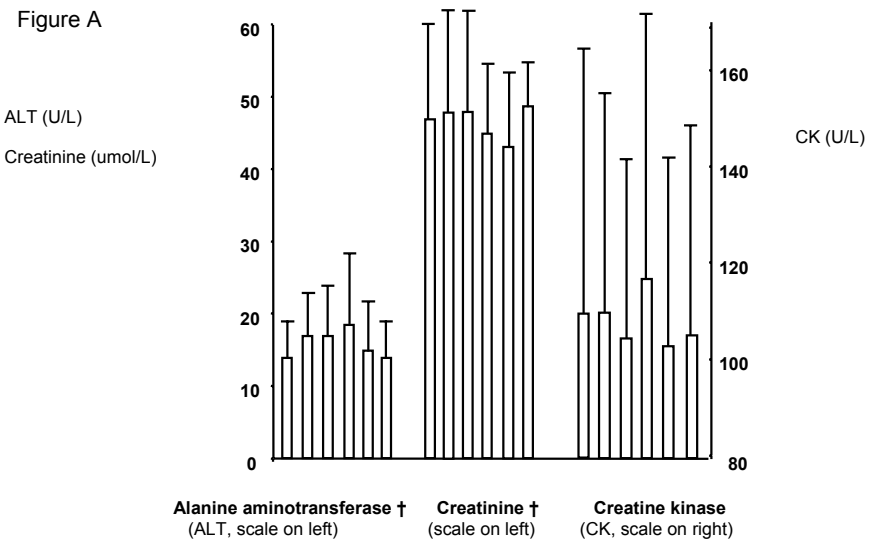

Figure B

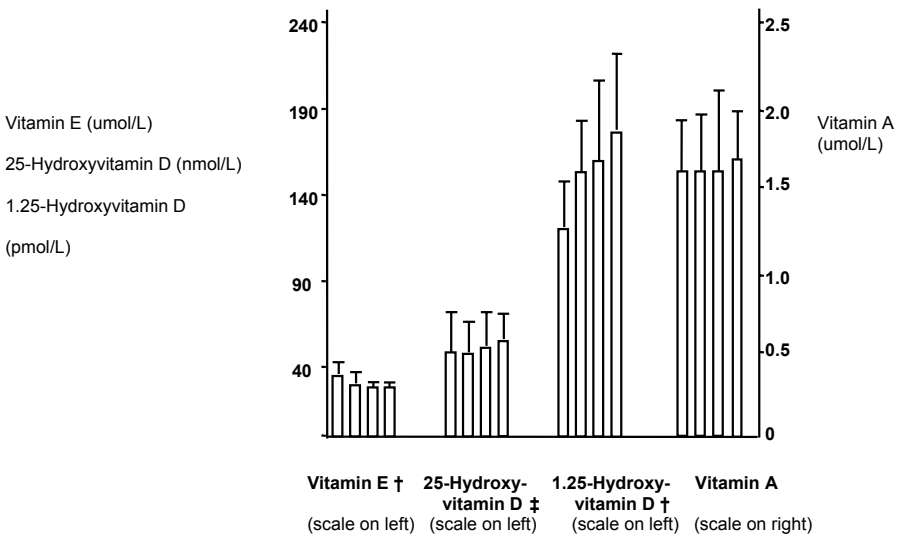

Figure 13. Serum alanine aminotransferase, creatinine and creatine kinase concentrations (mean and SD) before pravastatin treatment and at 2, 4, 6, 12, and 24 months (Figure A), and serum fat-soluble vitamin concentrations (mean and SD) before treatment and at 6, 12, and 24 months (Figure B) in children and adolescents with familial hypercholesterolemia. † Indicates that the changes in both the concentrations 0 to 12 months (30 patients) and 0 to 24 months (14 patients) are statistically significant ($P \leq 0.05$) in ANOVA. ‡ Indicates that the changes 0 to 24 months (14 patients) are statistically significant ($P \leq 0.05$) in ANOVA .

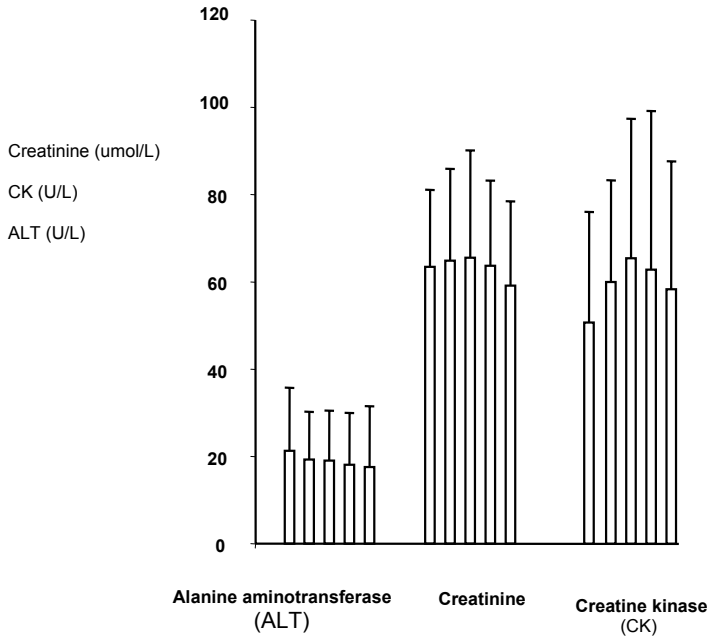

Figure 14. Serum creatine kinase (CK), creatinine, and alanine aminotransferase (ALT) concentrations (mean and SD) before and at 2, 4, 6, and 12 months of pravastatin (10 mg/d) in 19 pediatric cardiac transplant recipients. The changes were not statistically significant in ANOVA ($P \leq 0.05$).

Table 10. Growth in girls and boys with heterozygous familial hypercholesterolemia in up to 2 years of pravastatin treatment.

			Girls			Boys		
		Group	At baseline	Δ 0 to 1 y	Δ 1 to 2 y	At baseline	Δ 0 to 1 y	Δ 1 to 2 y
Chronological age	years	I A	7.1 ± 2.3	—	—	7.6 ± 2.0	—	—
		I B	9.7; 9.8	—	—	7.7; 10.3	—	—
		II	12.2 ± 0.7	—	—	9.8; 10.7	—	—
		IIi	15.9 ± 1.9	—	—	—	—	—
Height (hSDS)	SD	I A	0.1 ± 0.5	0.2 ± 0.2	-0.1; 0.0	0.0 ± 0.6	0.1 ± 0.3	0.2; 0.3
		I B	0.5; 0.6	0.0; 0.3	0.2; 0.3	-1.1; 1.9	0.1; 0.2	0.0; 0.2
		II	0.7 ± 0.7	-0.2 ± 0.2	-0.5 ± 0.3	-0.6; 0.5	0.6; 1.0	0.3
		III	0.8 ± 0.5	-0.1 ± 0.1	-0.1	—	—	—
Height *	cm	I A	122.2 ± 14.8	7.0 ± 1.0	5.4 ± 0.1	126.0 ± 12.0	5.7 ± 1.7	6.6; 7.1
		I B	139.7; 141.0	6.2; 8.3	7.6; 8.4	120.8; 151.8	6.5; 6.9	4.7; 6.7
		II	156.7 ± 3.7	4.1 ± 1.8	1.5 ± 1.2	134.5; 145.2	7.8; 11.6	6.5
		III	168.3 ± 4.7	0.5 ± 0.6	0.2	—	—	—
Weight for height index	%	I A	2.33 ± 15.7	0.5 ± 3.1	1.0; 5.0	10.4 ± 23.0	1.7 ± 4.7	-1.0; 5.0
		I B	-12.0; -1.0	-1.0; 9.0	-5.0; 1.0	2.0; 20.0	1.0; 8.0	5.0; 6.0
		II	-2.7 ± 11.9	2.7 ± 4.0	2.5 ± 3.3	-7.0; 58.0	0.0; 3.0	0.0
		III	19.7 ± 11.8†	4.3 ± 3.9	13.0	—	—	—
Body mass index	kg/m^2	I A	16.3 ± 2.5	0.4 ± 0.6	0.6; 1.0	17.7 ± 4.3	0.6 ± 1.0	0.0; 0.5
		I B	14.6; 16.6	0.4; 2.5	—	15.8; 21.2	0.5; 2.3	1.2; 1.3
		II	18.0 ± 2.0	0.8 ± 0.7	0.6 ± 0.6	15.2; 26.8	0.5; 2.5	0.5
		III	24.6 ± 4.0	0.4 ± 1.3	2.4	—	—	—
Bone age	years	I A	6.6 ± 2.2	1.3 ± 0.3	1.0; 1.2	7.1 ± 1.8	1.5 ± 0.5	0.6; 1.0
		I B	8.0; 8.8	1.2; 1.5	1.0; 1.5	7.0; 11.0	0.5; 1.0	1.0; 1.5
		II	12.8 ± 0.8	1.4 ± 0.4	0.7 ± 0.3	8.0; 13.5	0.0; 1.0	1.5
		III	15.6 ± 1.7	1.0 ± 0.7	1.0	—	—	—
Height for bone age	SD	I A	0.5 ± 0.4	0.03 ± 0.5	-0.1; -0.1	0.5 ± 0.7	-0.3 ± 0.4	0.4; 0.8
		I B	1.3; 2.4	-0.2; -0.1	0.0; 0.2	-0.4; 1.2	0.3; 0.7	-0.6; 0.0
		II	0.2 ± 0.9	-0.2 ± 0.2	-0.1 ± 0.2	-1.5; 1.1	0.4; 1.5	-0.1
		III	0.9 ± 0.4	-0.2 ± 0.2	-0.1	—	—	—

Data are mean ± SD and range, except for n = 1 and n = 2 (data are given as individual values in range).

Patients are divided into prepubertal (I A, prepubertal throughout the study, and I B, prepubertal at baseline), pubertal (II), and postpubertal (III), according to the gonadotropin-releasing hormone stimulation test response and clinical examination.

* The change is calculated by [change in height/change in age]

† the percentage is unavailable for one girl (baseline height 173 cm, weight 90 kg)

6.3.6. Development in HeFH children

The estradiol and testosterone concentrations appear in Table 11; we observed no abnormal changes, and at baseline, no signs of early maturation or delayed puberty. Pubertal development, as individually evaluated and related to age and gender, was normal in all patients. The measurements of uterus and ovaries or testicles, with reference values, appear in Table 11. The ovarian structure, related to age, was normal in all the patients examined. The testis volumes of pubertal boys (Group IB and II) were lower than the reference. This might partly be due to a difference in definition, as the GnRH stimulation test can detect central puberty before clinical signs.

Table 11. Development in girls and boys with familial hypercholesterolemia before pravastatin treatment and at one and two years.

		Group	Before treatment	At one year	At two years	Reference range‡
Girls						
Estradiol	mmol/L	I A	0.02 (A – 0.1)	A (A – 0.37)	A; A	<0.09
		I B	0.02; 0.03	0.03; 0.04	0.10; 0.12	<0.09 - 1.29
		II	0.15 (0.08 – 0.42)	0.18 (0.1 – 0.34)	0.10 (0.10 – 0.22)	0.09 - 1.29
		III	0.19 (0.11 – 0.88)	0.29 (0.14 – 0.55)	0.11	0.11 - 1.29
Volume of ovaries *	ml	I A	1.9 ± 1.6†	1.3 ± 0.2	—	<1.0 - 2.3
		I B	0.6; 1.1	1.2; 1.5	1.6	1.2 - 4.0
		II	6.1 ± 2.1	5.4 ± 1.0	6.7	2.0 - 20.0
		III	10.1	3.2	—	2.5 - 20.0
Length of uterus	cm	I A	2.9 ± 0.8	3.0 ± 0.5	—	2.5 - 4.0
		I B	3.3; 3.5	3.4; 3.8	3.7	2.5 - 8.0
		II	6.1 ± 0.9	6.1 ± 1.1	6.9	5.0 - 8.0
		III	8.4	6.6	—	5.0 - 8.0
Boys						
Testosterone	ng/mL	I A	B - C§	0.3 (D - 0.4)	0.1; 0.4	0.1 - 0.9
		I B	B; B	D; 0.3	0.2; 3.6	0.1 – 10.8
		II	0.4; 0.5	0.7; 1.2	4.4	0.3 – 10.8
		III	—	—	—	9.6 – 24.3
Volume of testicles *	mmmol/L	I A	0.6 ± 0.4	0.7 ± 0.4	0.4; 0.9	0.5 - 4.0
		I B	0.4; 0.9	0.9; 0.9	1.0‡	0.5 - 10.0
		II	0.9; 1.4	1.9; 7.5	4.2	2.0 -10.0
		III	—	—	—	15.0 - 25.0

Patients are divided into prepubertal (I A, prepubertal throughout the study, and I B, prepubertal at baseline), pubertal (II), and postpubertal (III), according to the gonadotropin-releasing hormone stimulation test response and clinical examination.

Data for testosterone and estradiol are median and range, except for n = 1 and n = 2 (data are individual values in range).

Data for volumes are mean ± SD and range, except for n = 1 and n = 2 (data are individual values in range).

A, B, C, and D indicate below detection (< 0.02nmol/L, < 0.1 nmol/L, < 0.8 nmol/L, and < 0.2 nmol/L, respectively).

* Data are means of the volumes for left and right ovaries/ testicles; † The ovaries in one pre-pubertal girl could not be visualized; § Only the range is given (several levels below detection); ‡ The testicles of the other boy were not measured.

‡ Sources: for estradiol, Hospital for Children and Adolescents, Helsinki; for ovaries and uterus, Garel et a.l[286]; for testosterone, Lashansky et al.[287] ;for testicles, Prader et al.[288];and Taranger et al. [289]

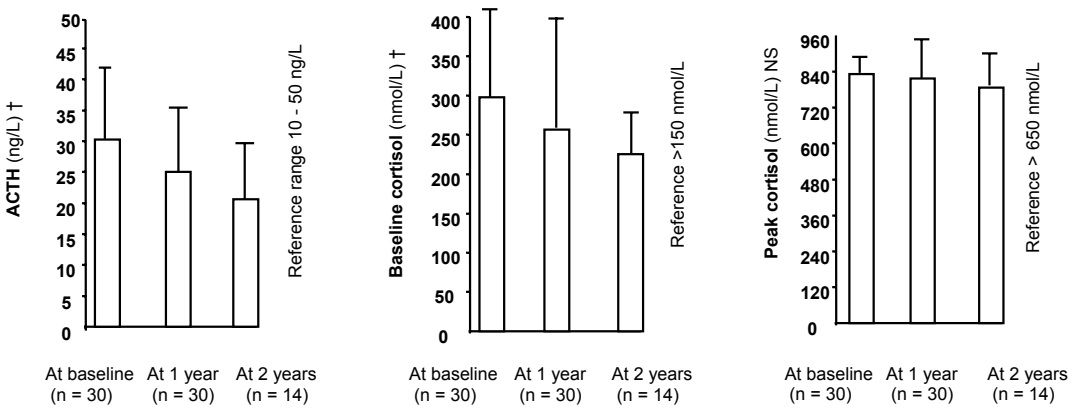

Figure 15. Serum adrenocorticotropic hormone (ACTH) concentrations and baseline and peak concentrations of cortisol during ACTH stimulation test in children and adolescents with familial hypercholesterolemia. † Indicates that the changes in both the concentrations 0 to 12 months (30 patients) and 0 to 24 months (14 patients) are statistically significant ($P \leq 0.05$) in ANOVA; whereas NS indicates that neither change was statistically significant. The reference values are those used in clinical practice at the Hospital for Children and Adolescents, Helsinki.

The ACTH concentrations and the results from the ACTH stimulation test in HeFH patients appear in Figure 15. The changes in ACTH and baseline cortisol levels were statistically significant in ANOVA. The ACTH concentrations (range 53 – 64 ng/L) at baseline in three patients and at one year in one patient were slightly higher than reference (10 – 50 ng/L). Their basal cortisol levels were, however, normal. At two years, the ACTH concentration (9 ng/L) in one patient was slightly lower than reference (10 – 50 ng/L), but the basal cortisol concentration (298 nmol/L) was normal. At one and two years, the basal cortisol concentrations (range 99 – 135 nmol/L) were below reference (> 150 nmol/L) in five and two patients, respectively. Their stimulated peak cortisol concentrations were, however, normal. We observed no clinical signs of hypocortisolism.

7. DISCUSSION

The purpose of this study was to contribute to our knowledge of the pharmacokinetics, safety, and efficacy of pravastatin in children. In children and adolescents with HeFH, the pharmacokinetic profile of pravastatin was found to correspond well to that previously reported for adults. Side-effects were mild and typically transient, compliance was satisfactory, and discontinuation rates resembled those with placebo.[54] In mild or moderate hypercholesterolemia, the cholesterol-lowering efficacy of pravastatin in general was sufficient, whereas in severe hypercholesterolemia, especially in the presence of enhanced cholesterol absorption, it was insufficient. In cardiac transplant recipients, plasma pravastatin concentrations were nearly ten-fold higher than those of the children with HeFH. Regardless of the high plasma concentrations, the short-term tolerability of pravastatin was good, and no serious side-effects occurred. The cholesterol-lowering efficacy of pravastatin was satisfactory, and 89% of the transplant recipients reached or maintained the target total cholesterol level of ≤ 5 mmol/L at one year of treatment. However, pravastatin failed to normalize the elevated serum triglyceride levels. Furthermore, recipients with low HDL cholesterol and a high ratio of apoB-100/apoA1 seem to be at increased risk for the development of accelerated coronary artery disease of the heart transplant (TxCAD). Altogether, our results were promising, and provide a rationale for the early pharmaceutical intervention of hypercholesterolemia in children.

7.1. Pharmacokinetics of pravastatin

Our results in the HeFH patients show that the pharmacokinetic profile of pravastatin in children over five years of age is similar to that previously reported in adults:[290, 291] pravastatin was absorbed rapidly, with the peak plasma concentrations (C_{max}) occurring one-half to four hours after ingestion. The AUC and C_{max} values of pravastatin in children corresponded well to those previously reported in adults.[211, 290, 291] Another study describing single dose pharmacokinetics with pravastatin 20 mg in children aged eight to 16 years is also currently available, with results similar to ours.[292] The pharmacokinetics of other statins in children remain currently unpublished. Due to marked differences in the way they are metabolized,[203, 205] the pharmacokinetic data of pravastatin cannot be applied to other statins.

As in adults, triple immunosuppressive medication greatly alters the pharmacokinetic profile of pravastatin, resulting in an approximately ten-fold increase in mean pravastatin plasma concentration. The exact mechanism of the interaction between pravastatin and cyclosporine is incompletely understood, but because cyclosporine inhibits drug transporters such MRP2[220] and OATP1B1,[221, 222] the interaction could be due to the inhibition of pravastatin transport.[210, 223, 293] Based on our results, we suggested that the inhibition of MRP2 could explain the increased bioavailability of pravastatin, since the inhibition of the hepatic uptake of pravastatin (e.g. OATPB1B) should decrease its (hepatic) clearance.[225] However, as the $t_{1/2}$ of pravastatin in our patients failed to increase, the inhibition of the hepatic OATP1B1 does not seem to explain the higher C_{max} and AUC values. Of note is that the plasma pravastatin concentrations were also high in the two patients treated with tacrolimus (without cyclosporine), suggesting that also tacrolimus could profoundly alter pravastatin pharmacokinetics. Since tacroliumus, like cyclosporine, is also a substrate for CYP3A4, P-glycoprotein, and OATP1B1, a similar pattern of interactions between tacrolimus and statins could be anticipated. However, because reports concerning major changes in the pharmacokinetics of statins during tacrolimus treatment are scarce,[294] and because recent reports by Lemahieu et al. and Ichimaru et al. found that tacrolimus has no effect on the pharmacokinetics of atorvastatin and simvastatin, respectively, the potential interaction between tacrolimus and pravastatin warrants further study.[223, 295]

The marked variation in pravastatin peak concentrations between individuals, approximately 34-fold in the HeFH group and 24-fold in the transplant recipient group, were noteworthy. In the HeFH group, the plasma concentrations of pravastatin were higher in younger and smaller patients who had received higher doses per body weight, but a similar correlation was absent in the cardiac transplant recipient group. Some have recently suspected that the polymorphism of some transporter proteins, differences in intragastric degradation (which for pravastatin can occur in the acidic stomach), or inter-individual variability in small-intestinal transit could explain some of the variability.[296-300] Due to the limited number of patients, the significant inter-individual variation in the pharmacokinetic profiles, and the existence of a variety of these other factors, which can affect the pharmacokinetic results, we were unable to study in detail the developmental aspects of pravastatin pharmacokinetics. Larger studies are thus needed to assess the

potential differences related to age, gender, and pubertal development in the pharmacokinetics and pharmacodynamics of pravastatin in children with HeFH and in cardiac transplant recipients, and to assess the mechanisms underlying the inter-individual differences in pravastatin pharmacokinetics. Importantly, the plasma concentrations of pravastatin in both patient groups approximately ten hours after administration were near zero, indicating that pravastatin does not accumulate significantly in children administrated once-daily.

7.2. Efficacy of pravastatin

The Finnish national Current Care (Käypä hoito) and the American National Cholesterol Education Program (NCEP) guidelines, as well as the International Panel on Management of Familial Hypercholesterolemia all recommend cholesterol-lowering dietary therapy as the primary treatment in HeFH children:[163-165, 170] The NCEP guidelines suggest that dietary therapy should begin with high-risk children, such as those with HeFH, over two years of age. Dietary therapy has proven beneficial as early as in the first year of life,[188-190] and large, randomized controlled trials have shown cholesterol-lowering dietary therapy in children to be safe, providing that the caloric intake is adequate.[191, 192] As with previous studies, the total and LDL cholesterol concentrations in the 30 children and adolescents with HeFH prior to treatment were considerably high, whereas the HDL cholesterol concentrations and triglycerides were mainly normal. The dietary intervention, consisting of a low-fat, reduced-saturated-fat, and low-cholesterol diet with the supplementation of plant stanol or sterol esters, lowered the total and LDL cholesterol levels of the subjects with HeFH by approximately 11% and 13%, respectively, but failed to normalize them.

The clinically important therapeutic goal in HeFH patients, besides lowering total and LDL cholesterol levels, is to reduce the incidence of CHD and cardiovascular mortality.
Several studies have clearly shown that adult patients with HeFH strongly benefit from statin therapy,[11, 301-303] and that intensive statin therapy in HeFH adults and in non-FH patients with CHD can slow the progression of or even regress coronary atherosclerosis.[11, 31, 242, 304] However, many important questions still remain incompletely answered: At what age should the cholesterol-lowering therapy be initiated and to what extent can atherosclerotic changes be reversed? What is the optimal therapeutic regimen? What is

the target level of cholesterol and should it be determined individually? Are the various regimens cost-effective? Is the treatment safe in the long-term? Due to the paucity of research data, several different guidelines concerning drug therapy in HeFH patients have been instituted: The International Panel on Management of Familial Hypercholesterolemia (2004) recommends that all men over 18 years and women over 30 years with HeFH receive cholesterol-lowering pharmacotherapy to reduce their LDL cholesterol level to between 2.6 and 4.1 mmol/L, defined individually according to major risk factors (age ≥ 30 in men and ≥ 45 in women, smoking, CHD in first-degree male relatives under 55 years or in first-degree female relatives under 65 years, LDL > 8.5 mol/L, HDL < 1.0 mmol/L, blood pressure > 140/90 mmHg, diabetes mellitus, lipoprotein a > 60 mg/dl) or clinical or subclinical atherosclerosis.[170] However, the Panel recommends pharmacotherapy for children with HeFH only in the presence of major risk factors. As in HeFH, the early signs of enhanced atherosclerosis, such as fatty streaks, carotid artery plaques, and increased carotid artery intima-media thickness may develop already in the early teens without any clinical signs or symptoms,[98-105] and because it remains unknown which changes can no longer sufficiently revert with therapy, many authorities currently consider drug therapy appropriate in children and adolescents with HeFH.[43, 54, 197] The NCEP guidelines recommend cholesterol-lowering drug therapy to HeFH children of over ten years who, after dietary interventions, have 1) serum LDL cholesterol > 4.1 ml/L and a family history of CHD, or 2) serum LDL cholesterol > 4.9 mol/L and no family history of CHD.[163, 164] The U.S. NCEP and the Finnish national Current Care (Käypä hoito) guidelines both recommend bile acid binding agents as the primary drug choice for HeFH children.[163-165] Results, however, have been modest due to poor compliance and insufficient efficacy.[180, 181, 198] Although the U.S. NCEP and the Finnish national Current Care guidelines do not recommend statins as the primary pharmaceuticals in this patient group, and although the International Panel on Management of Familial Hypercholesterolemia proposes that statin therapy be considered only in boys over ten years of age and in girls after puberty,[170] the U.S. Food and Drug Administration has approved pravastatin and atorvastatin for HeFH children over eight and ten years of age, respectively. In our study, the HeFH patients were 4.9 to 18.5 years of age (mean 10.1 ± 3.4 years). Patients at this age are generally capable of ingesting pravastatin in tablet form. If even younger patients required treatment (e.g. organ transplant recipients), a pravastatin mixture would make it easier to adjust the doses with greater accuracy. However, no statin dose recommendations based on weight or body surface area currently exist. It is important to emphasize that our study did not

attempt to determine the right age of onset of pharmacotherapy, which clearly warrants further study. While the decisions concerning life-long treatment are made, the psychological and emotional impact of the life-long genetic disease and the premature morbidity and mortality it disposes the patient and affected family members should not be underestimated.

In our children and adolescents with HeFH, pravastatin in increasing doses (maximum 10 to 60 mg) for one to two years lowered the total and LDL cholesterol values progressively and statistically significantly: at 2 months by 19% and 25%, at 6 months by 23% and 29%, and at 12 months by 27% and 33%, respectively; this trend corresponds well to the efficacy profile of pravastatin reported previously for hypercholesterolemic adults with and without HeFH.[185, 305] Because the doses were only increased if the patient had not reached the target total cholesterol level of 5 mmol/L, the study was not designed to determine the dose-response curve of pravastatin (patients with "a good response" would fall out from the higher doses). However, approximately 70 to 80% of the maximum efficacy was achieved with 10 to 20 mg. Interestingly, despite the use of high drug doses, only approximately one third of the patients reached the target cholesterol level of ≤ 5 mmol/L. In patients with a very high baseline cholesterol level, pravastatin may be insufficient, and other more potent cholesterol-lowering statins such as atorvastatin or rosuvastatin could be beneficial. Atorvastatin (10 to 20 mg) has been shown to reduce LDL cholesterol concentrations in HeFH children by approximately 40%.[51] While the efficacy and safety of atorvastatin has been evaluated in children,[44, 51] no data currently exist on the pediatric use of rosuvastatin.

Since insufficiently-treated hypercholesterolemic patients with high total and LDL cholesterol levels are likely to remain at an increased morbidity and mortality risk of cardiovascular disease, determination of factors predicting the responsiveness of these patients to therapy would be useful. To identify such factors, we studied baseline cholesterol metabolism by assessment of serum non-cholesterol sterols in seven participants who achieved the target level of 5 mmol/L total cholesterol by one year of treatment (Group A), and in nine patients who did not (Group B). Group B, with an insufficient response, exhibited higher baseline cholesterol concentrations and higher ratios of cholesterol absorption markers sitosterol and campesterol to cholesterol. Furthermore, high cholesterol absorption (ratio of cholestanol to cholesterol) was

associated with poor pravastatin response (low reduction of cholesterol at one year). This indicates that both the relatively weak maximum effects of pravastatin as well as the high intestinal cholesterol absorption of some individuals can contribute to insufficiency of therapy. Similar results exist for adult coronary patients receiving simvastatin among whom those with higher absorption and lower synthesis of cholesterol required larger statin doses.[306] Furthermore, a recent study by Miettinen et al. showed that the risk of recurrence of major coronary events among a subgroup of the Simvastatin Survival Study (4S) patients was greater in those with higher ratio of cholestanol to cholesterol.[307] This risk was 2.2-fold between the lowest and highest quartiles, a finding not applicable to total, LDL, or HDL cholesterol. [307] As in previous studies, statin therapy both in Group A and Group B increased the serum markers of cholesterol absorption.[73] From a clinical viewpoint, patients with high cholesterol levels caused by the high absorption capacity could benefit from a combination therapy with statins and pharmaceutical agents that inhibit cholesterol absorption (i.e. ezetimibe). In hypercholesterolemic patients, determinations of cholestanol and plant sterol ratios before statin therapy could therefore offer an objective way to evaluate cholesterol metabolism and possibly to predict potential treatment-related problems.

Hypercholesterolemia[58, 121, 122] and hypertriglyceremia[28, 125] are common concerns both in adult and pediatric cardiac transplant recipients; and among other factors, are thought to predispose to TxCAD.[23, 26-28, 30, 120] TxCAD is the main cause of poor graft function and death among cardiac transplant recipients, and the development of effective preventive therapy is a considerable challenge in the treatment of transplant recipients. Early attempts using bile acid binding agents and fibric acid derivatives to lower cholesterol concentrations in transplant recipients often led to an insufficient outcome, unacceptable side-effects, and disturbances in cyclosporine metabolism.[183, 308] The new cholesterol-lowering agent ezetimibe which inhibits cholesterol absorption, is likewise not currently recommended for cardiac transplant recipients due to the lack of safety data and the 12-fold increase in the plasma concentrations of ezetimibe following its co-administration with immunosuppressive medication.[32] In adult cardiac transplant recipients, promising results have been obtained with statins, particularly with pravastatin at a daily dose of 20 to 40 mg.[33] Besides lowering the total and LDL cholesterol levels, pravastatin and simvastatin have been shown to reduce the incidence of TxCAD, impaired endothelial function,

rejection, early myocardial infarction, and death.[32-37] Since accelerated TxCAD is also the main cause of poor long-term survival in pediatric cardiac transplant recipients,[16] to assess the tolerability, safety and efficacy of statins in this patient group is important. However, limited information on the safety and efficacy of statins in pediatric cardiac transplant recipients is currently available, since, besides this study, only a few retrospective reports with pravastatin and atorvastatin have been published.[55-58]

Surprisingly, the mean total and LDL cholesterol levels of the transplant recipients were quite similar to those of the healthy pediatric controls. However, in 32% and 26% of the recipients, the total and LDL cholesterol concentrations were higher than the target values of 5 mmol/L and 3 mmol/L, respectively; and in a high-risk population such as cardiac transplant recipients, even slightly increased cholesterol concentrations may be harmful. The baseline apoB-100 concentration and the apoB-100/apoA-1 ratio of the transplant recipients were significantly higher than those of the healthy controls. Furthermore, the serum HDL cholesterol concentrations of the transplant recipients were significantly reduced, and the triglyceride levels increased. The relationship between high serum LDL cholesterol concentrations and the incidence of CHD is well established in both transplant and non-transplant subjects. However, several studies have indicated that the level of apolipoprotein B-100 and the ratio of apoB-100/apoA-I are even better predictors of the risk of vascular disease than are LDL and HDL cholesterol, respectively.[309-313] Besides LDL, other lipoproteins that contain apoB, such as VLDL, are also known to promote atherosclerosis.[314] In our transplant recipients, pravastatin at an average dose of 0.3 mg/kg, roughly corresponding to the dose of 20 mg in adults, significantly decreased the apoB-100 concentration and the apoB-100/apoA-1 ratio of the recipients and brought them closer to that of the healthy controls. After one year, pravastatin (10 mg/day) had significantly decreased the total and LDL cholesterol concentrations of the transplant recipients, with mean reductions of 10% and 19%, respectively. Of the transplant recipients, 89% reached or maintained the target total cholesterol concentrations. However, pravastatin intervention failed to normalize the elevated triglyceride levels of the transplant recipients; in fact a slight increase in the serum triglycerides occurred during the one-year follow-up. Although the role of hypertriglyceridemia in atherosclerosis has been under debate, recent studies and meta-analyses suggest that elevated serum triglycerides are an independent risk factor for atherosclerosis and CHD.[314-317] It remains unclear, however, whether triglycerides themselves are atherogenic, or whether their elevation

merely reflects an increase in the concentrations of triglyceride-rich remnant lipoproteins known to promote atherosclerosis and increase the risk of CHD.[314] Persistent hypertriglyceridemia can aggravate low HDL cholesterol, and the combination of high triglyceride and low HDL cholesterol levels is known to be a powerful risk factor for CHD death, even in the absence of hypercholesterolemia.[315]

Those cardiac transplant recipients with angiographically detectable coronary abnormalities before pravastatin exhibited persistently lower serum HDL cholesterol concentrations than did those with apparently healthy coronaries. Furthermore, the apoB-100/apoA1 ratio of the patients with TxCAD remained higher throughout the study than that of the patients without TxCAD, whereas no significant differences occurred in their total or LDL cholesterol concentrations. Moreover, high LDL cholesterol concentrations,[143] low HDL cholesterol concentrations,[149-152, 318, 319] and high apoB-100/apoA-1 ratio[309-313] are also well-known risk factors for coronary atherosclerosis. Large experimental, epidemiological, and clinical studies have shown that low HDL cholesterol is an independent risk factor for atherosclerosis and CHD regardless of the serum levels of LDL cholesterol and triglycerides.[150, 151] Besides playing a key role in the reverse cholesterol transport process,[60, 76, 77] HDL particles are believed to have several other anti-inflammatory, antioxidant, antitrombotic, and antiproliferative properties.[77] HDL also seems to protect the endothelial cells from cytotoxic damage caused by remnants of triglyceride-enriched lipoproteins.[83]

Corticosteroids may cause insulin resistance, hypertriglyceridemia, and elevated VLDL cholesterol concentrations, whereas cyclosporine can increase serum LDL cholesterol concentrations.[123, 124] Dyslipidemias are, therefore, exceedingly common in adult and pediatric cardiac transplant recipients.[16, 17] Besides high triglycerides and low HDL cholesterol, other characteristics of metabolic syndrome that commonly occur in transplant recipients (i.e. small, dense LDL phenotype, abdominal obesity, and insulin resistance)[125] also impair endothelial function, enhance thrombosis, and promote atherosclerosis.[314] Therapeutic life-style changes, including regular physical exercise, smoking cessation, weight loss, and a health-promoting diet are therefore a vital part of treatment. In adult transplant recipients, promising results regarding the management of hypertriglyceridemia have been obtained with omega-3-fatty acids confined from fish oil.[183, 320] As hypertriglyceridemia is associated with low HDL cholesterol, omega-3-fatty acids may also

be of some benefit in the treatment of transplant recipients with low HDL cholesterol.[183, 320] In addition to non-pharmaceutical regimens, statins are commonly used nowadays as a routine post-transplantation therapy for the treatment of hypercholesterolemia. Whether all pediatric cardiac transplant recipients should receive statins regardless of cholesterol level currently remains uncertain. As in our recipients, pravastatin effectively reduces the elevated serum cholesterol levels, but often fails to correct the high serum triglycerides and low HDL cholesterol concentrations.[32] Rosuvastatin, with triglyceride-lowering and HDL cholesterol-elevating properties more potent than pravastatin, may thus be warranted in recipients with markedly elevated triglycerides and constantly low HDL cholesterol.[185], Thus far, however, only one short-term (6-weeks) study assessing the safety and efficacy of the combination therapy with rosuvastatin and immunosuppressive medication has been published in adult recipients,[321] and therefore further studies are required before rosuvastatin therapy can be recommended for the treatment of pediatric transplant recipients. Fibric acid derivatives and nicotinic acid increase the HDL cholesterol concentrations to a greater extent than do statins.[77, 322] On the other hand, the use of fibric acid derivatives in transplant recipients requires caution, especially if they are used concomitantly with statins, because of the increased risk of rhabdomyolysis.[308] Due to a high incidence of adverse events, nicotinic acid is rarely used in transplant recipients.[308] Altogether, effective statin therapy, steroid-free immunosuppression (whenever possible), regular exercise, weight control and a lipid-lowering diet with omega-3-fatty acids are advisable in reducing the metabolic stress that predisposes cardiac transplant recipients to premature atherosclerosis.[183, 320]

In adult cardiac transplant recipients, statins such as pravastatin and simvastatin have markedly improved both the patient and graft survival rates.[32-40] Although pediatric data are scarce, a retrospective analysis by Mahle et al. suggested that the post-transplantation use of pravastatin was associated with a lower incidence of TxCAD. Of our six patients with TxCAD prior to pravastatin, one patient died of severe vasculopathy and another underwent stent a operation during the four-year follow-up, which reflects the severity of the disease. Since the severe coronary abnormalities in both patients had already been diagnosed three and one years before the initiation of pravastatin, respectively, pravastatin therapy was obviously started too late in order to prevent coronary vasculopathy. In the remaining four, the coronary changes remained unchanged, or progressed only slightly. Two patients (of 13) with healthy coronaries at baseline developed TxCAD during

pravastatin intervention after a preceding grade I rejection. Altogether, the gradual progression of the coronary lesions in some patients indicates that pravastatin may be insufficient to completely suppress TxCAD in pediatric cardiac transplant recipients. It should be noted, however, that in the transplant recipients, due to potential safety concerns, the daily doses of pravastatin were not increased from 10 mg. Due to the limited number of patients, differences in the initiation-time of pravastatin and the lack of a placebo group, larger trials with a more homogenous patient population and a longer follow-up period are required to determine ultimately whether and at what dose pravastatin is sufficient to suppress the progression of TxCAD and to safely manage the post-transplantation dyslipidemia.

7.3. Safety of pravastatin

While no serious side-effects of pravastatin occurred in HeFH patients, the mild side-effects were mostly transient and occurred during the first weeks; thus they did not appear to be dose-dependent. In accordance with previous studies, headache and gastrointestinal symptoms were the most common side-effects. Of note, the same symptoms were also common before pravastatin. Since fat-soluble vitamins are transported in association with lipoproteins, cholesterol-lowering statin therapy has been feared to lead to vitamin deficiencies. Although a small statistically significant decrease in vitamin E levels was observed, the serum levels of fat-soluble vitamins remained satisfactory. Our results are therefore in line with those of previous studies in HeFH children, thus indicating that no significant reductions in fat-soluble vitamins occurred during statin therapy.[43, 54] The changes in CK, ALT, or creatinine concentrations were not clinically relevant, and no marked abnormalities that would have required the discontinuation of pravastatin therapy (Pravachol product information) occurred. Furthermore, compliance was satisfactory.

Since cholesterol plays many essential roles in growth and development,[59] the lack of long-term safety data has restricted the use of statins in children. The first study was that of Sinzinger et al. in 1992.[52] Currently, several studies addressing the short-term safety of different statins in children are available. [41, 43, 44, 46-54] Before the initiation of our study, only Stein et al. had launched data on the growth and pubertal development of adolescent males receiving lovastatin.[54] In 2002, de Jongh et al. published a similar study on

adolescents (females and males) treated with simvastatin.[47] Furthermore, after the completion of our study, Wiegman et al. reported the absence of adverse effects of pravastatin on growth and pubertal maturation in children and adolescents (8-18 years) during a two-year period.[43] As the pravastatin doses in the latter study were 20 mg in children of under 14 years, and 40 mg in adolescents of over 14 years, the doses in our study, especially in the younger children, were considerably higher. Also, unlike previously, we studied the growth and development of individual patients in relation to their age, pubertal stage and gender. To our knowledge, no previous study has reported on the growth and development of young children taking any statin. Nine of our patients were under eight years of age, and the youngest only 4.9 years old; also, in these patients pravastatin was safe and well-tolerated. Importantly, pravastatin treatment did not delay pubertal development or cause hormonal disturbances at any age: progressions in Tanner staging, estradiol and testosterone levels, and gonadal volumes were normal. Furthermore, growth was not adversely affected. The observed slight but statistically significant alterations in the plasma ACTH and baseline cortisol levels were most likely of little clinical relevance and no patient showed signs of hypocortisolism. In previous statin studies in children, the changes in cortisol concentrations have been non-significant.[43, 54] Adverse effects of pravastatin or simvastatin on adrenal function were neither observed in adults.[323-327] Even though the follow-up was among the longest of any statin study in children, an even longer follow-up period from early childhood to adulthood is required to ultimately conclude the long-term safety of pravastatin therapy in children.

Despite the higher plasma concentrations in the cardiac transplant recipients, pravastatin was well-tolerated, without significant short-term side-effects. In fact, we observed none of the more serious side-effects, such as rhabdomyolysis or hepatotoxicity, reported more commonly in adult recipients.[328] Overall, no clinically significant changes in glomerular filtration rate (determined by ^{51}Cr EDTA clearance) or plasma creatinine, ALT, or CK concentrations occurred, and we observed no signs of increased cyclosporine renal toxicity. Although headache is a common complaint in patients on cyclosporine, its frequency did not increase during the two-month follow-up period. Due to marked polypharmacy and the use of potentially hepato- and nephrotoxic substances, careful follow-up of hepatic and renal functions is crucial in cardiac transplant recipients. In pediatric transplant recipients undergoing immunosuppressive therapy, future trials

assessing the safety of pravastatin at daily doses exceeding 10 mg are still warranted. Although pravastatin did not adversely affect growth and pubertal development in patients with HeFH, growth and development during the combination therapy with immunosuppressive medication in pediatric recipients also remains to be determined.

7.4. Study limitations

An important limitation in our study was that, due to the relatively small number of HeFH patients treated at the Hospital for Children and Adolescents, Helsinki, this was an open clinical follow-up rather than a randomized, placebo-controlled, double-blind study. With the pediatric cardiac transplant recipients, the hospital's current treatment protocol includes the post-transplantation use of statins. Since recipients are at a very high risk for TxCAD,[16, 17] and since statins in adults have been shown to decrease cardiovascular mortality and morbidity after transplantation, [32-40] a placebo-controlled study in this patient group could be considered unethical. The relatively small number of participants in both patient groups, and especially in smaller subgroups, restricted the statistical power, and in some cases hindered the attainment of definite conclusions (i.e. the sufficiency of pravastatin for TxCAD prevention). Also, the variation in patient age, size and pubertal stage was considerable, which may limit the validity of the results in a given age group. Both children with HeFH and pediatric cardiac transplant recipients as a patient group are unique whereby the results from this study cannot be applied directly to all children (i.e. transplant recipients of other organs). Considering that the pharmaceutical intervention in both groups is likely to be life-long, larger trials with an even longer follow-up period are still warranted to confirm the promising results of our study.

8. SUMMARY AND CONCLUSIONS

In order to enable the pediatric use of pravastatin, my dissertation aimed to study the pharmacokinetics, safety, and, efficacy of pravastatin in children with heterozygous familial hypercholesterolemia and in pediatric cardiac transplant recipients receiving triple immunosuppressive medication.

The main findings of the study were as follows:

1 The pharmacokinetic profiles of pravastatin in children with HeFH and in pediatric cardiac transplant recipients corresponded well to those previously reported in adults with HeFH and in adult transplant recipients, respectively. The peak plasma concentrations of pravastatin in pediatric transplant recipients were nearly ten-fold higher than in children with HeFH, indicating that significant interactions between pravastatin and immunosuppressive medication occur.

2 In children and adolescents with HeFH, the cholesterol-lowering efficacy of pravastatin in slight or moderate hypercholesterolemia was satisfactory, whereas in severe hypercholesterolemia, especially in the presence of enhanced cholesterol absorption, it was insufficient. These patients with high baseline cholesterol levels together with high cholesterol absorption could benefit from a combination therapy of statins and ezetimibe, which inhibit both cholesterol synthesis and absorption, respectively.

3 In pediatric cardiac transplant recipients, pravastatin lowered the serum total and LDL cholesterol concentrations effectively, but failed to normalize the elevated triglyceride levels. Low HDL cholesterol concentrations, associated with high serum triglyceride levels, and high serum apoB-100/apoA-1 ratios were associated with the development of TxCAD. Altogether, effective statin therapy, steroid-free immunosuppression (whenever possible), regular exercise, weight control, and a lipid-lowering diet with omega-3-fatty acids should be favored in order to reduce the metabolic stress predisposing to premature atherosclerosis in cardiac transplant recipients.

4 In both groups, pravastatin was safe and well-tolerated. No serious side-effects occurred, and pravastatin did not increase the alanine aminotransferase, creatinine, or creatine kinase values to a clinically relevant extent. In children and adolescents with HeFH, pravastatin did not adversely affect growth or pubertal development.

9. ACKNOWLEDGEMENTS

This study was conducted in collaboration with the Hospital for Children and Adolescents, Department of Clinical Pharmacology, National Public Health Institute, Division of Internal Medicine, Division of Cardiology (Helsinki University Hospital), Department of Clinical Nutrition (Kuopio University Hospital), and the Department of Children and Adolescents (University of Oulu) during the years 2001-2006. I am grateful to all my colleagues who made this work possible. I also wish to express my gratitude to the heads of the Hospital for Children and Adolescents: Dr. Veli Ylitalo, and Professors Christer Holmberg and Michael Knip. I owe my sincere gratitude to Professor Markku Heikinheimo, head of the Pediatric Graduate School of the Hospital for Children and Adolescents, for his assistance.

I am also deeply grateful to my supervisor, Docent Marjatta Antikainen, for her time, support, encouragement, enthusiasm, and guidance. Her wide expertise on the treatment of lipid disorders in children was fundamental in this thesis work. Furthermore, it is a priceless resource to have a supervisor who always finds the time to listen and help with problems encountered – even during the Christmas holidays!

I thank Docents Matti Salo and Kalle Hoppu for reviewing this thesis and for helping me to improve it with their vast knowledge and expertise.

I warmly thank Professor Pertti Neuvonen for introducing me to the world of clinical pharmacology. He always finds the time to help with kindness, and his firm clinical and scientific expertise in medicine and in clinical pharmacology has made this project possible. I must say that I have learned greatly from him, and that I feel privileged to have worked with him.

I would like to express my sincere gratitude to Docent Tiina Laine for her expertise in pediatric endocrinology. Besides helping me to individually evaluate the growth and development of the patients, she encouraged me during difficult times. Her positive attitude towards life and pediatrics was a great inspiration to me.

I would also like to thank all my collaborators, Marjatta Antikainen, Pertti Neuvonen, Christer Holmberg, Eero Jokinen, Tatu Miettinen, Helena Gylling, Matti Jauhiainen, Christer Ehnholm, Raija Pahlman, Jouko Sundvall, Tiina Laine, Matti Nuutinen, Anna Föhr, Marjatta Lappi, Saila Piippo, Anna Ketomäki, Mikko Syvänne, Mikko Niemi, and Mikko Neuvonen, for their advice and encouragement. Matti Jauhiainen helped me to understand better the world of lipids. I would like to express my gratitude to Professors Christer Holmberg and Eero Jokinen for their expertise and advice on pediatric cardiac transplantation and pediatric cardiology.

I would also like to express my thanks to Dr. Päivi Miettinen and to Dr. Tero Saukkonen for their follow-up during my thesis work. I owe a special thanks to Dr. Eeva Salo and Dr. Eero Rahiala for enabling me to combine research with clinical practice. Furthermore, I want to thank the excellent clinicians of the Hospital for Children and Adolescents for teaching me how rewarding and fascinating pediatrics is.

I kindly thank Mr. Steven Stalter for editing this thesis. The technical assistance of the staff of the Hospital for Children and Adolescents, including nurses, laboratory assistants, secretaries and the Librarian, was also crucial.

I owe special thanks to my father, Professor Klaus Hedman, for his support and help with the text and the English language. In addition, I would also like to warmly thank my mother Lea, sisters Anne and Paula, grandma Helena, other relatives, as well as friends for their help and support during the years. I thank my precious dogs, Taiga and Tuisku, for bringing so much happiness into my life.

Finally, I wish to express my warm thanks to my dear husband and friend, Walter (who completed his thesis at the same time) for his support, love and care.

This work was supported by The Finnish Medical Foundation, the Foundation for Pediatric Research, the Biomedicum Helsinki Foundation, the Ida-Montin Foundation, The Clinical Drug Research Graduate School, and the Helsinki University Central Hospital Research and Education Fund. My sincere thanks also go to those families who participated in this study.

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