ARJA SIIRTOLA

Prevalence and Possible Causes of Dyslipidemia after Pediatric Solid Organ Transplantation

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ACADEMIC DISSERTATION
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“For nothing is hidden except for
the purpose of having it revealed.“

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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following five original publications, which are referred to in the text in their roman numerals I-V. Some additional unpublished data is also presented.

I Siirtola A, Antikainen M, Ala-Houhala M, Koivisto A-M, Solakivi T, Jokela H, Lehtimäki T, Holmberg C, Salo MK (2004): Serum lipids in children 3 to 5 years after kidney, liver, and heart transplantation. Transpl Int 17: 109-119.

II Siirtola A, Antikainen M, Ala-Houhala M, Solakivi T, Jokela H, Lehtimäki T, Holmberg C, Salo MK (2004): Studies of LDL particle size and susceptibility to oxidation and their association with glucose metabolism in children after heart transplantation. J Heart Lung Transpl 23: 418-426.

III Siirtola A, Antikainen M, Ala-Houhala M, Koivisto A-M, Solakivi T, Virtanen SM, Jokela H, Lehtimäki T, Holmberg C, Salo MK (2005): Insulin resistance, LDL particle size, and LDL susceptibility to oxidation in pediatric kidney and liver recipients. Kidney Int 67: 2046-2055.

IV Siirtola A, Ketomäki A, Miettinen TA, Gylling H, Lehtimäki T, Holmberg C, Salo MK, Antikainen M (2006): Cholesterol absorption and synthesis in pediatric kidney, liver and heart transplant recipients. Transplantation 81: 327–334.

V Siirtola A, Virtanen SM, Ala-Houhala M, Koivisto A-M, Solakivi T, Lehtimäki T, Holmberg C, Antikainen M, Salo MK (2008): Diet does not explain the high prevalence of dyslipidemia in pediatric renal transplant recipients Pediatr Nephrol 23: 297-305.

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ABBREVIATIONS

ALT	alanine aminotransferase
ApoA-I	apolipoprotein A-I
ApoB	apolipoprotein B
ApoE	apolipoprotein E
AZA	azathioprine
B-CsA	whole-blood cyclosporine trough level
β (beta)	regression coefficient
BMI SDS	body mass index standard deviation score
CAD	coronary artery disease
CETP	cholesterol ester transfer protein
CI	confidence interval
CsA	cyclosporine A
DU-prot	diurnal (24-hour) urinary protein excretion
Exp (B)	the change in the odds ratio of the dependent variable for a one-unit change in the predictor
ESRD	end-stage renal disease
GFR	glomerular filtration rate
GH	growth hormone
HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment for insulin resistance
HSDS	height standard deviation score
HTx	heart transplantation
IDL	intermediate density lipoprotein
LCAT	lecithin cholesterol acyltransferase
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
Lp (a)	lipoprotein (a)
LPL	lipoprotein lipase
LRP	liver receptor related protein
LTx	liver transplantation
MP	methylprednisolone
OGTT	oral glucose tolerance test
p	level of probability
PLTP	phospholipase transfer protein
Q1, Q3	25^{th} percentile, 75^{th} percentile
r	coefficient of correlation
R^2	coefficient of determination
RhGH	recombinant human growth hormone
RTx	renal transplantation
SD	standard deviation
SE	standard error of mean

TC	total cholesterol
TT	thromboplastin time
Tx	transplantation
Urea-N	urea nitrogen
VLDL	very low-density lipoprotein

ABSTRACT

Yearly, approximately 10-15 Finnish children receive a kidney, 5 a liver, and 5 a heart graft due to their end-stage disease. To date in Finland, more than 300 pediatric patients have undergone solid organ transplantation (Tx). The short-term prognosis is excellent with more than 90 % patient and graft survival, but long-term patient and graft survival has evolved more slowly. In adult solid organ recipients, a major cause of poor long-term prognosis is cardiovascular disease. In general population, the first vascular changes of cardiovascular disease begin to evolve in childhood. After iniation, the progression of the disease is dependent on the presence of risk factors, e.g. obesity, smoking, sedentary lifestyle, high blood pressure and dyslipidemia.

In the present study, we studied the prevalence of dyslipidemia in 71 pediatric kidney, 34 liver and 20 heart recipients for 3 to 5 years after Tx. In addition, we searched for associations between dyslipidemia and possible risk factors in a cross-sectional study of 50 kidney, 25 liver and 12 heart recipients. Controls were 181 healthy pediatric clinic outpatients selected for similar age and sex distribution. Patients were accepted as participants after surviving at least one year after Tx, being aged 3-17 years, and having acceptable renal function with glomerular filtration rate > 40 ml/min/m^2 and not using lipid-lowering drugs. The patients received triple immunosuppressive therapy with azathioprine, methylprednisolone, and cyclosporine A.

Tx improved serum lipid profiles in most pediatric solid organ recipients. However, serum triglyceride levels > 1.5 mmol/l were observed in approximately 50 % of the renal and heart recipients and in 30 % of the liver recipients. Low serum high-density lipoprotein cholesterol levels (< 1.0 mmol/l) were infrequent. Approximately 40 % of the renal recipients had a serum low density lipoprotein cholesterol concentration > 3.0 mmol/l. Increased frequency of insulin resistance, which was estimated by homeostasis model assessment, was seen in approximately 40 % of the solid organ recipients. Further, metabolic risk factors of cardiovascular disease seemed to cluster approximately in one quarter of the solid organ recipients. Presence of mild proteinuria (> 200 mg/d) in a renal graft, high trough concentration of cyclosporine A or high dose of methylprednisolone, obesity, older age, higher serum lipid levels before Tx and a kidney graft (vs. liver or heart) were associated with dyslipidemia after Tx.

In conclusion, dyslipidemia in the pediatric transplant recipients, though prevalent, was less severe and less prevalent than in many earlier studies mainly on adult recipients. In addition to maintaining good graft function with carefully monitored low dose immunosuppressive regimen, encouragement to active healthy lifestyle with weight control should be the aims of patient care after Tx.

TIIVISTELMÄ

Vuosittain keskimäärin 10–15 suomalaista lasta saa munuais-, 5 maksa- ja 5 sydänsiirteen edenneen munuaisten, maksan tai sydämen vajaatoiminnan vuoksi. Tällaisia elinsiirteen saaneita lapsia on ollut vuoden 2007 alkuun mennessä Suomessa yli 300. Elinsiirtojen lyhytaikainen ennuste on erinomainen: yli 90 % lapsista selviytyy toimivan siirteen kanssa yli vuoden, mutta siirteitä menetetään edelleen vuosien kuluttua siirrosta. Sydän- ja verisuonisairaudet ovat elinsiirteen saaneilla aikuisilla merkittävä lisääntyneen kuolleisuuden syy. Tämän valtimonkovettumataudin alkavia muutoksia nähdään jo terveilläkin lapsilla. Veren rasva-arvojen poikkeavuudet eli dyslipidemia (erityisesti korkea LDL-kolesteroli) on keskeinen valtimonkovettumataudin riskitekijä.

Tutkimuksessamme seurasimme dyslipidemian esiintyvyyttä 71 munuais-, 34 maksa- ja 20 sydänsiirteen saaneella lapsella 3-5 vuoden ajan elinsiirron jälkeen. Dyslipidemian mahdollisia riskitekijöitä selvitimme lisäksi poikkileikkauksena 50 munuais-, 25 maksa- ja 12 sydänsiirteen saaneella lapsella. 181 tervettä polikliinisella sairaalakäynnillä ollutta samanikäistä lasta olivat verrokkeina. Potilaat hyväksyttiin tutkimukseen, jos heidän ikänsä oli 3-17 vuotta, elinsiirrostaan oli kulunut vähintään vuosi, siirteensä toimi hyvin ja heidän munuaistoimintaa kuvaava glomerulussuodattumisensa oli vähintään 40ml/min/m^2. Elinsiirteen saaneiden lasten hylkimisenestolääkityksenä olivat atsatiopriini, metyyliprednisoloni ja siklosporiini.

Elinsiirto korjasi useimpien lasten seerumin rasva-arvoja. Kuitenkin siirron jälkeen seerumin triglyseridipitoisuus oli korkea (> 1.5 mmol/l) 50 %:lla munuais- tai sydänsiirteen saaneista ja 30 %:lla maksasiirteen saaneista lapsista. Matala HDL-kolesteroli-pitoisuus (< 1.0 mmol/l) oli harvinainen. Lisäksi 40 %:lla munuaisiirteen saaneista oli korkeahko seerumin LDL-kolesterolipitoisuus (> 3.0 mmol/l). Noin 40 %:lla elinsiirteen saaneella lapsella arvioimme olevan insuliiniresistenssiä ja neljänneksellä lapsista oli kolme tai useampi metabolisen oireyhtymän piirre. Kuuluminen munuaissiirreryhmään, valkuaisainevirtsaisuus (> 200 mg/vrk), lihavuus, vanhempi ikä, korkea siklosporiinin veren paastopitoisuus, korkea metyylipredinisoloniannos ja dyslipidemia jo ennen elinsiirtoa olivat yhteydessä dyslipidemiaan.

Vaikka seerumin rasva-arvojen poikkeavuudet olivat tavallisia, ne vaikuttivat kuitenkin olevan lähempänä sekä tavoitteita että vertailulasten arvoja kuin useimmissa aiemmissa, erityisesti aikuisilla tehdyissä tutkimuksissa. Siirteen hyvä toiminta käyttäen pieniannoksista hylkimisen estolääkitystä ja aktiivinen terveellinen elämäntapa (normaalipainoisuus; ravinto, jossa vähän tyydyttynyttä rasvaa ja kolesterolia, mutta riittävästi monityydyttymätöntä rasvaa; riittävä liikunta) ovat tärkeä osa elinsiirron jälkeistä hoitoa.

1. INTRODUCTION

The first renal transplantation (RTx) was carried out on a pediatric recipient in Finland in 1985. Today, end-stage kidney, liver and heart disease in children are treated with solid organ transplantation (Tx) whenever appropriate. In Finland, pediatric patients receive approximately 10-15 renal (RTx), 5 liver (LTx) and 5 cardiac grafts (HTx) every year. Thus far, more than 300 Finnish children have undergone solid organ Tx (until Dec 31st, 2006 a total of 349, 202 RTx, 96 LTx, 51 HTx, personal communication, Ulla Sandholm, April 23rd 2007). Surgical techniques and post-operative pharmacological therapies have evolved since the first pediatric solid organ Tx was done. Accordingly, both patient and graft survival has become excellent with approximately 90 % 1-year survival [299, 320, 328]. In Finnish pediatric renal recipients, 7-year patient survival is approximately 96 % and graft survival approximately 80 % [375]. In Finnish pediatric liver and heart recipients, 5-year patient survival is approximately 80 % and 75 % respectively [325, 367]. The majority of these patients live normal lives with a long expected lifespan. Internationally, 10-year graft and patient survival is for pediatric RTx, LTx and HTx approximately 70 %. Long-term patient and graft survival has evolved more slowly than short-term survival. [56]

In adult renal recipients, cardiovascular disease is the main cause of death, especially in long-term survivors [278]. After RTx and LTx, the incidence of symptomatic arterial disease is 3- to 4-fold compared with the incidence in general population [1, 216, 222]. The prevalence of atherosclerosis in adult renal recipients is comparable to that of end-stage renal disease (ESRD) patients during the pre-dialysis phase [218]. Further, recent practice guidelines for the treatment of dyslipidemia after RTx reported the 10-year cumulative risk of coronary heart disease to be at least 20 % in adult renal recipients [228]. Thus, adult renal recipients are at a risk of subsequent cardiovascular disease similar to that in patients with previous cardiovascular disease. In addition to atherosclerotic vascular disease, chronic rejection is another major cause of long-term solid organ recipient and graft losses [422, 446]. In grafted heart, both features of atherosclerosis and in particular, features of chronic allograft vasculopathy or chronic rejection (i.e. changes in the vascular wall due to immune response between the recipient and the graft) are seen. Accordingly, coronary occlusive disease is the major cause of death and late graft loss after HTx.[323]

In general population, a considerable amount of data supports the causative role of circulating lipids, especially high low-density lipoprotein cholesterol (LDL-C), but also high total cholesterol (TC), high triglyceride, and low high-density cholesterol (HDL-C) concentration (i.e. dyslipidemia) in the progression of atherosclerosis [272, 433]. Other major risk factors of cardiovascular disease are

family history, male sex, age, obesity, smoking, sedentary lifestyle and high blood pressure [57, 108]. Atherosclerosis manifests as a symptomatic disease in adulthood, but dyslipidemia as a risk factor of atherosclerosis and incipient atherosclerotic vascular lesions is seen already in youth [306, 307, 463].

The reported prevalence of dyslipidemia in adult solid organ recipients (on various combinations of immunosuppressive agents) varies widely after RTx, from 16 to 70 % [291]. From 16 % to 50 % of liver recipients have had hypercholesterolemia post-LTx and approximately 40 % of them have had hypertriglyceridemia [324]. Serum LDL-C and triglyceride levels have remained high at 6 months after HTx in 64 % and 41 % of heart recipients respectively, although the patients received postoperative dietary counseling [47]. As in adults, dyslipidemia seems to complicate pediatric solid organ Tx [96, 300, 421]. Also, the risk factors of atherosclerosis contribute to increased cardiovascular morbidity and mortality in solid organ recipients as in general population [194, 222, 451].

The underlying causes of post-Tx dyslipidemia are e.g. influence of pre-Tx diseases and lipid levels, weight gain and obesity, age, gender, the presence of diabetes, impaired renal function, urinary protein excretion, unfavorable diet, and the use of cyclosporine A (CsA), glucocorticoids, and antihypertensives (beta-blockers and diuretics) [25, 60, 242].

The purpose of this study was to examine the prevalence of dyslipidemia in pediatric renal, liver and heart recipients with comparison to healthy controls of similar age and sex distribution. An additional aim was to assess the prevalence of features of metabolic syndrome. Further, associations between possible risk factors of dyslipidemia and serum lipid and insulin levels were studied.

2. REVIEW OF THE LITERATURE

2.1. Lipoprotein metabolism

2.1.1. Structure of lipids and lipoproteins

Lipids are organic solvent soluble carbon compounds and include fatty acids, triglyceride, and cholesterol. Fatty acids consist of a carboxyl group and a hydrocarbon tail. They are called saturated if there are no double bonds between carbon atoms and mono- or polyunsaturated if carbon-to-carbon double bonds exist. A triglyceride molecule consists of three mainly saturated fatty acid chains esterified to glycerol. A cholesterol molecule consists of a sterol nucleus with one hydroxyl group and a double bond, and side chain of eight carbon atoms attached to the nucleus. Triglycerides and fatty acids supply cells with a source of energy. Cholesterol is important in cell membrane and plasma lipoprotein structure and a precursor of all other steroids in the human body. Serum triglyceride and cholesterol are mainly derived from two sources: they are synthesized in eukaryotic human cells, especially in the hepatocytes, and ingested of from food and bile in the small intestine.[68]

In circulation, lipids are transported in lipoproteins, which consist of a phospholipid core, cholesterol, triglyceride and apolipoproteins characteristic of each lipoprotein. Lipoproteins are classified according to increasing density and decreasing size to chylomicrons and chylomicron remnants derived from intestinal lipids (exogenous lipid pathway), and to very low density (VLDL), intermediate density (IDL), low-density (LDL), and high-density (HDL) lipoproteins of hepatic origin (endogenous lipid pathway).[68, 69] One apolipoprotein B (ApoB-100) of hepatocyte origin is the major protein constituent of VLDL and LDL [401]. Apolipoprotein A-I (ApoA-I) is the major protein constituent of HDL [400]. Lipoproteins also carry other apolipoproteins like apolipoprotein E (ApoE) in very low-density lipoproteins and their degradation products [104]. Apolipoproteins form the structure of lipoproteins, are enzyme co-factors [like apolipoproteins A-I and C-II for lecithin-cholesterol acyl transferase (LCAT)] or inhibitors [like apolipoproteins A-II and C-III for lipoprotein lipase (LPL)], and ligands for receptor mediated uptake of lipoproteins [68]. Genetic apolipoprotein polymorphisms influence the function of the apolipoproteins. As an example, ApoE is coded in the three codominant alleles ε2, ε3 and ε4 resulting in the respective isoforms (E2, E3, E4) and six different phenotypes. The isoform E4 has been related to enhanced cholesterol absorption resulting in elevated serum TC and LDL-C concentrations and smaller LDL particle size

while the apoE2 isoform is associated with lipoprotein remnant metabolism and is more frequent in patients with hypertriglyceridemia and with hypertriglyceridemia and hypercholesterolemia. [176, 237, 285, 466, 467]

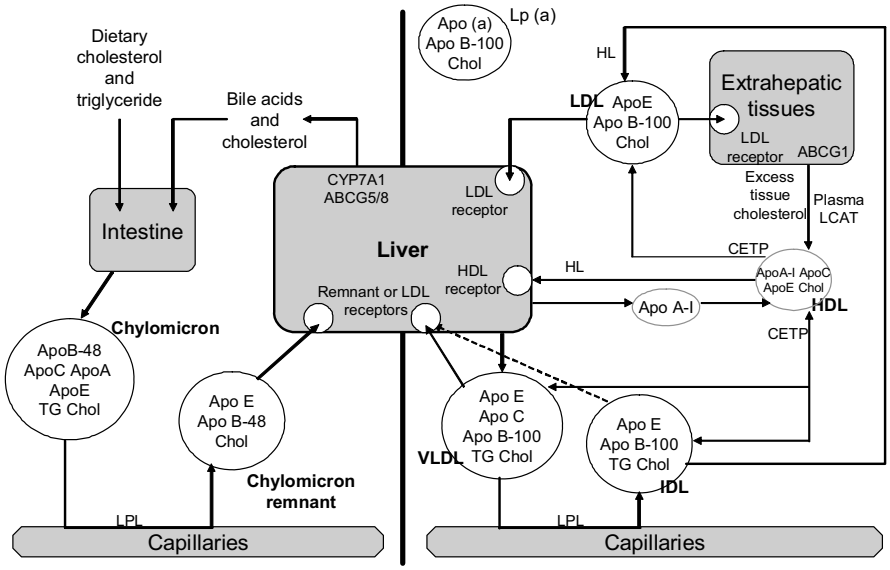
2.1.2. Exogenous lipid pathway

Adults in most Western cultures consume daily approximately 140 g of dietary lipids, of which lipid approximately > 90 % is triglyceride and 0.5 g cholesterol [59]. Children consume dietary lipid relative to their size, e.g. children aged 6 to 11 approximately 50 % of adult amount of dietary lipid [244].

Dietary lipids in the intestine rise exogenous lipid pathway (Fig. 1). Absorption of intestinal lipids varies from very efficient to low or absent absorption (coefficient of absorption, triglycerides: > 90 %; cholesterol: 25 %–75 %; plant sterols, sitosterol: 4 %–8 %, campesterol: 9 %–18 %, sitostanol: 0%) [59, 171, 313, 345, 440, 458]. As plant sterols are only received from food, their concentrations adjusted to concentrations of plasma cholesterol approximate the absorption efficiency of cholesterol [236, 237, 346]. The variable absorption of cholesterol is due to the cholesterol content of the meal, genetic differences in absorption efficiency and differences in serum cholesterol concentrations [175].

After absorption, enterocytes secrete the lipids in the form of chylomicrons into lymphatic vessels and further into circulation. LPL is localized on the endothelial cells of muscles and adipose tissue and catalyzes degradation of circulating chylomicrons into chylomicron remnants [241]. Hepatocytes take chylomicron remnants from circulation through the action of liver receptor related protein (LRP, remnant receptor) [68].

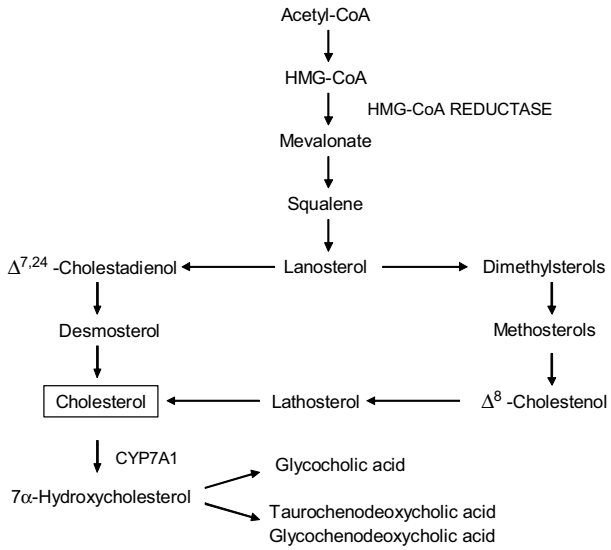
Figure 1. Exogenous and endogenous lipid pathway (modified from Walldius and Jungner, 2004 [469])



2.1.3. Endogenous lipid pathway

The endogenous lipid pathway originates from the liver. Liver is the main site of cholesterol synthesis with the conversion of acetyl coenzyme through synthetic cascade to cholesterol (Fig. 2). Hepatic cholesterol derives from circulating lipoproteins or newly synthesized cholesterol. Serum concentrations of cholesterol precursors (Δ^8-cholestenol, lathosterol, desmosterol) relative to serum concentration of cholesterol reflect the activity of the synthetic cascade of cholesterol.[61, 67, 346]

Figure 2. Pathway of cholesterol synthesis [67]



Hepatocytes secrete 70–80 % of intracellular cholesterol as lipoproteins to the blood stream. The rest of the cholesterol is excreted into bile. The hepatic production rate of VLDL, the major carrier of triglyceride in plasma, is controlled by the availability of fatty acids, synthesis and degradation of ApoB-100, and hormones (insulin, steroids, and thyroxin).[68, 458] VLDL releases triglyceride for cellular use through the action of LPL. The VLDL particles further transform to IDL and LDL after releasing triglyceride and core proteins for cellular use and HDL [257]. The majority of VLDL degradates to LDL with concurrent increase in the clearance of LDL [390]. Accordingly, an increase in serum triglyceride concentrations does not usually increase serum LDL-C concentrations markedly [235]. Concentration of LDL in plasma is regulated through the availability of cholesterol and other lipids, the efficiency of enterohepatic circulation, hydrolysis and transfer of circulating lipids and clearance mediated by LDL receptor [79, 314].

In so-called reverse cholesterol transport, HDL particles transfer cholesterol from peripheral cells back to circulation and into the liver [377]. Discoid HDL of hepatocyte origin evolves into larger atheroprotective HDL and collects cholesterol through the action of LCAT [68]. Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) further control the composition of lipoproteins and reverse cholesterol transport. CETP mediates the exchange of triglycerides of VLDL, IDL and LDL for cholesteryl esters of HDL. Similarly, PLTP mediates exchange of phospholipid for cholesteryl esters between VLDL, IDL, LDL and HDL.[67, 68]

The size of lipoproteins and abundance of lipids influences their catabolic rates. Larger sized VLDL and LDL particles are removed from the circulation but smaller sized VLDL and LDL have a prolonged residence time [379, 412]. Increased plasma triglyceride concentration is associated with diminished lipolysis of VLDL and LDL and increased construction of small-dense LDL particles [351]. Hepatocyte synthesis and availability of apoproteins and catabolic rate of HDL determine HDL concentration [366]. HDL particles abundant in triglyceride have a high catabolic rate [68].

Lipoprotein (a) [Lp (a)] is a small species of lipoproteins consisting of LDL and an apolipoprotein a which is attached to LDL by a disulfide bond. The size of the Lp (a) isoform is mainly genetically determined and further determines its synthetic and clearance rates [246].

2.1.4. Lipids, growth and maturation

Serum lipid levels change with childhood growth and maturation. Cholesterol concentrations are low at birth but until the age of one year increase to an average concentration of TC of 3.9 mmol/l, LDL-C of 2.6 mmol/l, and HDL-C of 1.4 mmol/l. From 1 to 12 years the serum cholesterol levels remain fairly constant, and TC and LDL-C levels are in general slightly lower in pre-pubertal boys than girls. During puberty there is a decrease in TC, LDL-C and HDL-C levels in both boys and girls. The decrease in serum HDL-C levels is more pronounced in boys and thereafter boys continue to have slightly lower HDL-C levels than girls. Also, boys have somewhat lower mean triglyceride concentrations than the girls until the age of 12, but after that their values are reversed. After puberty, TC, LDL-C and TG increase to adult levels.[149, 330, 481] In Finnish children and adolescents, average serum TC, LDL-C, HDL-C and triglyceride levels have been 5.1 mmol/l, 3.3 mmol/l, 1.5 mmol/l and 0.8 mmol/l, respectively [481].

2.2. Insulin, growth and maturation

Insulin is an anabolic polypeptide hormone that is synthesized in the endoplasmic reticulum of pancreatic beta cells. Pancreatic secretion of insulin parallels blood glucose concentration. Insulin is essential for the utilization of glucose and fatty acids by peripheral tissues and the liver. It facilitates hepatic glycogen and fatty acid synthesis and inhibits glucose output.[55] In adipose tissue, insulin stimulates the synthesis and activity of LPL. Hepatic lipase activity is positively associated with insulin concentrations [68].

Fasting insulin concentrations are lowest before puberty. They increase during puberty and after puberty decrease to near pre-pubertal levels [386]. As with insulin concentrations, pubertal children have more resistance to the action of insulin than children before or after puberty [17, 120].

2.3. Altered lipoprotein and insulin metabolism

2.3.1. Definition of dyslipidemia

Dyslipidemia refers to either increased (for example high LDL-C or triglyceride levels) or decreased (for example HDL-C levels) serum lipid concentrations and/or to alterations in the composition of lipoproteins. The term "dyslipidemia" implies an idea that the above alterations are harmful. Most often levels of dyslipidemia have been derived from fasting lipid profiles.

Cut-off concentrations of serum lipids for dyslipidemia vary. A recent guideline, the European Third Task Force of European Cardiovascular Disease Prevention in Clinical Practice, set the limits of dyslipidemia at TC > 5.0 mmol/l, LDL-C to > 3.0 mmol/l, HDL-C to < 1.0 mmol/l, and triglyceride to > 1.7 mmol/l [108]. These cholesterol concentration limits have also been approved in the Finnish Current Care Guidelines (The Working group of Finnish Medical Society Duodecim 2004), though in the Finnish Guidelines the upper limit of normal triglyceride was slightly higher (2.0 mmol/l) [454]. In the third report of National Cholesterol Education Program (NCEP III), a TC level >200 mg/dl (5.2 mmol/l) and a LDL-C level > 130 mg/dl (3.4 mmol/l) were considered elevated [457]. After solid organ Tx, cut-off levels of dyslipidemia have sometimes been higher than in the guidelines above, like a TC level higher than 6.4 mmol/l [192]. In recent reports on pediatric heart recipients, low limits of NCEP III (coronary heart disease equivalent) or other limits of similar magnitude have been used [421].

2.3.2. *Insulin resistance and definition of metabolic syndrome*

The term insulin resistance describes a situation of increased need for pancreatic insulin secretion in order to obtain adequate glucose disposal in the tissues. In hyperinsulinemia, changes in lipoprotein metabolism are seen. Hepatic VLDL synthesis is enhanced. The action of LPL exhibits resistance to the action of insulin. The action of hepatic lipase is high. [68] As a result, increased triglyceride and decreased HDL-C levels, and small-dense LDL particles emerge into circulation [198, 371, 391].

A precise estimate of the individual sensitivity to insulin action is obtained by the euglycemic insulin clamp technique [113]. This method is complex and not suitable for clinical use or epidemiological studies. One simple estimate for insulin resistance is the homeostasis model assessment index: HOMA = [fasting glucose (mmol/l) x fasting insulin (mU/l)] / 22.5 [298]. HOMA is based on the assumption that normal adults have a HOMA of 1. HOMA has been a valid estimate of insulin resistance in general population and in adult renal and liver recipients [65, 347, 357].

Hyperinsulinemia and insulin resistance coincide with high serum triglyceride and/or increased ApoB levels and small-sized LDL particles [308, 351, 383]. These metabolic abnormalities have been named an atherogenic metabolic triad. This atherogenic metabolic triad clusters with a low HDL-C concentration, abdominal obesity, a high fasting plasma glucose concentration or impaired glucose tolerance, increased blood pressure, and microalbuminuria (see Appendix 1) [12, 44, 382]. The cluster of metabolic alterations is associated with increased risk of atherosclerosis and accordingly has been named metabolic syndrome [178, 382]. Many researchers accept insulin resistance as an underlying cause of other metabolic features of the syndrome [19], but others emphasize the accumulation of visceral fat [12, 131, 457]. In adult renal recipients, obesity, central distribution of body fat, and presence of glucocorticoid therapy have been the main determinants of insulin resistance [347].

The research on the metabolic syndrome has mostly focused on adults. Thus, the definitions of metabolic syndrome are not directly applicable to children. However, childhood obesity (weight $> 95^{th}$ percentile of the reference population) is considered an important risk factor for metabolic alterations and the future risk of atherosclerosis, although children predisposed to gain weight as adults may have even larger risk of future atherosclerosis [95, 365, 436].

2.3.3. *Dyslipidemia and cardiovascular disease*

It is assumed that serum LDL-C level > 2.0 mmol/l is needed for the progression of atherosclerosis [332]. Further, a small LDL diameter, generally considered a diameter of LDL particle below 25.5 nm, and easy oxidizability of LDL seem to reinforce its atherogenicity [37, 267]. Small-dense LDL particles may be more easily internalized into the sub-intimal space of the vascular wall and they also have longer residence time in circulation than larger particles [92, 350, 438]. Further, small-

dense LDL particles are prone to oxidation, which may promote atherosclerosis via increased affinity of oxidized LDL to macrophage scavenger receptors [45, 137, 180]. The lag time of oxidative modification of LDL in vitro reflects the total antioxidant status of LDL and has been used as an estimate of LDL oxidizability [137].

In addition to increased serum LDL-C level, increased serum triglyceride and decreased serum HDL-C concentrations contribute to increased risk of atherosclerosis [30, 88, 167, 220, 266, 272, 288]. An increased serum triglyceride level is not a strong independent contributor of atherosclerosis, but together with other features of metabolic syndrome it is a significant risk factor [184, 268]. High concentrations of lipoprotein (a) [Lp (a)] (> 75 nmol/l i.e. ~ 225 IU/l in white adults, values higher than the 75th percentile of the reference population, although with some debate) are associated with a mild increase in cardiovascular risk in general population but this has not been observed after Tx [21, 159, 397].

2.3.4. Life-style risk factors for dyslipidemia

2.3.4.1. Obesity and distribution of body fat

Obesity is an excess of body fat that frequently results in significant impairment of health [148]. BMI is used for estimating the severity of obesity. An increased BMI correlates with an increased risk for cardiovascular disease in adolescent and young adult men [305]. BMI values reveal childhood obesity only after adjustment to a valid reference population as they change substantially with childhood growth [101, 481]. At the age 1, the median of BMI is approximately 17 kg/m^2. It decreases to 15.5 kg/m^2 at the age 6. Then, it increases to 21 kg/m^2 by age of 20. Major causes of obesity are excess caloric intake and inadequate physical activity [148].

In general adult and pediatric population, an increased BMI, and especially the amount of visceral fat, is associated with hypertriglyceridemia, increased production of VLDL particles, low HDL-C levels, hyperinsulinemia and insulin resistance [120, 150, 270, 418, 453]. After Tx, use of glucocorticoids and impaired action of GH in deteriorating renal function cause additional risk for excess weight gain and accumulation of visceral fat [62].

2.3.4.2. Diet

Quality of diet influences serum lipid levels in many ways. A large amount of ingested total calories not only predisposes to obesity but also directly increases serum triglyceride levels [106]. High intake of total calories is often associated with high intake of saturated fat and cholesterol, which may raise serum LDL-C levels in adults and children and be associated with an unfavorable LDL-C/HDL-C [18,

[170, 250, 330, 457]. Dietary Guidelines for Americans states that for each isocaloric 1 percent increase in the intake of saturated fats (E %) replacing the intake of carbohydrates, serum LDL-C concentrations increase by 0.03 to 0.05 mmol/l [99, 185, 309]. Accordingly, substituting dietary saturated fat with polyunsaturated fat decreases serum LDL-C and HDL-C concentrations and may also improve insulin sensitivity [76, 170, 201]. Despite the decrease in serum HDL-C concentrations, reverse cholesterol transport has still been efficient enough [128].

Serum triglyceride levels increase and serum HDL-C levels decrease when carbohydrate replaces fat as a source of energy [106, 170]. Within ingested carbohydrates, greater intake of dietary soluble fiber (e.g., beta-glucans, pectin, and guar) decreases serum LDL-C concentrations and improves insulin sensitivity [135]. In addition to energy yielding nutrients, higher intake of calcium (from dairy products) has been associated with better insulin sensitivity and attenuation of postprandial lipemia [283, 284].

As a mechanism between diet and dyslipidemia, excess dietary fat, saturated fat and cholesterol provide abundance of lipids for the synthesis of lipoproteins and also decrease the clearance of LDL via the LDL receptor pathway [106, 170]. Dietary cholesterol promotes ApoA-I gene expression, saturated fat counteracts ApoA-I and HDL clearance, while polyunsaturated fat decreases hepatic ApoA-I production [76, 428]. Nevertheless, a diet rich in cholesterol increases the amount of hepatic cholesterol 7 alphahydroxylase [53]. Dietary cholesterol abundance results in the accumulation of cholesterol in circulation and tissues despite changes in cholesterol metabolism in the opposite direction [170]. Excess energy and low fiber intake (especially soluble fiber) and high ability of ingested carbohydrates to increase blood glucose (glycemic index) seem to be associated with the development of insulin resistance [106].

2.3.4.3. Other risk factors: sedentary lifestyle and smoking

Healthy active lifestyle consists of adequate amount of physical exercise, i.e. at least a half an hour of physical activity on most of the days at 60-75% of the average maximum heart rate. On the other hand, sedentary life-style is associated with low HDL-C levels and an unfavorable ratio of HDL-C to LDL-C. [108]

Smoking is an important risk factor for cardiovascular disease [108]. As an influence on serum lipids, it may increase LDL-C and triglyceride levels and decrease HDL-C levels [105]. Smoking may also decrease serum LCAT activity and promote the accumulation of cholesterol in peripheral tissues [124].

2.4. Solid organ transplantation

The first successful RTx was done between identical twins in 1954 [109, 174]. Since then, worldwide more than 50.000 Tx have been performed every year. Approximately 3.000 of these are performed on pediatric patients [The

Collaborative Transplant Study, European Liver Transplant Registry, Organ Procurement and Transplantation Network (OPTN)]. [102, 138, 337] In 2006, a total of 293 transplantations were done in Finland. Of these 26 were done on pediatric recipients. [197]

2.4.1. Pre-transplant diseases

In Finland, pediatric patients with ESRD are on dialysis before RTx. Most often peritoneal dialysis is used. If massive proteinuria complicates ESRD, nephrectomy is performed prior to RTx. Approximately one third of the Finnish pediatric patients receive a living related donor graft and the rest a cadaver graft. Finnish children's most common cause of ESRD and need for RTx is congenital nephrotic syndrome. The majority of congenital nephrotic syndrome is due to NPHS1 gene defect. Other causes behind the need for RTx are, for example, urethral valve, polycystic kidney disease, prune-belly syndrome, neuroblastoma and congenital nephritis.[375]

Pediatric LTx is most often done due to either acute or chronic liver disease with progression to liver failure [16]. In Finland, children with childhood hepatic cancer without extrahepatic malignant disease are also accepted for LTx after chemotherapy [264]. Common causes of liver failure are cholestatic diseases (e.g., extrahepatic biliary atresia), metabolic diseases (e.g. tyrosinemia) or acute hepatic failure [139, 232]. An uncommon cause of LTx has been homozygous familial hypercholesterolemia.

Common causes of pediatric end-stage heart disease and a need for HTx are various congenital heart defects and restrictive or dilative cardiomyopathies. Cardiomyopathy emerges from multiple etiologies (myocarditis, chemotherapy, chronic arrhythmia, structural or metabolic abnormalities).[71]

2.4.2. Long-term pharmacological therapy after transplantation

In Finland, immunosuppressive therapy mainly consists of azathioprine (AZA), methylprednisolone (MP) and CsA (triple therapy), in addition to anti-thymocyte globulin during the first weeks after HTx. In selected patients, CsA may be replaced with tacrolimus. AZA has gradually given way to mycophenolate mofetil.[261, 262, 263] In addition, some other regimen may be used according to the need of the recipients, such as antihypertensives, antiepileptics or RhGH.

2.4.2.1. Azathioprine and mycophenolate mofetil

AZA and mycophenolate mofetil are anti-proliferative agents and act through inhibiting purine synthesis, which is necessary for the proliferation of cells, especially of leukocytes and lymphocytes. Mycophenolate mofetil has replaced AZA as a more selective and potent inhibitor of lymphocyte proliferation. Either

AZA or mycophenolate mofetil do not seem to disturb lipid metabolism.[182, 231, 296, 302, 315]

2.4.2.2. Glucocorticoids

Synthetic glucocorticoids are derived from cortisol (steroid hormone). All glucocorticoids act in nuclei after binding to glucocorticoid receptors in cytoplasm [97]. In addition to immunosuppressive properties, glucocorticoids have numerous other actions. As their effects on glucose and lipoprotein metabolism, they stimulate gluconeogenesis (particularly in the liver) and lipolysis and inhibit glucose uptake in muscle and adipose tissues [38, 75, 133, 214, 276, 296, 393]. They inhibit insulin secretion in the short term but in the long-term predispose to hyperinsulinemia and decreased insulin sensitivity [132, 269, 276]. Individuals have variable sensitivity to the effects of glucocorticoids and the duration of action varies between the individuals [472]. Within an individual, glucocorticoids impact on glucose metabolism for a shorter time than on the immune system [38]. A clinical implication of this variability is alternate day dosing [90, 154, 302].

2.4.2.3. Cyclosporine A and tacrolimus

CsA is a lipophilic polypeptide and an inhibitor of lymphocyte proliferation (calcineurin inhibitor). CsA was introduced in the late 1970's [83]. Since 1993 an advanced microemulsion formulation of the drug has been available. This formulation of CsA is absorbed more consistently and more independently of bile production than the former [248]. Young children have faster metabolism of CsA and may need more frequent dosing [490]. Tacrolimus is a macrolide lactone antibiotic and a calcineurin inhibitor similar to CsA. It is used as an alternative of CsA [415].

2.4.2.4. Growth hormone

Growth hormone (GH) is an anabolic polypeptide which is synthesized in the anterior pituitary gland. It acts through GH binding receptor on the surface of the cells. After Tx, in case of significantly delayed growth and possible GH resistance, which may be seen especially after RTx, recombinant human growth hormone (RhGH) is given to aid the child to grow as close as possible to his target height.[146] In addition to stimulating growth, RhGH stimulates lipolysis, protein synthesis, opposes insulin action, reduces hepatic uptake of glucose and glucose synthesis, and thus increases serum glucose (after the first hour of administration of RhGH) and triglyceride concentrations. Long-term RhGH therapy is associated with elevated serum insulin levels, which return to normal after cessation of the therapy. [72, 388] In adults and children with growth hormone deficiency, serum lipid profiles improve after initiation of RhGH but serum

Lp (a) levels increase [136, 271, 277, 444]. Long-term RhGH therapy favors flux of fatty acids to muscle instead of adipose tissue and induces LDL clearance [151, 277].

2.4.2.5. Antihypertensives

Approximately 50 % to 80 % of adult renal and heart recipients have hypertension and need antihypertensive therapy [203, 413]. Calcium channel blockers have been considered especially suitable agents for post-RTx hypertension as they may also protect renal graft against CsA induced graft dysfunction [239]. Other agents (angiotensin converting enzyme inhibitors, beta blockers, diuretics) may also be used depending on the pathophysiology of hypertension [302].

2.4.3. Outcome and long-term complications

The short-term prognosis of pediatric solid organ Tx is excellent with over 90 % patient and graft survival, but there is still room for improvement of the long-term prognosis [303, 328, 376]. For living related and cadaver donor pediatric RTx (1-17 years), actuarial 5-year graft survival has been 85 % and 75 % respectively, and 5-year patient survival has been approximately 95 % and 93 %, respectively [338, 339, 340, 341]. Five-year pediatric patient survival has been 80-85 % for LTx and 75 % for HTx [102, 140, 342, 343]. In Finnish pediatric patients, the corresponding figures are of a similar magnitude, or possibly even slightly better [375].

In adults, atherosclerotic cardiovascular disease is the most common cause of death with functioning graft after RTx [226, 228, 460]. The incidence of cardiovascular disease in adult renal recipients is approximately 3- to 4-fold to that of the general population [1, 3, 216, 228]. Also, after HTx cardiovascular complications remain the major cause of death and graft loss [156, 191, 215]. In long-term survivors of pediatric transplantation (> 10 years), cardiopulmonary disease is a major cause of death in pediatric renal recipients (in 17 % of the patients) and coronary artery vasculopathy in pediatric heart recipients (in 31 % of the patients) [70, 328]. Other major causes for long-term patient and graft losses are late acute and chronic rejection, infections, late surgical complications and malignancies [232, 320, 328]. Main risk factors for post-Tx cardiovascular disease are old age, hypertension, diabetes, smoking, dyslipidemia and deteriorating renal function [50, 228, 387].

2.4.3.1. Atherosclerosis and graft vasculopathy

Both atherosclerotic cardiovascular disease and chronic rejection affect the blood vessel walls, the latter mainly within the graft. Atherosclerotic lesions are typically eccentric with focal intimal thickenings. These lesions of fat accumulation develop over decades after an initial vascular wall injury in childhood and adolescence [57, 134, 326]. After emerging, the first lesions, reversible

fatty streaks, progress into more raised lesions through infiltration of foam cells (monocytes that in situ develop to macrophages filled with fatty droplets) on the intima layer if the risk factors like abundant LDL-C are present [332, 360].

Vascular lesions of chronic rejection are intima-medial, concentric, diffuse, and habitually distal. They are seen in the vasculature of the transplanted organ and are called graft vasculopathy. They may appear within weeks after transplantation.[491] These two categories of vascular changes have similarities: a high LDL-C and triglyceride level, a low HDL-C level, and disturbances in glucose and insulin metabolism increase the risk of atherosclerosis [2, 8, 116, 194, 222, 226, 227, 336, 385, 387] and may increase the risk of graft vasculopathy in adult renal and heart recipients [112, 156, 209, 215, 221, 293, 362, 468, 486]. However, immunological risk factors are more important in the progression of transplant vasculopathy than the metabolic risk actors above [66, 73, 304, 335]. Thus far, the definite role of lipids in transplant vasculopathy has not been ascertained [227, 302].

2.5. Dyslipidemia in end-stage disease and after transplantation

2.5.1. Kidneys and lipid metabolism

The role of the kidneys in lipid metabolism is not yet thoroughly known, although dyslipidemia accompanies urinary protein excretion and declining renal function. Some lipid regulating mechanisms may take place in kidneys. As suggested mechanisms, two members of the liver receptor binding protein family, megalin and cubilin, are expressed in the proximal tubule. Cubilin binds macromolecules like albumin and ApoA-I and thus might enhance reabsorption of ApoA-I, and endocytosis of HDL in concert with megalin.[181, 317, 318, 493]

2.5.1.1. Proteinuria and nephrosis

Nephrotic syndrome is characterized by massive loss of intermediate molecular weight proteins into urine. By contrast, circulating lipoprotein levels [VLDL, LDL, Lp (a)] increase and a pronounced increase in serum triglyceride and LDL-C concentrations is seen. Serum HDL-C concentration is reduced or unchanged. [20, 252, 439, 464, 477, 478] The magnitude of the changes in lipid concentrations is relative to the degree of protein losses in urine. In patients with various degrees of chronic kidney disease and proteinuria, serum LDL-C and triglyceride levels have increased after protein excretion has exceeded 0.3 g/d, but serum HDL-C concentration has decreased already with protein excretion of 0.06-0.3g/d compared to protein excretion less than 0.06g/d [395]. In peritoneal dialysis, protein losses to dialysate mimic urinary protein excretion and corresponding influence on serum lipid profiles is seen [35]. Proteinuria and

nephrotic episodes after RTx increase triglyceride-rich lipoprotein levels in serum and accordingly serum triglyceride and TC levels similarly to primary nephrotic disorder [63]. However, the association of post-Tx lipids levels with the magnitude of proteinuria has not been so obvious [485].

Nephrotic range proteinuria is associated with a relative elevation of hepatic HMG-CoA reductase activity with a relative reduction of cholesterol 7-hydroxylase activity, LDL receptor deficiency, a decrease in LRP (remnant receptor) expression, urinary losses of LCAT and concomitant deficiency of LCAT, deficient LPL and hepatic lipase activities, and an increase in CETP activity [91, 153, 164, 230, 240, 292, 321, 411, 478, 482, 489]. As a result, triglyceride accumulates in HDL particles, which enhances their removal from the circulation. In addition, HDL concentration decreases due to urinary wastage of lipid poor HDL/ApoA-I [411]. Consequently, the clearance of triglyceride-rich lipoproteins decrease, and cholesterol accumulates in circulation [110].

2.5.1.2. Declining renal function and end-stage renal disease

In ESRD, hypertriglyceridemia is seen in approximately 30 %–75 % of patients often with low serum HDL-C levels [32, 34, 41, 48, 89, 172, 205, 361]. Serum LDL-C levels are normal or somewhat elevated and a greater amount of small-sized LDL particles is seen [32, 35, 89, 115, 172, 205]. Serum Lp (a) levels increase relative to reduction in renal function [63, 246, 403, 404]. These changes are seen after a decrease in glomerular filtration rate (GFR) below 52 to 60 ml/min/1.73 m^2 [58, 394]. A further reduction in GFR is associated with a more pronounced increase in serum triglyceride concentration [32].

Altered lipid levels in ESRD seem to be a consequence of a decrease in the hepatic synthesis of apolipoproteins A-I and A-II, a reduction in serum apolipoprotein C-II/C-III, and decrease in activities of LCAT, LPL and hepatic lipase, and an increase in serum CETP activity. As a result, triglycerides instead of cholesterol esters accumulate in HDL particles, catabolism of triglyceride-rich HDL increases, but the clearance of other triglyceride-rich lipoproteins decreases. [27, 28, 33, 477] Also, LDL clearance rates have been decreased in ESRD patients on dialysis treatment, possibly due to deficient LDL receptor binding [196].

2.5.1.3. Dyslipidemia after renal transplantation

Both adult and pediatric RTx is associated with increases in serum TC, LDL-C, and HDL-C levels and a decrease in serum triglyceride levels compared to pre-Tx levels [26, 48, 125, 210, 419, 420, 476]. In adult renal recipients, serum TC levels are typically around 6-7 mmol/l and serum triglyceride levels approximately 2 mmol/l [26, 48, 86, 125, 210, 476]. Though, the prevalence of severe post-Tx dyslipidemia seems to have diminished parallel to evolving post-Tx care. Dyslipidemia appears 3 to 6 months after RTx, persists or improves slightly in further follow-up in adults and children [86, 94, 316, 476]. Low HDL-C concentrations are rare after

RTx and accumulation of larger sized HDL particles has been reported [217]. Lp (a) concentrations have been increased or comparable to the reference population [186, 208].

The reported prevalence of hypertriglyceridemia in adult renal recipients varies from 15 % to 46 % and that of hypercholesterolemia from 10 % to 70 % [48, 52, 90, 116, 187, 291, 409, 462, 476]. Small dense LDL has been seen in 26 % to 58 % of adult renal recipients [40, 160]. Of pediatric renal recipients, approximately 18 % to 63 % have had an elevated triglyceride concentration of > 1.5 mmol/l and 55 to 69 % have had an elevated LDL-C concentration of > 3.0 mmol/l (Table 1) [316, 373, 410, 416, 419, 471]. At 5 years after RTx, 14 % of pediatric renal recipients have had an elevated triglyceride (> 2.2 mmol/l) and 41 % of them an elevated TC (> 5.1 mmol/l) [94]. As an estimate of cholesterol synthesis, higher serum lathosterol to cholesterol ratios compared to controls have been seen in adult renal recipients [442].

Some studies suggest significant correlations between renal graft function (estimated with serum creatinine level, creatinine clearance or GFR) and plasma lipid and apolipoprotein concentrations [triglyceride, TC, HDL-C, ApoB, apolipoprotein (a)] [1, 223, 247, 485]. An increase in serum TC has been observed in renal recipients with a GFR below 54 ml/min/1.73 m^2 and a low HDL-C level with a GFR below 30 ml/min/1.73 m^2.[1] By contrast, other studies have not revealed any associations between estimates of renal function and dyslipidemia [26, 81, 210, 361].

2.5.2. Liver and lipid metabolism

Liver plays a crucial role in lipid metabolism. It facilitates the digestion and absorption of lipids through the production of bile. Fatty acid, cholesterol, triglyceride, and plasma lipoprotein synthesis is expressed in the liver. Hepatic LDL receptors provide the primary pathway for plasma cholesterol clearance. Features of glucose metabolism are related to lipid metabolism and the liver is also essential in glucose metabolism as uptake of glucose, glycogen synthesis and gluconeogenesis take place in the liver.[155]

In liver insufficiency, serum cholesterol and apolipoprotein concentrations, and amount of circulating lipoproteins decrease [331, 406, 480]. In cholestasis, children usually have hypercholesterolemia, but they may also have fat malabsorption and hypolipidemia [280, 300, 426].

2.5.2.1. Dyslipidemia after liver transplantation

With well-functioning liver graft, adult LTx with CsA based immunosuppression is associated with increases in TC, LDL-C, and triglyceride levels [93, 163, 206]. HDL-C levels have been increased, decreased or unchanged [295, 324]. Of adult liver recipients, 11 % to 70 % have had a high triglyceride level (most often > 1.7 mmol/l) and 7 % to 43 % an elevated serum TC level (> 5.0–6.4 mmol/l) [93, 143,

[163, 206, 295, 324]. Both an increased triglyceride and TC level have been reported in 12 % of patients [163]. Hyperlipidemia prior to LTx seems to predispose to hyperlipidemia after LTx [163]. Post-LTx hypertriglyceridemia often occurs by the first month and hypercholesterolemia by the sixth month, and alterations in serum lipid levels seem to persist (at least the first year) [163]. On the other hand, a considerable number of patients (34 % to 55 %) have had similar serum lipid values similar to those of the controls [163, 295].

After pediatric LTx, 41 % of children have had an elevated level of triglyceride (> 1.6 mmol/l), 57 % an elevated level of total cholesterol (> 4.4 mmol/l), and 19 % an elevated LDL-C level (approximately > 3.4 mmol/l) [300]. In more recent studies, pediatric liver recipients have had mild hypertriglyceridemia ~ 1.2 mmol/l, a relative decrease in the amount of mature HDL particles and hypocholesterolemia with average TC concentration of ~ 3.4 mmol/l [169, 202].

After LTx, hepatocyte failure, deficient bile acid production and/or bile duct function may occur and further impact on serum lipid levels [300]. As a liver graft expresses the genes of the donor, it may have a significant impact on serum lipid levels of a liver recipient [279].

2.5.3. Heart and lipid metabolism

The heart does not have a major role in lipid metabolism, though some preliminary data suggest that atrial natriuretic peptide promotes mobilization of lipids through activation of LPL and/or hormone-sensitive lipase [258]. Obvious decline in heart function is associated with hypolipidemia [140, 432].

2.5.3.1. Dyslipidemia after heart transplantation

In adult heart recipients on CsA based immunosuppression, increases in serum TC, LDL-C, HDL-C and often also triglyceride levels are seen [9, 54, 103, 140, 168, 234, 432]. Lp (a) concentrations decrease from pre- to post-HTx [140]. In part, the increase in serum lipid levels seems to be due to improved heart function and concomitant improvement of nutritional status [140, 168, 432]. However, by 10 years post-HTx, the majority (> 90 %) of surviving adult heart recipients have hyperlipidemia according to the International Society of Heart and Lung Transplantation registry [451]. In another group of adult long-term (> 10 years) HTx survivors on CsA or tacrolimus based immunosuppressive protocols, 40 % were introduced to lipid-lowering therapy due to significant dyslipidemia [413]. The prevalence of hypertriglyceridemia adult heart recipients on CsA and glucocorticoid-based immunosuppression has been 41 % and that of an elevated LDL-C 40 % to 64 % [47, 54, 117]. However, in some patient groups serum TC levels have also been similar prior to and after HTx [168, 432]. Of pediatric heart recipients on CsA-based immunosuppression, 50 %–90 % had an increased serum triglyceride or a decreased HDL-C level or both compared to the recommendations (NCEP III) [23, 96, 421]. However, recent studies have shown that the serum LDL-C level has been

similar between the pediatric heart recipients and the reference population, although, 25 % to 50 % of the pediatric heart recipients have had the LDL-C concentrations sub-optimal for the efficient prevention of atherosclerosis (> 2.8 mmol/l) [23, 96, 407, 421].

Hypercholesterolemia develops within the first 6-24 months after HTx [9, 254], but the severity of hypercholesterolemia tends to diminish after the first 6-12 months post-HTx [9]. Triglyceride levels have remained fairly constant after HTx [103, 168]. Potential loss of heart function in episodes of cardiac graft rejection has been associated with small but constant increase in serum triglyceride and cholesterol levels possibly due to rejection therapy and enhancement of immunosuppressive therapy [9].

2.6. Risk factors for dyslipidemia after transplantation

2.6.1. General

Hypothyroidism and diabetes cause secondary dyslipidemia in general population [280]. However, hypothyroidism is rare in pediatric recipient population [87]. Diabetes with typical lipid alterations may occur after pediatric solid organ Tx, but it does not affect the majority of pediatric recipients [162]. These risk factors are not discussed here.

The main risk factors for a high triglyceride level after RTx (and HTx) are obesity, the degree of impairment of renal function and glucocorticoid therapy [1, 9, 187, 190, 228, 234, 310, 452, 476].The main risk factors for a high LDL-C level impairment of renal function, presence of proteinuria, and therapy with CsA [11, 228, 282]. Major risk factors for low HDL-C levels are similar to those of high triglyceride levels, except the presence of glucocorticoid therapy (in more detail below and in Table 2).

2.6.2. Age and gender

Older recipients may be at higher risk of post-Tx dyslipidemia than younger ones, though this has not always been seen [23, 421]. In adult solid organ recipients, female gender has been a risk factor to high TC or triglyceride levels possibly due to women's greater sensitivity to glucocorticoids [2, 9, 60, 187, 281, 372]. Gender differences have not been reported in children [94].

Table 1. Average serum lipid concentrations and proportion of patients with elevated values in pediatric renal, liver, and heart recipients receiving CsA and corticosteroid based immunosuppression

Organ	Study	N	Age, years, mean (SD, range)	Years post-Tx, mean (SD, range)	Estimated percentage of patients with elevated values; mean (SD) concentrations (mmol/l)			
					TC > 5.0 mmol/l	LDL-C > 3.0 mmol/l	HDL-C < 1.0 mmol/l	TG > 1.5 mmol/l
RTx	Van Gool 1991	20	13.6 [e] (5.3–20)	NA	**66 %**; 5.62 (1.47)	**60 %**; 3.33 (1.29)	**14 %**; 1.42 (0.39)	**63 %**; 1.79 (0.87)
	Querfeld 1993 [a]	23	14.8 (5.9)	3.6 (3.3, 0.1–12.3)	**67 %**; 5.53 (1.22)	**73 %**; 3.67 (1.09)	**28 %**; 1.16 (0.28)	**62 %**; 1.74 (0.82)
	Sharma 1994	12	10.3 (4.9)	4.8 (1.7)	**74 %**; 5.52 (0.80)	**55 %**; 3.15 (1.12)	**5 %**; 1.84 (0.52)	**18 %**; 1.16 (0.37)
	Milliner 1994 [b]	54	14.4	1.0	**66 %**; 5.65 (1.61)	**65 %**; 3.35 (0.88)	**17 %**; 1.37 (0.39)	**54 %**; 1.60 (1.11)
	Singh 1996	18	14.8	> 1.0	**74 %**; 6.20 (1.84)	**80 %**; 4.19 (1.43)	**23 %**; 1.21 (0.29)	**63 %**; 1.99 (1.51)
	Singh 1998	29	14.5 (4-18)	1.0–1.25	**NA** ; 5.50	**NA** ; 3.61	**NA** ; 1.21	**NA** ; 1.47
	Silverstein 2000 [c]	62	15.4 (4.7, 3.0–22.8)	6.7 (3.1)	**61 %**; 5.30 (1.12)	**69 %**; 3.58 (1.14)	**16 %**; 1.41 (0.41)	**61 %**; 1.78 (1.00)
	Chavers 2003	29	13.2 (5.7, 5.8–22.8) est.	5.0	**57 %**; 5.21 (1.24)			**51 %**; 1.51 (0.68)
LTx	Hyams 1989	6	9.7 (4.2–17.5)	3.1 (1-7.2)	**0 %**; 4.04 (0.41)	**17 %**; 2.44 (0.7)	**100 %**; 0.75 (0.14)	**67 %**; 2.33 (1.49)
	McDiarmid 1992 [d]	102	6 (1-18) [e]	2.1 [e] (0.5–6.1)	**36 %**; 4.57 (1.16)			**64 %**; 1.78 (0.80)
	Granot 1996	10	7.5 (3-14)	3.9 (1.5-7)	**9 %**; 3.85 (0.86)	**16 %**; 2.27 (0.72)	**50 %**; 1.00 (0.23)	**38 %**; 1.30 (0.66)
HTx	Chin 2000	28	11.1 (3-21.6) [e]	3.7 [e] (0.1–12.1)	**19 %**; 4.22 (0.90) est.	**30 %**; 2.57 (0.82)	**39 %**; 1.11 (0.38)	**31 %**; 1.18 (0.63)
	Penson 2001 [f]	22	16.9 (10–21)	3.2	**43 %**; 4.67 (1.78)	**43 %**; 2.76 (1.34)	**32 %**; 1.21 (0.46)	**49 %**; 1.46 (1.25)
	Seipelt 2004	50	9 (6.3)	1.1 (0.4)	**50 %**; 5.00 (1.57)	**54 %**; 3.12 (1.11)	**31 %**; 1.19 (0.39)	**56 %**; 1.69 (1.28)
	Singh 2006 [g]	535	NA	1.0	**0 %**; 4.02 (0.08) est.	**0 %**; 2.37 (0.05) est.	**0 %**; 1.16 (0.03) est.	**0 %**; 1.24 (0.06) est.

ABBREVIATIONS: est. = estimated, HDL-C = serum high-density lipoprotein cholesterol, HTx = heart transplantation, LDL-C = serum low-density lipoprotein cholesterol, n = number of patients, NA= not available , LTx = liver transplantation, RTx = renal transplantation, SD = standard deviation, TC = serum total cholesterol, TG = serum total triglyceride. NOTES: The studies are characterized by the first author. All lipid values are displayed in mmol/l. To convert mmol/l to mg/dl, multiply cholesterol by 38.67 and for triglyceride by 87.5. [a]Cyclosporine A in 87 % (n = 20) of patients, [b]Cyclosporine A in 46 % (n = 32) of patients, [c]LDL-C determined for 49 children, [d]non-fasting lipid profile, [e]a median (range) displayed, [f]Cyclosporine A in 41 % (n = 9) and tacrolimus in 59 % (n = 13) of patients, [g]number of children is 535, 361, 396, 499 for TC. LDL-C, HDL-C and TG analyses, respectively.

2.6.3. *Genetic risk factors*

Data on genetic risk factors on post-Tx dyslipidemia is sparse. Inherited predisposition to familial combined hyperlipidemia (an elevated serum total cholesterol or triglyceride level, or both; a prevalence of 1 %–6 % in Western populations) [443], increases the renal recipients' tendency to have hyperlipidemia [5]. In adult renal recipients, higher average plasma TC, triglyceride, LDL-C, ApoB, HDL-2, and insulin concentrations, and an elevated HOMA index forinsulin resistance have been seen in apo E4 isoform carriers compared to carriers of other apoE polymorphisms [5, 219]. In adult renal recipients, ApoE polymorphisms were not associated with dyslipidemia or impaired glucose tolerance [385]. Apolipoprotein E2 isoform carriage has been associated with hypertriglyceridemia and apolipoprotein A-I promoter region polymorphisms with plasma triglyceride and LDL-C levels in adult heart recipients [165, 166].

2.6.4. *Pre-transplant diseases*

Pre-Tx kidney or liver diseases do not significantly influence post-transplant lipid values after the first post-Tx year, rather lipid profiles have unified after Tx [20, 142, 322, 380, 437]. Although, dyslipidemia during ESRD predisposes to altered lipid levels also after RTx [94, 187]. Secondary predisposition to dyslipidemia may be seen due to deteriorating renal function in structural or functional kidney abnormalities, like bladder failure in posterior urethral valves, or in systemic diseases [229, 260, 262, 392]. The recurrence of primary disease in approximately 5 % to 30 % of the renal and liver recipients produces similar changes in serum lipid levels as the original disease [126, 127, 329, 358]. In adult heart recipients, ischemic heart disease as a cause of end-stage heart disease and transplantation has been associated with increased prevalence of postoperative dyslipidemia [9, 273, 293].

Childhood cancer survivors with or without bone marrow transplantation have had elevated serum triglyceride levels and decreased serum HDL-C levels, obesity, hyperinsulinemia and glucose intolerance years after their diagnosis [319, 445, 447]. GH deficiency due cancer therapy agents may cause these metabolic alterations [107, 122, 334, 445], though this has not always been proved [447].

2.6.5. *Pharmacological therapy*

2.6.5.1. *Triple therapy*

Combined use of AZA, CsA and glucocorticoids is associated with elevated levels of triglyceride, LDL-C, and apoB [1, 54, 60, 125, 224, 234, 254, 361, 372, 378, 405, 449, 476]. Both CsA and glucocorticoids seem to have an independent impact on dyslipidemia [199]. However, the prevalence of dyslipidemia in adult renal

recipients or in young liver or heart recipients using low-dose triple therapy has not always exceeded the prevalence of dyslipidemia in the reference population [96, 169, 202, 211, 421]. In pediatric renal recipients, the use of CsA with prednisone has been associated with elevated serum LDL-C, TC and triglyceride levels, though an uninfluenced HDL-C/TC [419].

2.6.5.2. Glucocorticoids

Glucocorticoids favor accumulation of VLDL and IDL and respectively triglyceride in circulation, especially in high doses [223, 356, 372, 421, 449]. Glucocorticoid therapy decreases clearance of triglyceride containing lipoproteins via reduced action of LPL and plasma LCAT and induces hepatic synthesis of triglyceride via stimulation acetyl-CoA carboxylase and free fatty-acid synthethase [42, 213, 251, 315]. It increases plasma HDL-C concentration and ApoA-I messenger RNA levels, reduces plasma CETP activity and alters structure of HDL [276, 431, 447].

According to the mechanisms above, the use of glucocorticoids would be expected to be associated with elevated serum triglyceride levels. Indeed, lower serum triglyceride concentrations have been seen in the solid organ recipients with steroid-free medication than in those with steroids [473], but more often no significant decrease or even an increase in serum triglyceride levels has been noted [6, 63, 187, 195, 276, 297, 301, 352, 355, 381, 425, 427, 430, 474].

Many authors have suggested that the use of glucocorticoids increases serum LDL-C levels in adult and pediatric solid organ recipients more than other immunosuppressive regimens [23, 26, 54, 111, 200, 254, 419, 421, 449, 485]. An increase in serum HDL-C level often parallels the increase in serum LDL-C level [111, 125, 142, 224, 396, 449]. Therefore, in renal and liver recipients, steroid withdrawal results in lower levels of serum TC, LDL-C and HDL-C [6, 188, 199, 207, 297, 353, 370, 425, 430, 435, 473, 474], although the reduction in TC or LDL-C levels has not always been seen or sustained long-term [64, 195, 352, 424, 427]. A recent trial suggested an improvement of LDL/HDL-C after steroid withdrawal [474]. Moreover, lower ApoB levels have been seen after steroid withdrawal (or replacement with AZA) [199, 255, 276, 359].

The data on the dose dependent effects of glucocorticoids on lipid metabolism are contradictory. Actual and cumulative steroid doses have not correlated with triglyceride levels in renal or liver recipients [85, 476]. On the other hand, actual glucocorticoid doses have correlated positively with serum triglyceride or HDL-C levels in adult renal recipients, and with serum LDL-C levels in pediatric heart recipients [5, 23, 361, 372]. Also, cumulative steroid doses have correlated with serum LDL-C levels in adult heart recipients [54]. Glucocorticoid therapy is a major risk factor for metabolic syndrome after RTx [348]. Consequently, steroid withdrawal has decreased the number of patients having either insulin dependent or non-insulin dependent diabetes [195, 435].

2.6.5.3. Cyclosporine A and tacrolimus

Both CsA and tacrolimus may favor accumulation of LDL-C in circulation [39, 45, 46, 296]. The use of CsA may diminish hepatic LDL-receptor expression and inhibit bile acid synthesis, which has been seen in vitro and in pediatric liver recipients [11, 202, 488]. In rat, CsA also stimulates transcription of HMG-CoA reductase gene and hepatic secretion of VLDL triglyceride through stimulation of transcription of fatty acid synthase gene [488]. It may influence hepatic lipase activity, though the data is inconsistent [119, 441]. LPL activity did not seem to change markedly with the use of CsA [26, 119]. A decrease in reverse cholesterol transport due to cholesteryl ester saturation in HDL at the site of the graft has been seen in adult CsA-treated heart recipients [31].

The impact of CsA as contributing to post-Tx dyslipidemia has been derived from the comparison of patient groups on various immunosuppressive protocols, or of the comparison of the same patients with CsA and after conversion from CsA to tacrolimus or to other immunosuppressive regimen without assessing true bioavailability. In comparison of CsA to tacrolimus, tacrolimus may save the use of steroids which has been ignored in many reports [349]. In these clinical studies, use of CsA has increased serum LDL-C (or TC) levels and often also serum triglyceride levels but not decreased serum HDL-C levels after adult RTx, LTx or HTx [4, 43, 100, 117, 141, 157, 182, 192, 199, 224, 243, 256, 289, 290, 450, 479, 484]. Accordingly, higher ApoB levels and an unfavorable TC/HDL-C have been seen in patients treated with CsA compared to patients on AZA and prednisone or tacrolimus [9, 29, 43, 100, 256, 289, 290, 402, 479]. On the other hand, in some large studies on approximately 200 to 400 adult renal recipients, no independent contribution of CsA to dyslipidemia has been seen compared to AZA + glucocorticoid based therapies and HDL-C/TC has apparently been unaffected [1, 26, 211, 336, 389].

Some data suggests an association between CsA blood trough concentration (B-CsA) and dyslipidemia (an increased LDL-C, ApoB, possibly also triglyceride and decreased HDL-C) in renal graft recipients [7, 256, 483], but more often no dose-dependent influence of CsA has been reported [40, 43, 60, 100, 117, 141, 173, 192, 211, 274, 327, 455, 479]. Increased, uninfluenced, and decreased Lp (a) concentrations have been seen [40, 78, 192, 253]. LDL susceptibility to oxidation has been elevated and reported to decrease after a switch from CsA to tacrolimus in adult renal recipients [22, 29, 100, 479]. CsA Neoral formulation is fortified with dl-alpha tocopherol and therefore might provide protection against oxidation [475]. Moreover, pro-oxidant effect of CsA has not been seen in vitro [121].

Dyslipidemia has also been seen in pediatric solid organ recipients on CsA-based immunosuppressive therapy [123, 144, 145, 421]. This has in general been less frequent and less severe than in adult studies and the role of CsA on this dyslipidemia has been inconsistent. CsA as monotherapy after pediatric RTx or compared with tacrolimus after pediatric HTx has not been an independent risk factor for increased LDL-C, HDL-C, triglyceride or Lp(a) levels [96, 356, 419]. In contrast, the use of CsA has been associated with a high LDL-C level but as well with a high HDL-C level after pediatric HTx [421]. CsA blood levels have been

positively associated with serum LDL-C levels in pediatric heart recipients but not with serum TC levels in pediatric liver recipients [23, 300].

2.6.5.4. Growth hormone and antihypertensives

In pediatric renal recipients, initiation of RhGH has not led to changes in serum triglyceride, LDL-C, or HDL-C concentrations, but to an increase in postprandial insulin response and consistently elevated fasting insulin concentrations without a change in blood glucose concentrations [51, 161, 177, 193]. Thus, RhGH therapy seems to aggravate pre-existing hyperinsulinemia and promote insulin resistance in prepubertal solid organ recipients [72, 177, 388].

Administration of ß-blockers may increase serum triglyceride and decrease serum HDL-C levels, while diuretics may increase serum LDL-C levels and possibly also serum triglyceride levels [225]. A negative influence of antihypertensive treatment on lipid metabolism has been reported, particularly in the case of beta-blocker or diuretic use after RTx, LTx or HTx [5, 9, 26, 60, 85, 389]. The use of angiotensin-converting enzyme inhibitors or calcium channel blockers has not been associated with dyslipidemia after Tx [222].

2.7. Insulin resistance in end-stage disease and after transplantation

Glucose intolerance and insulin resistance accompany ESRD in both adults and children already in the early stages of the disease [114, 147, 179, 287, 414]. RTx improves the situation, although an increased fasting insulin concentration and an increased insulin response to hyperglycemia have been described in adult renal recipients on glucocorticoid-based immunosuppression [133, 276]. Metabolic syndrome according to NCEP III criteria has been reported in 23 % of renal recipients at one year post-RTx and in 38 % of them after at least 18 months follow-up after RTx [364]. Of adult renal recipients at a median of 6.8 years post-transplant, 75 % had a HOMA index higher than 1, i.e. possible insulin resistance, compared to 50 % of the controls [24]. In a sample of 12 pediatric renal recipients, 25 % had an increased HOMA index [162]. LTx improves low insulin sensitivity of end-stage liver cirrhosis, though mildly increased insulin response to high glucose levels persists after LTx [311]. Insulin resistance, according to an elevated 2-hour insulin response in OGTT has been seen in 36 % of adult heart recipients and cluster of metabolic risk factors of cardiovascular disease (high plasma glucose and insulin concentrations in response to OGTT, high fasting plasma triglyceride, and LDL-C concentrations) more frequently in heart recipients than in controls [233, 468].

Major risk factors for post-Tx insulin resistance are obesity, increasing age and sedentary lifestyle. Obese renal and heart recipients are prone to hypertriglyceridemia and possibly also to hypercholesterolemia [1, 187, 234, 310, 452,

[476]. In adult RTx patients with CsA and prednisone, glucose intolerance has been seen [133, 344]. Use of CsA may increase insulin resistance after LTx [141].

2.8. Dietary interventions after transplantation

In adult solid organ recipients, dietary interventions with restrictions on the intake of total energy and fat, saturated fat and cholesterol (American Heart Association step I and/or II diets, see Appendix 2) have decreased serum LDL-C concentrations, although the patients concomitantly also lost weight [189, 492]. Moreover, the American Heart Association step I diet enhanced with an increased intake of monounsaturated fat and alimentary fiber has reduced serum triglyceride and LDL-C concentrations in adult renal recipients [49]. On the other hand, when weight and serum creatinine concentrations have remained stable, dietary restriction of total and saturated fat, and cholesterol have not influenced serum triglyceride or cholesterol concentrations, or have reduced serum triglyceride levels only slightly in lean adult renal recipients [265, 459].

Few reports have focused on diet and serum lipids after pediatric solid organ Tx. Within pediatric renal recipients, ingested total proteins have been positively associated with serum TC levels [80]. Ingested monounsaturated fat has been positively associated with plasma TC, LDL-C glucose and insulin levels, and HOMA [14]. Short-term dietary intervention has reduced serum TC concentrations in 12 hyperlipidemic pediatric renal recipients [118]. The dietary variables studied have not seemed to influence the serum lipid profiles in pediatric liver recipients [300].

Table 2. Studies of risk factors for dyslipidemia in adult and pediatric transplant recipients (multiple regression or covariate analysis)

Organ	Study	Patients	N	Lipid variable	Risk factor						
					Sex	Age	Obesity	Renal function/ proteinuria	Immunosuppression	Other agents	Other
RTx	**Bittar 1990**	**Adult**	275	**Low HDL-C**	Male +	–	x	Creatinine+	CsA –	Other antihypertensives –	Post-Tx time –
				High TG	–	Old age +	x	Proteinuria +	CsA –	Beta blocker + Diuretics + Other antihypertensives –	Albumin + Post-Tx time –
	Divakar 1991	**Adult**	62	**High LDL-C**	Female +	–	–	–	AZA dose + CsA –	Diuretics Other antihypertensives –	Short post-Tx time + Diabetes –
				Low HDL-C	–	–	–	Creatinine+	CsA + (vs. AZA et prednisone)	Antihypertensives –	Post-Tx time – Diabetes –
				High TG	–	–	–	–	CsA –	Diuretics + ACE inhibitors + Other antihypertensives –	Post-Tx time – Diabetes –
	Kuster 1994	**Adult**	35	**High LDL-C**	x	–	–	–	B-CsA +	Antihypertensives –	Post-Tx time – Diabetes –
				Low HDL-C	x	–	–	–	B-CsA +	Antihypertensives –	Post-Tx time – Diabetes –
				High TG	x	–	–	–	B-CsA –	Diuretics +	Post-Tx time – Diabetes +
	Aakhus 1996	**Adult**	406	**Low HDL-C**	Male +	–	+	Creatinine+	CsA –	Beta blockers +	Hypertension – Coronary artery disease – Diabetes –
				High TG	–	Young age +	+	Low creatinine clearance +	CsA –	Beta blockers + Diuretics +	Diabetes + Hypertension – Coronary artery disease –
HTx	**Becker 1988**	**Adult**	92	**High LDL-C**	–	Old age +	x	x	Cumulative prednisone dose + Low cumulative CsA dose +	x	Post-Tx time +
				Low HDL-C	–	Young age +	x	Creatinine+	Cumulative prednisone dose + Low cumulative CsA dose +	x	Post-Tx time –
				High TG	–	Old age +	x	Creatinine+	Cumulative prednisone dose + Low cumulative CsA dose +	x	Glucose + Post-Tx time –
	Singh 2006	**Pediatric**	361	**High LDL-C**	Female +	Old age +	–	x	CsA + vs. tacrolimus Steroid –	x	Race –
			396	**Low HDL-C**	–	Young age +	–	x	Tacrolimus + vs. CsA Steroid –	x	White race +
			499	**High TG**	–	–	–	x	Steroid +	x	Race –

ABBREVIATIONS: ACE = angiotensin-converting enzyme, AZA = azathioprine, CsA = cyclosporine A, HDL-C = high-density lipoprotein cholesterol, HTx = heart transplantation, LDL-C = low-density lipoprotein cholesterol, N = number of patients, RTx = renal transplantation, TG = triglyceride. NOTES: The studies are characterized by the first author.
NOTES: "+" = variable was a significant risk factor, "–" = variable was not a significant risk factor, "x" = data not included in analysis or data of possible inclusion not provided.

3. AIMS

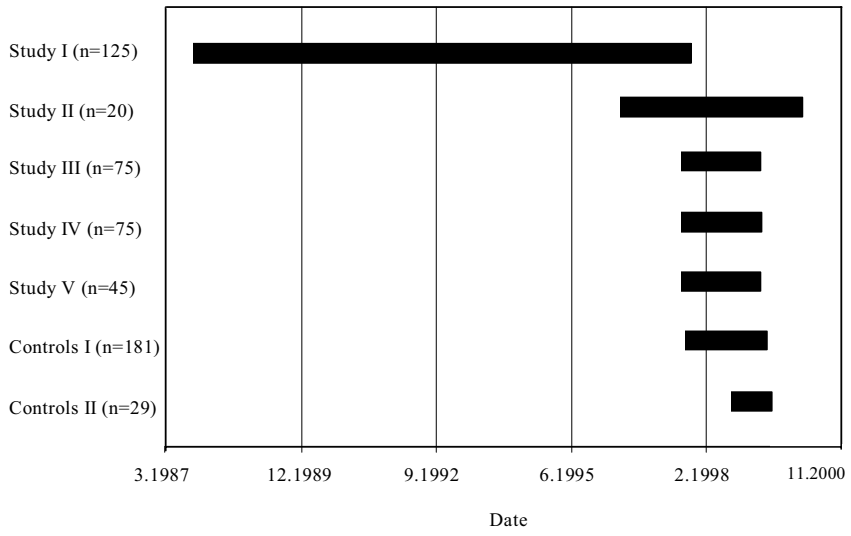
1. To describe the serum lipid profiles from 1 to 3-5 years after pediatric renal, liver or heart transplantation with a stable graft function and to compare the values with those in healthy controls (Study I).
2. To study the prevalence of apolipoprotein E4 isoform carriage and high Lp (a) concentration, and susceptibility of LDL to in vitro oxidation in pediatric renal, liver or heart recipients (Studies II and III).
3. To investigate the association of cholesterol synthesis and absorption efficiency with serum cholesterol concentrations in pediatric renal, liver or heart recipients (Study IV).
4. To investigate the prevalence of features of metabolic syndrome in pediatric renal, liver or heart recipients (Studies II and III).
5. To characterize the intake of foods and nutrients in pediatric renal recipients and to investigate the associations of nutrient intakes with serum lipid and insulin concentrations (Study V).
6. To evaluate associations between serum lipid levels and possible risk factors for dyslipidemia (Studies I-V).

4. PATIENTS AND METHODS

4.1. Patients

Children who received a kidney (Studies I, III-V), a liver (Studies I, III-IV) or a heart transplant (Studies I-II, IV) in Helsinki University Hospital between October 1987 and January 1998 were included in the present study (Fig. 3). In Study I, 71 pediatric renal, 34 liver and 20 heart recipients were followed up for at least three years after Tx (latest Tx done in October 1997). Correspondingly, 57, 31, and 13 of the children were followed up for 5 years. The patients for Study I were selected from all 130 children who had survived for at least 3 years after Tx. Four renal recipient children were excluded because of missing data and one heart recipient because he was switched to a lipid-lowering agent 1.5 years after HTx.

Figure 3. Children's enrollment dates in Studies I-V



Cross-sectional data for Studies II-V was collected on children with at least one year survival after Tx, age 3-17 years and GFR > 40 ml/min/1.73 m^2 during their follow-up visits to either Helsinki or Tampere University Hospitals between September 1997 and May 1999 (Fig. 3). In Studies II-IV, 50 renal, 25 liver, and/or 12 heart recipients were studied for features of metabolic syndrome, LDL characteristics, Lp (a) concentration and ApoE phenotype. An additional 11 heart recipients were enrolled on Study II during their annual follow-up visits between June 1996 and February 2000.

The respective participation rates in the cross-sectional study were 88.0 %, 92.6 % and 92.3 % in renal, liver and heart recipients. Of the renal recipients in Studies III-IV, 47 adequately completed a food record (participation rate 81.8 %). Two of them had diurnal urinary protein excretion > 200 mg/24 hours and were excluded from analysis of Study V. Results of the 45 renal recipients are described.

All patients had acceptably functioning grafts and were euthyroid. The distribution of pre-Tx diagnoses in all studies is shown in Table 3.

Table 3. Number and diagnoses of patients enrolled in Studies I-V.

Organ	Diagnosis	Study			
		I	II	III-IV	V
Kidney	Congenital nephrosis (NPHS1)	38	-	31	28
	Urethral valve	10	-	5	3
	Nephronophthisis	5	-	2	2
	Polycystic kidney disease	3	-	3	3
	Prune-belly syndrome	2	-	1	1
	Vesico-ureteral reflux	2	-	0	0
	Glomerulonephritis	2	-	2	2
	Alport´s syndrome, mega-ureter, bilateral dysplastic or multicystic kidneys, Denys-Drash syndrome, renal insufficiency due to prematurity	6	-	6	6
	Vaginal cancer, neuroblastoma, juvenile nephronophthisis	3	-	0	0
Liver	Biliary atresia	12	-	9	-
	Tyrosinemia	7	-	7	-
	Hepatitis (one neonatal)	5	-	1	-
	Hepatoblastoma	4	-	4	-
	Wilson´s disease	2	-	1	
	Hepatocellular carcinoma, homozygous familial hypercholesterolemia, alpha1-antitrypsin deficiency	3	-	3	-
	Hepatic adenoma	1	-	0	-
Heart	Congenital heart defect	9	10	6	-
	Dilative cardiomyopathy	5	7	4	-
	Restrictive cardiomyopathy	6	6	2	-

All children with ESRD were on peritoneal dialysis before RTx. Bilateral nephrectomy was undertaken prior to Tx in all except one patient with congenital nephrosis (NPHS1), and uni- or bilateral nephrectomy in 8 patients with other diagnoses. In Study I, 35 % of the renal recipients (40 % in Studies III-V) had the graft from a living related donor. In Study I, three of the renal recipients and four of the liver recipients had a second graft.

All the patients received immunosuppressive triple therapy with CsA, MP, and AZA with few exceptions (i.e. tacrolimus instead of CsA and AZA in two to five patients in Studies I, III-V). In Study I, one boy was weaned off steroids during the first post-RTx year due to exceptional side-effects. Nephrotic syndrome (proteinuria > 40 mg/m^2 per hour with edema and an albumin concentration < 25 g/l) occurred during the first 3 years post-Tx in 8 renal recipients (6 NPHS1, 1 urethral valve, 1 chronic glomerulonephritis), but all except one of them were in remission at the time of the all the lipid tests. The boy with renephrosis received cyclophosphamide instead of AZA at the time of one serum lipid sampling. Mild proteinuria (> 200 mg/d) occurred at least once in 20 % of the renal and in 12 % of the liver recipients during the first three post-Tx years.

The B-CsA trough level was maintained between 80 and 120 µg/l after the first year in the RTx group and between 100 and 200 µg/l in the LTx and HTx groups. Since 1994, CsA was given in a microemulsion composition. The CsA dose was individually adjusted according to trough levels and renal function tests to maintain sufficient immunosuppression and to avoid nephrotoxicity. Preschool children took CsA in three daily doses and older children in two. MP was given on alternate days after the first 3-6 months post-Tx. Acute rejection after RTx or LTx was treated with MP at 1.5 mg/kg orally, followed by 3 mg/kg per day, for 5 days or until the blast cell reaction in a fine-needle aspirate subsided in kidney or liver grafts. After HTx, acute rejection was diagnosed according to the clinical picture, changes in ultrasound examination, and a cellular reaction in the endomyocardial biopsy (EMB). Rejection was treated with MP 1.5 mg/kg orally followed by 3mg/kg/d, for 5 days or until EMB was normalized.

Antihypertensives were initiated if ambulatory-measured blood pressure exceeded age-specific reference values. Calcium-channel blockers were mostly used. All except seven pediatric renal recipients in Studies III-V and 3 controls (controls I, see below) were on their usual diets (i.e. without specific dietary counseling). These seven renal recipients were advised a diet low in total and saturated fat due to excess weight gain (2 patients, one control), elevated serum cholesterol concentrations after Tx (3 patients) or due to a family history of elevated serum cholesterol concentrations (2 patients, 2 controls). In Study V, a similar proportion of renal recipients and controls (19.1 % vs. 11.2 %, p=NS) had taken supplementary vitamins, calcium or iron. The renal recipients in Study V received calcium and iron supplementation more often than the controls, [calcium: 13.3 % vs. 2.8 %, $p = 0.004$, iron: 4.4 % vs. 0 %, $p = 0.04$].

Socio-economic group classification was done and based on the classification of occupational status according to the Central Statistical Office of Finland (Statistics Finland 1989, further referred to the Statistical Standards and Studies

No 40 of the United Nations 1987) [384, 434]. The highest personal socio-economic status of the mother or father was considered to be the socio-economic status of the family. Socio-economic status and the length of maternal education were comparable between the patients and the controls. The solid organ recipients' mothers had less often vocational or other secondary school training [27.7 % (n=13) and 36.5 % (n=65) respectively], or college or university degree [38.2 % (n=18) and 48.9 % (n=87), respectively, $p = 0.034$ for the difference in the frequency distributions] than the control (I) children's mothers. Socio-economic variables did not significantly influence on the dietary pattern.

4.2. Controls

In Studies I-III, the control group (I) consisted of 181 children (112 boys and 69 girls) studied between November 1997 and August 1999. Of these children, 178 completed a food record and were controls in Study V. The controls were patients from the pediatric outpatient clinic or were minor pediatric or otorhinolaryngologic surgery patients in Tampere University Hospital. The group of controls was matched with the patients for age and sex. The controls/parents were interviewed by telephone before enrollment in the study. The inclusion criteria were: no regular medication, acute infection or inflammatory or metabolic disease. The participation rate of the controls was 49.3 % (48.5 % in Study V).

In Study IV, the control group (II) consisted of 29 healthy children aged from 5.0 to 16.3 years studied between September 1998 and September 1999. Ketomäki et al. (2003) has described recruitment and clinical characteristics of the controls II [238].

4.3. Ethics

Children gave either verbal or written informed consent and their parents gave written informed consent and all were volunteers for the study. The ethical committees of Tampere and Helsinki University Hospital approved the study.

4.4. Methods

4.4.1. Clinical characteristics

The following clinical data were collected: sex, age at Tx, preceding nephrectomy, indication for Tx, living related donor vs. cadaveric graft, first or

second graft, actual doses of AZA, CsA, MP, and cumulative dose of CsA and MP from Tx to 3 years; the use and type of antihypertensives used, the use of anti-epileptics, hydrocortisone substitution and RhGH; the occurrence and number of acute rejections, height, weight, renal function [GFR, serum creatinine, serum urea nitrogen, diurnal urinary protein excretion (dU-prot)], liver tests [serum alanine aminotransferase (ALT), plasma thromboplastin time (TT), serum total bilirubin, serum albumin and protein], fasting blood glucose and insulin, coronary narrowing in angiography (HTx), and heart function (clinical echocardiography). Data on dyslipidemia or early-onset cardiovascular disease (men < 55 years, women < 65 years) in first-degree or second-degree relatives were elicited by questionnaire in a subgroup of 80 patients and 180 controls. Pubertal staging was estimated according to Tanner's classification (1 = pre-pubertal, 2 - 4 = pubertal, 5 = post-pubertal). Blood pressure was measured on the right upper arm of the sitting patient by an electronic sphygmomanometer or in case of unsuccessful electronic measurement by manual mercury manometer. The mean of the three measurements was used in analysis. Blood pressure was considered high if it exceeded the 95th percentile of the age, sex and height specific reference values (Update on the 1987 Report on High Blood Pressure in Children and Adolescents [465]).

4.4.2. Anthropometry

Height was measured with a Harpenden stadiometer (Holtain, Crymych, Dyfed, UK) and weight on electronic scales at noon (in controls ± 3 hours). Body mass index (BMI) was calculated according to the formula: weight (kg)/height^2 (m^2). BMI standard deviation score (BMI SDS) was calculated according to the following equation: (individual BMI – mean BMI for age)/SD and height standard deviation score (HSDS) according to the following equation: (observed height – mean height for age)/SD. SD represents the standard deviation for a general Finnish population of the same chronological age and gender [333, 429]. BMI SDS was considered elevated if it exceeded the age and sex adjusted the 95th percentile of the reference population.

Biceps, triceps, subsclapular and suprailiac skin folds were measured with a Harpenden skin fold caliper (John Bull, British Indicators Ltd., St. Albans, Herts, UK) according to the Anthropometric Standardization Reference Manual [183]. Waist circumference was measured with a tape in the middle of the distance between the lowest rib and the iliac crest. Hip circumference was measured at the level of trochanter major. Mid-upper arm circumference was measured with the arm relaxed and hanging freely at the midpoint between the tip of the acromial process and the tip of the olecranon process fiberglass tape [82]. Fat body mass was calculated using Brook's equation for children aged 1-11 years, Durnin and Rahaman's equations for children aged 12-16 years and Durnin and Womersley's equation for children older than 16 years [77, 129, 130]. The percentage of fat body mass relative to weight was calculated using Siri's equation [423].

4.4.3. Food records

Forty-seven of the renal, 24 of the liver and 12 of the heart recipients completed a 6-day food record, which included 2 weekend days and macronutrient, cholesterol and fiber intake was analyzed (Study IV). As those results did not reveal marked differences between the solid organ recipient groups the intake of foods and nutrients was described in detail only for the renal recipients. The controls and/or their parents completed a 3-day food record, which included one weekend day. The author entered the record days in a blank document. The days were aimed to be within a week before or after the lipid sampling. The child and/or the parents were asked to record the type and quantity of all the foods and drinks consumed. The record was checked in an interview with the child and/or parent by the author. Simple models of foods (e.g., different sized potatoes), volume and household measures and pictures of food amounts and pictures of dietary fat packages on the market at the time were used for checking the food record data. Complementary data from parents or caregivers was in some cases requested by telephone (e.g., in cases when the type of fat used in preparing the dish was not reported). A separate blank food record document was sent with the child to the day-care center or school. Data on day-care meals and school lunches was checked by telephone from the kitchen personnel of the day-care centres and schools. The nutrient intake from supplements was included in the intake of nutrients. Food record data were analyzed with a Finnish nutrient software program (Nutrica 3.1 Fin, The Social Insurance Institution of Finland, Turku, Finland).

4.4.4. Laboratory analyses

Blood samples for lipid, B-CsA trough levels, glucose and insulin were taken after at least a 12-hour overnight fast, while blood samples for other laboratory tests were taken the previous day. A 24-hour urine sample was collected within a week of the fasting blood sample. B-CsA was determined by specific monoclonal radioimmunoassay [374]. GFR was determined by ^{51}Cr-EDTA clearance [74]. If the distribution space deviated more than 10 % from the expected extracellular fluid volume, the result was ignored (e.g., too high distribution volume would lead to a falsely high GFR). DU-prot, determined from 24-hour urine output, creatinine, ALT, thromboplastin time (expressed as a percentage of the normal mean) and total bilirubin were determined by routine laboratory methods. A result above the age-specific and gender-specific reference value of the laboratory was considered abnormal.

4.4.4.1. Serum lipids, apolipoproteins and lipoproteins

In Study I, lipid analyses were performed on fresh samples according to laboratory routine. TC, HDL-C and triglyceride concentrations were analyzed

enzymatically (Reagena, Roche Diagnostics, Basel, Switzerland). LDL-C was calculated according to Friedewald's formula, though not if triglyceride values exceeded 4.0 mmol/l [152]. In Study I, did the serum triglyceride level exceed 4.0 mmol/l in 23.2 % (n = 29) of patients before Tx, in 2.4 % (n = 3) at 1 year post-Tx, and in 0.8 % (n = 1) at two and five years post-Tx. HDL-C was determined after precipitation of other lipoproteins by dextran sulphate and $MgCl^2$ until February 1997, and thereafter directly i.e. without precipitation of other lipoproteins. During follow-up, the calibrator for TC and triglyceride analyses was changed. The effect of changes on calibrators and methods was corrected by a regression equation. In Studies II-V, the fasting serum triglyceride, TC and HDL-C concentrations as well as ApoB and A-I (ApoA-I) concentrations were analyzed using Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The inter-assay coefficient of variation of the total cholesterol assessment was 1.4 %, of triglyceride 1.0 %, of HDL cholesterol 3.7 %, of ApoA-I 3.8 %, and of ApoB 3.5 %. We considered serum TC > 5.0 mmol/l, serum LDL-C > 3.0 mmol/l, serum HDL-C < 1.0 mmol/l and serum triglyceride > 1.5 mmol/l as elevated [for cholesterol Executive summary of European Guidelines [108], for triglyceride > 95th percentile in Porkka et al. (1994) [363]]. Lp (a) was determined radioimmunologically [158]. ApoE phenotypes were determined by isoelectric focusing and immunoblotting as described [275].

4.4.4.2. Low-density lipoprotein particle size

For the estimation of LDL particle size EDTA-plasma samples were subjected to non-denaturing gradient gel electrophoresis as described by Krauss and Burke (1982) [249]. However, the 2-16 % polyacrylamide gels were cast in-house according to the instructions given by Pharmacia (Uppsala, Sweden). To calibrate for particle hydrated diameter we used the HMW calibration kit (Pharmacia) supplemented with LDL particles whose peak particle diameter was 25 nm. The LDL was isolated by ultracentrifugation as described and kept at -70°C in 0.15M NaCl/1mM EDTA solution containing 0.6 % saccharose [212]. After 24-hour electrophoresis the gels were first stained with Oil Red O for lipids and thereafter a filter paper soaked with Coomassie Brilliant Blue was placed on the standard lane to stain the proteins. The gels were scanned with a laser densitometer (LKB, Ultroscan 2202) connected to an integrator (Hewlett-Packard, 3309A). LDL peak particle diameters were determined from a calibration curve constructed from the migration distances and log-transformed diameters of the standards. A control plasma sample (peak particle diameter 27.00 nm) stored at –70°C was included in every gel. The inter-assay coefficient of variation during this study was 1.0 %. LDL particle size was determined for all renal, 24 liver and all heart recipients, and for 95 controls, respectively, representing the whole control group according to age and sex.

4.4.4.3. Copper-induced oxidation of low-density lipoprotein

Plasma LDL was isolated by single-step non-equilibrium density gradient ultracentrifugation at 100,000 rpm for 30 min at 10ºC as described in Chung et al. (1986) [98]. The isolated LDL fraction was desalted by passing it through a gel filtration column (Econo-Pac 10 DG, Bio Rad Laboratories, CA, USA). The protein concentration of the eluate was measured using bovine serum albumin as standard. The LDL preparations were diluted with phosphate buffered saline to contain 0.05 g/l protein (0.1 micromol/l LDL). Oxidation was started by adding 10 µl of freshly prepared 0.167 mmol/l CuSO4 to 1.0 ml of LDL solution in a 1 cm quartz cuvette. Oxidation was determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously (at 1 min intervals for 5 h) monitoring the change in absorbance at 234 nm at 37ºC, as described [368]. We used a Perkin Elmer Lambda Bio 10 spectrophotometer (Überlingen, Germany) equipped with an 8-position automatic cell changer. Several indices were obtained from the absorbance versus time curves. The lag time (min) was determined from the intercept of lines drawn through the linear portions of the lag phase and propagation phase. The rate of propagation (Prorate, micromol/l of dienes/min) was obtained from the slope of the absorbance curve during the propagation phase using the molar absorptivity of $\varepsilon_{234\ nm}$ of 29,500 l/mol/cm for conjugated dienes. The maximal concentration of dienes formed (MaxDC, nmol/mg LDL protein) was calculated from the difference in absorbance at zero time and at diene peak. In every oxidation run, one reference LDL isolated from reference plasma stored at –70ºC was used to control the whole procedure. The intra-assay CV of lag time measurements was 1.5 % and the inter-assay was 3.2 %. The susceptibility of LDL to oxidation was determined respectively for 46 kidney, all liver and heart recipients, and 88 controls representing the whole control group for age and sex. Maximum storage time was 30 months.

4.4.4.4. Absorption efficiency and synthesis of cholesterol

Serum sterols and TC concentrations (for calculating ratios) were measured by gas-liquid chromatography on a 50 m long capillary column (Ultra 2, 5890, Hewlett Packard, Wilmington, DE) [312]. Because non cholesterol sterols are mainly transported in cholesterol-containing particles in serum, the absolute concentrations were adjusted for serum cholesterol concentration (10^2 × mmol/mol cholesterol) and are expressed as ratios in the text. The ratios of cholestanol, campesterol, and sitosterol are expressed in text as absorption markers of cholesterol, and those of the precursor sterols (Δ^8-cholestenol, desmosterol and lathosterol) as synthesis markers of cholesterol.

4.4.4.5. Glucose and insulin

Blood glucose was analyzed by enzymatic and serum insulin by radioimmunological method. Insulin resistance was estimated by HOMA [insulin resistance = (fasting insulin × fasting glucose)/22.5] [298]. We compared our patients' HOMA indices to the mean of the 95th percentile of 9, 13, 16 year-old Canadian healthy children [15]. Our patients were divided into sub-groups according to age and gender (we used as separator between the age groups the median between the Canadian age cohorts, i.e., younger than 11 years vs. 11-14.49 years vs. 14.5 years and older). Oral glucose tolerance tests (OGTT) were performed after a 12 h fast and an oral dose of glucose 1.75 g/kg (maximum 75 g) was given. Samples for blood glucose and serum insulin measurements were collected at 0, 30, 60, 90, 120, and 180 min. Criteria for hyperinsulinemia were diagnosed as serum fasting insulin exceeding 20 mU/l and or as a peak insulin concentration exceeding 150 mU/l [461]. Impaired glucose tolerance was determined according to WHO criteria: blood glucose 5.6 - 6.0 mmol/l after an overnight fast or 6.7 - 9.9 mmol/l at 120 min in the OGTT [487].

Features of metabolic syndrome with adequate prognostic value for future cardiovascular disease have not been validated for children. For this dissertation the European Group for the Study of Insulin Resistance (EGIR) definition of metabolic syndrome [44] was modified in order to assess the clustering of risk factors. The results for the following definition are presented: insulin resistance (defined as fasting insulin values > 75th percentile among reference population); in addition any two of the following features BMI SDS >2 SD, triglyceride >2.0 mmol/l and/or HDL-C < 1.0 mmol/l or treatment for these lipid abnormalities, either systolic or diastolic blood pressure > 95th percentile of the sex, age and height adjusted reference values or blood pressure therapy, fasting blood glucose > 5.6 mmol/l or 2 hour blood glucose in post-glucose challenge > 6.7 mmol/l.

4.4.5. Statistical analysis

The normality of the distribution of the variables was tested by one-sample Kolmogorov-Smirnov goodness-of-fit test. If the distribution was skewed, log transformation was applied when appropriate. The values are presented as means and 95 % confidence intervals (CI) and/or range or SD (normal distribution), median and 25th percentile (Q1) and 75th percentile (Q3) (skewed or discrete distribution) or number (n) and percentages of subjects (frequency distribution). MP dose is presented as daily dose, which equals the dose on alternate days divided by 2.

Differences in means between groups were tested with the analysis of variance (ANOVA) or t-test for normally distributed continuous variables or Mann-Whitney or Kruskal-Wallis test for skewed or discrete variables. To evaluate whether the changes in variables over time were statistically significant, we used ANOVA for repeated measures (normal distribution) or the Wilcoxon test (skewed or discrete distribution). The significance of differences in the

categorical variables was tested by the χ^2-test. In cases where the frequencies in the cells were low, Fisher's exact test was used instead. We used the McNemar test to evaluate the significance of changes within time in categorical variables. Univariate linear and logistic regression, and multiple forward stepwise logistic and linear regression models were applied in Studies I and III-V in order to explain variations in lipid variables as dependents. Of the multiple linear regression models, the model with the highest R^2 was presented as the final result. The variables in the multiple regression models did not show a strong correlation ($r < 0.4$, in Study V: $r < 0.6$). A variable was included in the multiple model if its significance was < 0.05 and removed if the significance was > 0.1. Otherwise, p values < 0.05 were considered statistically significant. Computations were carried out with SPSS for Windows version 10.1 (SPSS, Chicago, Ill, USA).

5. RESULTS

5.1. Clinical characteristics

5.1.1. Age, anthropometry and medications

Table 4 shows clinical characteristics of the three recipient categories in Studies II-IV, which approximate the clinical characteristics in Studies I (at 3 years post-Tx) and V. In Study I, 70 % of the renal recipients were male, likewise 47 % of the liver and 45 % of the heart recipients (difference: $p = 0.024$). Heart recipients transplantation were performed at on average at an older age than renal or liver transplantation (median age at RTx 3.8 years, at LTx 3.6 years, and at HTx 12.3 years, $p = 0.004$), while in Studies II-V, patients and controls had a similar distribution of age, gender, and pubertal development. The relative height of the solid organ recipients was smaller than that of the controls I (Table 4). Waist to hip circumference ratios were larger and triceps skin folds thinner in the renal recipients than in the controls (Table 5).

Table 4. Clinical characteristics of 50 pediatric renal, 25 liver and 12 heart recipients and 181 controls (Studies II-IV)

	Kidney (n=50)	Liver (n=25)	Heart (n=12)	Controls I (n=181)
Gender, Male/Female, n	33/17	15/10	6/6	112/69
Age, years, median (range)	10.8 (4.3 – 17.2)	9.3 (3.9 -17.9)	9.6 (3.0-17.7)	9.1 (3.2 - 18.7)
Age at Tx, years, median (range)	3.2 (1.1 - 15.3) [a]	2.0 (0.4 -15.9)	3.0 (1.0-7.0)	
Years post-Tx, years, median (range)	5.1 (1.0 - 11.0)	7.0 (1.0 -11.0)	2.0 (1.0-7.0)	
Height SDS, mean (SD)	–1.3 (1.0) [b]	–1.7 (1.0)	–1.0 (1.2)	0.2 (1.0)
BMI SDS, mean (SD)	0.3 (1.2)	0.5 (1.4)	0.5 (2.0)	0.3 (1.3)

ABBREVIATIONS: BMI=body mass index, n=number of patients, SD=standard deviation, SDS=standard deviation score, Tx=transplantation; STATISTICS: [a] p=0.008; [b] p < 0.001; kidney group vs. controls, liver group vs. controls, heart group vs. controls: p < 0.001.

Table 6 shows the average dosages of CsA and MP in Study I. The average three-year dosages were approximately equal to the respective values in Studies II-V. At the one year post-Tx all the recipient categories had higher average doses of CsA and MP and the annual cumulative dose of MP, and the renal and liver recipients also had a higher frequency of antihypertensive use than at the three years ($p < 0.01$ for all). RhGH therapy was used three years post-Tx on

23.9 %, 17.6 % and 15.0 % of the renal, liver and heart recipients respectively (Table 6).

Table 5. Clinical characteristics of 45 renal recipients and 178 controls (Study V)[a]

	Kidney (n=45)	Controls I (n=178)	P value
Aged 3-9 years/10-13 years/14-18 years, n	21 / 15 / 9	100 / 44 / 34	0.445
Waist circumference, SDS, median (Q_1, Q_3)	–0.06 (–0.23, 0.34)	–0.14 (–0.37, 0.21)	0.089
Waist to hip circumference ratio, mean (SD)	0.92 (0.08)	0.85 (0.06)	<0.001
Biceps skin fold, SDS, median (Q_1, Q_3)	–0.04 (–0.30, 0.33)	0.09 (–0.15, 0.42)	0.062
Triceps skin fold, SDS, median (Q_1, Q_3)	–0.07 (–0.28, 0.04)	0.03 (–0.13, 0.18)	<0.001
Subscapular skin fold, SDS, median (Q_1, Q_3)	–0.10 (–0.25, 0.11)	–0.06 (–0.21, 0.14)	0.313
Mid-upper arm circumference, SDS median (Q_1, Q_3)[b]	–0.20 (–0.43, 0.08)	–0.04 (–0.34, 0.36)	0.147

ABBREVIATIONS: n=number of patients, p=probality value of significance, Q_1= 25th percentile, Q_3=75th percentile, SD=standard deviation SDS=standard deviation score. NOTES:[a] The renal recipients were studied at median age of 10.6, a median of 5.2 years post-transplant and the controls at median age of 9.1 years. [b] Mid-upper arm circumference was measured in 45 renal recipients and 166 controls.

Table 6. Medication at one and three years post-Tx in 71 pediatric renal, 34 liver, and 20 heart recipients studied for dyslipidemia (Study I)

	Kidney (n=71)		Liver (n=34)		Heart (n=20)	
Years after Tx	**1 y**	**3 y**	**1 y**	**3y**	**1y**	**3 y**
CsA, mg/kg/day, median	8.0	5.4	8.7	5.3	6.9	5.7
(Q_1, Q_3)[a]	(5.6; 10.6)	(4.2; 6.8)	(5.3; 10.5)	(3.4; 6.6)	(5.0; 9.5)	(4.2; 6.6)
B-CsA, microg/l , median	110	93	184	130	194	164
(Q_1, Q_3)[b]	(83; 147)	(77; 113)	(109; 225)	(80; 160)	(150; 244)	(137; 196)
MP, mg/kg/day, median	0.2	0.1	0.2	0.1	0.1	0.1
(Q_1, Q_3)[a]	(0.1; 0.2)	(0.1; 0.1)	(0.1; 0.2)	(0.1; 0.1)	(0.1; 0.2)	(0.1; 0.1)
MP, mg/kg/year, median	87	46	99	40	90	33
(Q_1, Q_3)[a, c]	(78; 101)	(35; 52)	(92; 115)	(31; 53)	(82; 97)	(29; 48)
Antihypertensives, % (n)[d]	49.3 (35)	26.8 (19)	32.4 (11)	8.8 (3)	30.0 (6)	20.0 (4)
B-blockers or diuretics, % (n)	19.7 (14)	11.3 (8)	8.8 (3)	0 (0)	15.0 (3)	5.0 (1)
RhGH, % (n)[e]	0 (0)	23.9 (17)	0 (0)	17.6 (6)	5.0 (1)	15.0 (3)

ABBREVIATIONS: B-CsA=blood cyclosporine trough level, CsA=cyclosporine A, MP=methylprednisolone, n=number of patients, Q_1=25th percentile, Q_3=75th percentile, RhGH=recombinant human growth hormone, Tx=transplantation, y=year. STATISTICS: [a]Change between one and three years: p < 0.001 for kidney, liver and heart transplant group. [b]Change between one and three years: p < 0.01 for kidney and liver patients and difference between groups at one and three years, p < 0.001. [c]Difference between the groups at one year: p=0.003. [d]Difference between one and three years, kidney transplant group: p < 0.001, liver transplant group: p=0.008. [e]Difference between one and three years all groups together, p < 0.001.

5.1.2. Graft function

Renal recipients had a lower GFR, higher serum creatinine and serum urea nitrogen concentrations, while liver recipients had higher ALT concentrations

than other solid organ recipients ($p < 0.001$, Table 7). After 3 years post-Tx follow-up, 35 % of the renal recipients had a GFR below 60 ml/min per 1.73 m^2 (liver: 9 %; heart 10 %, $p < 0.001$). Serum urea nitrogen concentrations above the normal range were found in 88 %, 40 % and 50 % of the kidney, liver and heart recipients respectively ($p < 0.001$). Impaired liver function (serum thromboplastin time < 70 % of the normal mean) was seen in 3 % of the renal, in 18 % of the liver and in 20 % of the heart recipients. Abnormal serum ALT concentrations were found in 21 % of the liver recipients vs. none of the renal and heart recipients ($p < 0.001$). Elevated serum total bilirubin concentrations were seen in 0 %, 9 % and 7 % in the renal, liver and heart recipients respectively.

Table 7. Graft function of the 71 renal, 34 liver, and 20 heart recipients (Study I)

	Kidney (n=71)		Liver (n=34)		Heart (n=20)	
Years after Tx	**1 y**	**3 y**	**1 y**	**3y**	**1y**	**3 y**
GFR, ml/min/1.73 m^2, mean	76	69	104	98	104	93
(range)[a]	(26 - 130)	(26 - 144)	(48 - 182)	(38 - 154)	(33 - 161)	(52 - 155)
DU-prot > 200 mg/d, % (N)	11.3 (8)	11.3 (8)	2.9 (1)	8.8 (3)	5.0 (1)	0 (0)
ALT,U/l, median (Q_1, Q_3)[a]	15 (13, 20)	14 (10, 16)	29 (19, 56)	24 (18, 39)	17 (12, 29)	13 (11, 15)
Bilirubin, µmol/l, median (Q_1, Q_3)[b]	7 (5, 10)	8 (5, 10)	10 (6, 15)	10 (8, 14)	8 (6, 13)	9 (6, 11)
TT, %, median (Q_1, Q_3)[c]	107 (95, 131)	109 (94, 124)	87 (75, 102)	89 (72, 108)	87 (65, 102)	81 (75, 106)

*ABBREVIATIONS: ALT=alanine amino transferase, dU-prot=24-h urinary protein excretion, GFR = glomerular filtration rate, n=number of patients. Q_1=25th percentile, Q_3=75th percentile, TT=thromboplastin time, Tx=transplantation, y=year. STATISTICS: Time: $p < 0.001$, group: $p < 0.001$, time*group: NS (ANOVA for repeated measures), [b]Change in total bilirubin between one and three years: p=0.042 for LTx group [c]Difference between groups: $p < 0.001$ at one and three years.*

5.2. Food consumption and intake of nutrients

5.2.1. General

Reported intakes of total calories, saturated fat and cholesterol did not differ between the recipient groups, and controls, Table 8. The renal recipients reported receiving more energy from dietary fat and less from carbohydrate than the liver recipients [fat: mean (SD): 34.1 (4.4) E % vs. 31.2 (4.9) E %, $p = 0.013$; carbohydrate: 49.0 (4.9) E % vs. 53.2 (5.3) E %, $p = 0.002$].

5.2.2. Food and nutrients in the renal recipients

Average daily dietary calories per kg of body weight of the patients and the controls were similar and diminished similarly from younger to older children

[caloric intake, mean (SD), patients vs. controls: 3-9 years 0.31 (0.09) MJ/kg vs. 0.31 (0.06) MJ/kg; 10-13 years 0.22 (0.07) MJ/kg vs. 0.20 (0.06) MJ/kg; 14-18 years 0.15 (0.05) MJ/kg vs. 0.15 (0.04) MJ/kg]. The amount and type of ingested fat was similar among the transplant recipients and controls I. Those seven renal recipient children, who were assumed to follow a low fat and low saturated fat diet received a median 33.2 E % of dietary fats (range: 25.2 E %–36.0 E %) and a median 13.1 E % of saturated dietary fats (range: 10.8 E %–14.7 E %).

Table 8. Intake of energy yielding nutrients and cholesterol in 45 pediatric renal, 24 liver recipients at a median of 5.2, 7.0 and 2.0 years post-transplant respectively, and in 178 controls

Energy yielding nutrients	Kidney (n=45)	Liver (n=24)	Heart (n=12)	Controls I (n=178)
Total calories, MJ, median (Q_1, Q_3)	6.9 (5.6, 8.5)	6.9 (6.1, 7.6)	7.2 (5.0, 9.6)	7.5 (6.4, 8.8)
Protein, E%, mean (SD)	16.7 (2.5)	15.6 (2.8)	16.9 (2.7)	16.0 (2.7)
Carbohydrate, E%, mean (SD)	49.1 (4.9) [a]	53.2 (5.3)	50.5 (3.8)	50.7 (5.7)
Fat, E%, mean (SD)	34.1 (4.3) [b]	31.2 (4.9)	32.6 (3.7)	33.2 (4.9)
Saturated fat, E%, mean (SD)	14.4 (2.4)	13.2 (3.3)	13.9 (2.5)	14.1 (2.8)
Monounsaturated fat, E%, mean (SD)	11.5 (1.7) [b]	10.1 (2.0)	10.9 (1.7)	11.1 (2.2)
Polyunsaturated fat, E%, mean (SD)	4.8 (1.3)	4.6 (1.4)	4.6 (1.1)	4.6 (1.5)
Dietary cholesterol, mg, median (Q_1, Q_3)	195 (154, 269)	207 (157, 254)	201 (146, 229)	221 (174, 278)
Fiber, g/MJ, median (Q_1, Q_3)	1.8 (1.5, 2.1)	1.8 (1.5, 2.1)	1.7 (1.4, 2.2)	1.9 (1.6, 2.2)

ABBREVIATIONS: N=number of patients, Q_1=25th percentile, Q_3=75th percentile, E%=percent of total caloric intake, SD=standard deviation. STATISTICS: [a] $p < 0.01$ between solid organ recipients [b] $p < 0.05$ between solid organ recipients.

Table 9. Median (25th, 75th percentile) daily intakes of vitamins and minerals in 45 pediatric renal recipients and 178 controls

Nutrients	Renal recipients		Controls I	
	Boys (n=28)	Girls (n=17)	Boys (n=109)	Girls (n=69)
Vitamin D, µg	4.1 (2.5, 5.4) [b]	2.7 (2.0, 3.7)	2.8 (2.0, 4.2)	2.9 (2.1, 5.1)
Riboflavin (vitamin B 2), mg	2.1 (1.4, 2.8)	2.1 (1.6, 2.6)	2.1 (1.6, 2.6)	1.7 (1.3, 2.1)
Folic acid, µg	214 (178, 246)	155 (137, 181)	202 (169, 276)	179 (147, 228)
Vitamin C, mg [a]	84 (65, 126)	46 (28, 96) [c]	94 (60, 141)	103 (70, 148)
Calcium, g	1.2 (0.8, 1.4)	1.2 (0.9, 1.4) [d]	1.1 (0.9, 1.5)	1.0 (0.7, 1.2)
Magnesium, mg	252 (210, 315)	239 (205, 276)	278 (239, 352)	228 (198, 271)
Potassium, g [a]	2.7 (2.3, 3.5) [b]	2.8 (2.3, 2.9)	3.3 (2.8, 3.9)	2.7 (2.2, 3.2)
Iron, mg	8.7 (7.4, 11.8)	6.5 (5.6, 9.2)	9.7 (7.7, 12.0)	8.0 (6.3, 9.9)
Selenium, µg	72 (58, 88)	58 (56, 73)	70 (56, 90)	60 (48, 74)

ABBREVIATIONS: n=number of patients. STATISTICS: [a] Pediatric renal recipients vs. controls: $p < 0.01$, [b]Renal recipient boys vs. control boys: $p < 0.01$, [c] Renal recipient girls vs. control girls: $p < 0.01$, [d] Renal recipient girls vs. control girls: $p < 0.05$. NOTES: The values presented include vitamin and mineral supplements.

The renal recipients consumed less fruits and berries than the controls [patients and controls, median (25^{th}, 75^{th} percentile): 28 (15, 49) g/MJ and 52 (32, 68) g/MJ respectively, $p = 0.002$]. Accordingly, the renal recipients received less vitamin C than the controls [median (25^{th}, 75^{th} percentile) 78 (46,121) mg vs. 101 (64, 147) mg, $p = 0.011$, Table 9]. The male renal recipients received fewer calories from proteins and more from carbohydrates, and received less dietary cholesterol per MJ and more fiber per MJ than the female recipients ($p < 0.05$ *for all*).

5.3. Lipid profiles

5.3.1. Triglyceride and associations

Hypertriglyceridemia characterized post-Tx dyslipidemia in all of the three Tx recipient categories in both the follow-up and in the cross-sectional study for up to 11 years after Tx, although average serum triglyceride levels decreased in the renal and liver recipients from pre- to post-Tx (Fig. 4). The prevalence of high triglyceride post-Tx was approximately 50 % in the renal and heart recipients, and 30 % in the liver recipients compared to a prevalence of 7 % in the controls ($p < 0.001$).

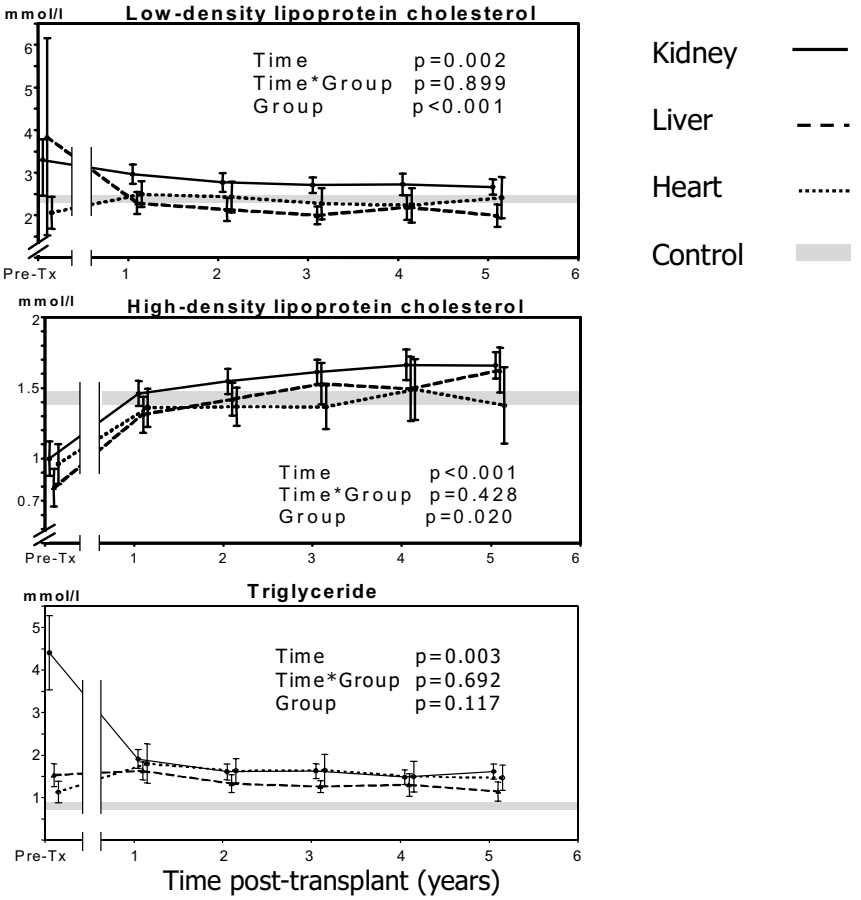
Table 10. Mean (SD) serum lipid and lipoprotein concentrations in 50 pediatric renal, 25 liver, and 12 heart recipients at a median of 5.1, 7.0 and 2.0 years post-transplant respectively, and controls I (Studies I-III) and II (Study IV)

Lipid variable	Kidney (n=50)	Liver (n=25)	Heart (n=12)	Controls I (n=181)	Controls II (n=29)
Total cholesterol, mmol/l	4.99 (0.99) [a, c, d, e, f]	4.25 (1.02)	4.10 (0.82)	4.18 (0.74)	4.20 (0.51)
LDL cholesterol, mmol/l	2.70 (0.78) [a, c, d, e, f]	2.16 (0.77)	2.02 (0.63)	2.39 (0.61)	2.20 (0.53)
HDL cholesterol, mmol/l	1.62 (0.34) [a, d, e, f]	1.56 (0.45)	1.33 (0.31)	1.42 (0.30)	1.33 (0.20)
Triglyceride, mmol/l	1.48 (0.70) [a, e, f]	1.18 (0.48) [h]	1.65 (0.69) [i, j]	0.80 (0.42)	0.87 (0.41)
HDL-C/TC, %	33.2 (7.3)	36.8 (8.3)	32.8 (7.0)	34.6 (7.6)	31.9 (6.0)
Apolipoprotein A-I, g/l	1.53 (1.25) [a, d, e]	1.46 (0.31) [k]	1.24 (0.22)	1.39 (0.22)	Nd
Apolipoprotein B, g/l	0.83 (0.20) [b, c, e]	0.70 (0.18)	0.72 (0.17)	0.73 (0.16)	Nd
Lipoprotein (a), U/l [k]	65 (21, 143)	67 (32, 161)	29 (< 17; 116)	85 (27; 238)	Nd
Diameter of LDL, nm [l]	26.9 (0.5) [c]	26.8 (0.4)	26.7 (0.5)	26.6 (0.4)	Nd
Lag time, min [m]	78 (8)	76 (11)	78 (11)	77 (8)	Nd
Oxidation rate, microM/min [m]	0.44 (0.04) [c]	0.40 (0.06)	0.43 (0.04)	0.43 (0.04)	Nd
Max. dienes formed, nmol/mg [m]	569 (53) [c]	522 (64)	557 (41)	547 (43)	Nd

ABBREVIATIONS (Table 10): HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, n=number of patients, Nd=not determined, SD=standard deviation, TC=total cholesterol. STATISTICS: [a] Tx groups vs. control I, p < 0.001; Tx groups vs. control II, p < 0.001, [b] Tx groups vs. control I, p=0.002, [c] Kidney vs. liver group, p < 0.05, [d] kidney group vs. heart, p < 0.05, [e] kidney group vs. control I, p < 0.05, [f] kidney group vs. control II and control II, p < 0.05, [g] liver group vs. control I, p < 0.05, [h] heart group vs. control I, p < 0.05, [i] heart group vs. control II, p < 0.05, [j] liver vs. heart group, p < 0.05. NOTES: [k] Values for lipoprotein (a) are median (25th percentile, 75th percentile). [l] LDL particle size was analyzed for 24 children in the liver recipient and for 95 children in the control group, [m] LDL susceptibility to in vitro oxidation was analyzed for 46 children in the kidney recipient and for 88 children in the control group.

All three recipient categories had a similar decrease in the triglyceride concentrations from 1 to 3 years post-Tx. In the renal recipients, the prevalence of high triglyceride concentrations decreased from 94 % before RTx to 61 % at one year (significance of change: $p < 0.001$) and further to 45 % at 3 years after RTx. After Tx, variations in actual triglyceride concentrations explained from 19 to 33 % of the variations in subsequent triglyceride concentrations.

Figure 4. Serum lipid values of 71 pediatric renal, 34 liver and 20 heart recipients 1 to 5 years post-transplant (presented as mean and 95 % confidence intervals of the mean; the text in the figure represents the results of ANOVA for repeated measures within the solid organ recipients from 1 to 3 years post-Tx, figure modified from Study I)



In Study I, variation in the renal recipients' triglyceride levels was explained by variation in the age at Tx (*OR = 1.2, CI 95 %: 1.1–1.3, p = 0.007*). In Study III, variations in renal recipients' triglyceride concentrations were explained by variations in HDL-C concentrations ($\beta = -0.45$, $p = 0.013$), BMI SDS ($\beta = 0.11$, $p = 0.031$) and diurnal urinary protein excretion ($B = 0.53$, $p = 0.031$, $R^2 = 0.308$).

In liver recipients, the therapy with RhGH was associated with a high triglyceride level at three years post-Tx (*OR = 17.3, CI 95 %: 1.7–172.0, p = 0.015*). However, these liver recipients already had a tendency to high triglyceride concentrations before the initiation of RhGH therapy (difference from the other liver recipients: $p = 0.082$). In the liver recipients, no significant model was found for triglyceride concentration in Study III.

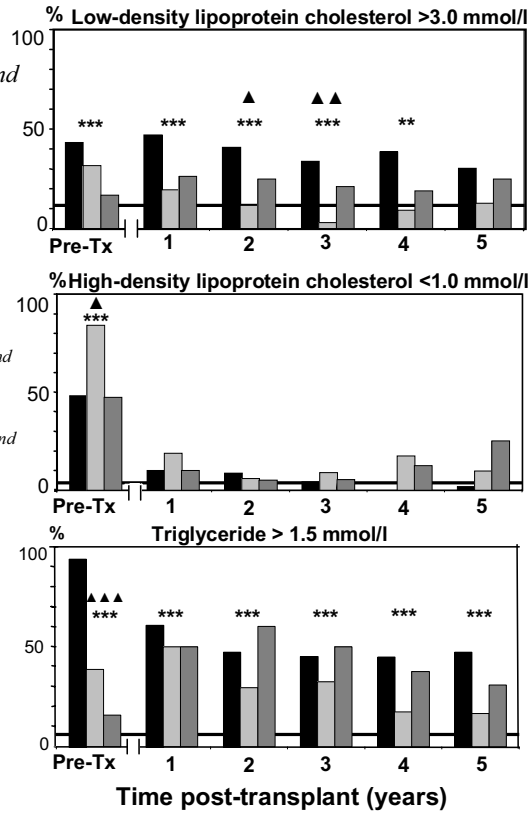


Figure 5. Frequencies of dyslipidemias in 71 pediatric renal, 34 liver and and 20 heart recipients 1 to 5 years post-transplant and in 181 controls (figure modified from Study 1).

*Abbreviation for figure 2: RTx: filled column; LTx: light gray column; HTx: dark grey column; Control group: horizontal line behind the columns. ▲ p<0.05, ▲▲p<0.01, ▲▲▲p<0.001: significance of difference in frequencies of dyslipidemia between the three Tx and control groups (cross-tabulation). * p<0.05, ** p<0.01, *** p<0.001 significance of difference in frequencies of dyslipidemia between the Tx groups (cross-tabulation). Group comparisons were calculated in each time point separately. Post-Tx p values were multiplied by 5 to avoid a multiple comparison problem.*

5.3.2. Cholesterol and associations

Average LDL-C levels decreased in the renal and liver recipient groups and average serum HDL-C levels increased in all three recipient groups from pre- to post-Tx. The serum LDL-C concentrations remained stable after Tx (Fig. 4). An elevated LDL-C post-Tx was observed in approximately 40 % of the renal and in

approximately 20 % of the heart recipients respectively but in approximately 10 % of the liver recipients and controls I (Fig. 5). All three recipient categories had a similar increase in the HDL-C concentrations from 1 to 3 years post-Tx (Fig. 4) and low HDL-C concentrations were rare. The liver recipients showed the most obvious improvement of HDL/TC compared to the renal and heart recipients (*Time: $p < 0.001$, Time*group: $p = 0.03$, group: NS*). In the cross-sectional sample of renal, liver and heart recipients, a high LDL-C of > 3.0 mmol/l was seen in 28 %, 8 % and 8 % (*NS*), and further a high LDL-C of > 2.5 mmol/l was seen in 68 %, 28 % and 25 % respectively ($p = 0.005$ for the difference between the groups). At 3 years post-Tx, 39 %, 59 % and 42 % of the renal, liver and heart recipients respectively had HDL-C, LDL-C and triglyceride levels within the normal range.

Within the renal recipients, pre-Tx TC and HDL-C concentrations explained approximately 10 % of the variation in the respective lipid concentrations at 3 years. After Tx, the variations in the preceding lipid concentrations predicted from 23 % (the liver recipients' TC concentration) to 74 % (the heart recipients' HDL/TC) of the variations of subsequent lipid concentrations. In multiple regression analysis in both renal and heart recipients, high MP doses explained low HDL-C levels (RTx: *OR = 6.3, CI 95 %: 1.9–21.3, $p = 0.003$*, HTx: *OR = 19.5, CI 95 %: 1.3–292.7, $p = 0.032$*, Study I). LTx without pre-Tx liver failure (n=7) was an independent determinant of high TC and further, hepatic cancer as an indication of LTx (n = 5 vs. other indications) was significant in explaining a high TC level [*OR = 29.3 (95 % CI: 2.4-357.9), $p = 0.008$*]. Cumulative doses of CsA and MP were not associated with serum lipid profiles.

In the 45 renal recipients (Study V), statistically significant partial correlation coefficients adjusted for total calories were seen, e.g., between serum LDL-C concentration and intake of dietary fiber (*$r = –0.33$, $p = 0.025$*) and between serum HDL-C concentration and intake of carbohydrate and magnesium (*$r = -0.37$, $p = 0.013$* and *$r = –0.38$, $p = 0.009$* respectively). In multiple linear regression analysis variation in the energy-adjusted intake of fats [*$\beta = 0.01$ (SE = 0.01), $p = 0.029$*] and BMI SDS [*$\beta = –0.10$ (SE = 0.04), $p = 0.012$*] explained variation in the serum concentration of HDL-C (*$R^2 = 0.24$*).

5.4. Low-density lipoprotein particle size and susceptibility to in vitro oxidation

Small dense LDL (< 25.5 nm) was not seen among the major LDL bands. Variation in LDL particle size distributions between the patient categories was slight. The renal recipients had statistically significantly larger LDL particle size than the controls I (*$p < 0.001$*), Table 10. In the 45 renal recipients (Study V) variation in BMI SDS explained variation in the LDL particle size [*$\beta = –0.17$ (SE = 0.06), $p = 0.010$, $R^2 = 0.15$*]. The lag time of LDL susceptibility to in vitro oxidation did not differ between the solid organ recipients and the controls. The

LDL oxidation rate was highest in the renal and lowest in the liver recipients ($p = 0.002$, Table 10).

5.5. Apolipoprotein E phenotype and lipoprotein (a)

ApoE isoform E4 carriage was seen in 28 % of the renal, in 26 % of the liver and in 42 % of the heart recipients, and in 28 % of the controls (difference: *NS*). The apolipoprotein E phenotype was not associated with lipid or lipoprotein concentrations, or ratios of non-cholesterol sterols in any of the patient categories. The heart recipients with ApoE4 isoform carriage (n=5) had smaller LDL particle size than the heart recipients homozygous for ApoE3 isoform (n=7, 26.4 and 26.9 nm respectively, $p = 0.041$). The limit for the 75th percentile of serum Lp (a) concentrations derived from the values in the controls was 232 U/l. Lp (a) values above this limit were found in 16 %, 20 % and 17 % of the renal, liver and heart recipients respectively (difference: *NS*). The Lp (a) concentrations did not correlate with estimates of renal function.

5.6. Absorption efficiency and synthesis of cholesterol

The kidney recipients had lower ratios of Δ8-cholestenol ($p = 0.031$), similar ratios of lathosterol and higher ratios of desmosterol ($p = 0.02$) compared to the controls (Table 11). The liver and heart recipients had higher ratios of desmosterol and lathosterol than the controls ($p < 0.05$ for all). The liver recipients had lower ratios of campesterol than the kidney recipients and the controls ($p = 0.002$) and lower ratios of sitosterol than the heart recipients and controls ($p = 0.007$).

Table 11. Mean (SD) ratios of non-cholesterol sterols and cholestanol to cholesterol in 50 pediatric renal, 25 liver, and 12 heart recipients at a median 5.1, 7.0 and 2.0 years post-transplant respectively, and in 29 controls (Study IV)

	Kidney (n=50)		Liver (n=25)		Heart (n=12)		Controls II (n=29)		P value
Estimates of cholesterol synthesis									
Δ^8-cholestenol to cholesterol	9	(5)	11	(4)	12	(5)	12	(5)	0.015 [c]
Desmosterol to cholesterol	64	(12)	75	(14)	69	(14)	55	(11)	<0.001 [a, c, d, e]
Lathosterol to cholesterol	133	(50)	145	(44)	154	(59)	108	(29)	0.008 [d, e]
Estimates of cholesterol absorption									
Campesterol to cholesterol	332	(118)	250	(80)	296	(89)	357	(100)	0.002 [a, d]
Sitosterol to cholesterol	167	(70)	136	(54)	199	(65)	177	(57)	0.007 [b, d]
Cholestanol to cholesterol	156	(57)	137	(40)	131	(22)	151	(28)	NS

ABBREVIATIONS (Table 11): n=number of patients, P value=P value of significance, SD=standard deviation, NS=non-significant. STATISTICS: [a] kidney vs. liver, $p < 0.05$, [b] liver vs. heart, $p < 0.05$, [c] kidney vs. control, $p < 0.05$, [d] liver vs. control, $p < 0.05$, [e] heart vs. control, $p < 0.05$. NOTES: Ratio is defined as the concentration of particular sterol or cholestanol (10^2 x mmol) to the concentration of cholesterol (mol).

Among all recipients, estimates of cholesterol synthesis correlated inversely with estimates of cholesterol absorption but not with GFR or serum creatinine concentrations. In the liver recipient group serum TC concentration and serum thromboplastin time were positively associated with the ratios of Δ8-cholestenol ($r = 0.452$, $p = 0.023$ and $r = 0.420$, $p = 0.037$ respectively), and lathosterol ($r = 0.467$, $p = 0.019$ and $r = 0.423$, $p = 0.035$ respectively). In the multiple regression model, lower ratios of cholestenol, desmosterol and lathosterol and higher ratios of campesterol and cholestanol were independently associated with RTx compared to LTx or HTx (most signicantly for the ratio of desmosterol: $\beta = 9.53$, $SE = 2.95$, $p = 0.002$ and the ratio of campesterol: $\beta = -0.24$, $SE = 0.07$, $p = 0.002$).

5.7. Other than lipid features of metabolic syndrome

5.7.1. Insulin and insulin resistance

The kidney, liver and heart recipients had higher average serum fasting insulin concentrations and than the controls ($P < 0.001$, Table 12). Hyperinsulinemia, according to fasting levels over the 75th percentile in our controls I (> 5 mU/l in pre-pubertal, > 10 mU/l in pubertal, and > 11 mU/l in post-pubertal children), was found in 66 % (n=33) of the renal, 60 % (n=15) of the liver, and 67 % (n=8) of the heart recipients, $p < 0.001$. When the HOMA indices of our patients were compared to values derived from Canadian pediatric normal population, an elevated HOMA was seen in 40 % (n=20), 40 % (n=10) and 42 % (n=5) of the renal, liver and heart recipients, respectively.

5.7.2. Clustering of risk factors

In Study IV, 28.0 % of the renal, 8.0 % of the liver and 41.7 % of the heart recipients and none of the controls used antihypertensive medication ($p < 0.001$). Either elevated blood pressure or antihypertensive medication was noted in 78.0 % of the renal, 56.0 % of the liver and 66.7 % of the heart recipients, and in 18.9 % of the controls ($p < 0.001$). Both an increased triglyceride concentration and HOMA index higher than 1 was seen in 22 % of our renal, 4 % of our liver and in 39 % of our heart recipients. Clustering of at least three metabolic risk factors according to the modified definition of metabolic syndrome (EGIR) was seen in 24.0 %, 12.5 % and 33.3 % of the renal, liver and heart recipients respectively (difference: NS).

In the 45 renal recipients (Study V), the final multiple logistic regression model showed the HOMA index to be associated with pubertal stage [*OR = 7.2 (95 % CI: 1.5-35.7), p = 0.015*] and the intake of riboflavin [*OR = 0.3 (95 % CI: 0.1-0.9), p = 0.033*].

Table 12. Other than lipid features of metabolic syndrome in 50 pediatric renal, 25 liver, and 12 heart recipients at a median of 5.1, 7.0 and 2.0 years post-transplant respectively, and control group I (n=181) and II (n=29)

Metabolic variable	Kidney (n=50)	Liver (n=25)	Heart (n=12)	Controls I (n=181)	Controls II (n=29)
Insulin, mU/l, median (Q^1, Q^3)	10 (7; 16) [a, b]	9 (5; 15)	13 (3; 20)	5 (3; 7)	7 (4; 9)
Pre-pubertal (N=24/15/7/112)	8 (4; 11) [a]	6 (4; 11)	3 (3; 19)	4 (3; 5)	Nd
Pubertal (N=21/8 /4/54)	14 (10; 19) [a]	13 (5; 36)	17 (13; 24)	7 (5; 10)	Nd
Post-pubertal (N=5/2/1/15)	9 (8; 14)	(9; 18)	13	6 (5; 14)	Nd
High fasting insulin, % (n)	10.0 (5)	12.0 (3)	16.7 (2)	1.7 (3)	Nd
Hyperinsulinemia in OGTT, % (n)	3.9 (2)	8.0 (2)	8.3 (1)	Nd	Nd
HOMA, median (Q^1, Q^3)	2.2 (1.4; 3.3)	1.8 (0.9; 2.9)	2.5 (0.6; 4.5)	Nd	1.4 (0.8; 1.9)
Pre-pubertal (N=24/15/7)	1.6 (0.8; 2.4)	1.2 (0.9; 2.2)	0.7 (0.4; 4.3)		Nd
Pubertal (N=21/8/4)	2.9 (2.2; 4.4)	2.9 (2.0; 7.0)	3.6 (2.5; 5.1)		Nd
Post-pubertal (N=5/2/1)	1.7 (1.6; 2.9)	2.7 (1.7; 3.7)	2.9		Nd
High glucose concentration, % (n) [c]	18.0 (9)	32.0 (8)	41.7 (5)	Nd	Nd
High body mass index, % (n)	10.0 (5)	16.0 (4)	25.0 (3)	8.8 (16)	Nd
High systolic blood pressure, % (n)	60.0 (30) [a]	48.0 (12)	25.0 (3)	14.4 (26)	Nd
High diastolic blood pressure, % (n)	22.0 (11) [a]	28.0 (7)	25.0 (3)	8.3 (15)	Nd

ABBREVIATIONS: HOMA= homeostasis model assessment index for insulin resistance (Matthews et al. 1985)[298], N=number of patients, Nd=not determined, OGTT=oral glucose tolerance test. STATISTICS: [a]Tx groups and control I, p < 0.001, [b]Tx groups and control II, p=0.01. NOTES: [c]An elevated concentration of fasting blood glucose or impaired glucose tolerance according to 2-hour post glucose challenge (WHO criteria).

6. DISCUSSION

6.1. Prevalence of dyslipidemia after transplantation

6.1.1. Triglyceride

Dyslipidemia in our patients was less frequent and less severe than that reported in most adult studies, and also somewhat less severe than in some pediatric studies [228, 300, 322, 373, 407, 410, 416, 420, 471]. On the other hand, other studies on pediatric solid organ recipients have described an approximately similar prevalence of dyslipidemia that reported here [94, 96, 169, 204, 356, 421].

After Tx, hypertriglyceridemia, which was the main lipid abnormality in our recipients, was found in approximately 50 % of the renal and heart recipients, and in approximately 30 % in the liver recipients. RTx improved the lipid profiles, as before RTx, the renal recipients had severe hypertriglyceridemia, typical of patients with end-stage kidney disease and on dialysis. The liver recipients had mild hypertriglyceridemia both before and after LTx, while the heart recipients had more severe hypertriglyceridemia after HTx. These lipid patterns were in line with the literature on end-stage disease and follow-up after Tx.

The most common lipoprotein abnormalities that are seen in hypertriglyceridemia are higher concentrations of chylomicron remnants, VLDL remnants, and IDL lipoproteins, likewise lower levels of HDL. In addition, circulating LDL particles change and become lipid-depleted, smaller in size and denser. [398, 403]

6.1.2. Cholesterol and low-density lipoprotein particle size

Before RTx, our renal recipients had hypercholesterolemia, similarly to the patients with end-stage kidney disease and on dialysis studied before [32, 373]. Our renal recipients' pre-RTx hypercholesterolemia was probably due to the influence of ESRD on serum lipids but presumably also due to the use of peritoneal dialysis. Patients with end-stage liver disease had their serum cholesterol levels from high (as in biliary atresia or in familial hypercholesterolemia) to low (as in hepatitis with hepatocyte failure), as expected. Our heart recipients had hypocholesterolemia before HTx, as also reported in adult heart recipients [47, 140, 432].

Many studies have reported a high LDL-C level, though often concurrently with a high HDL-C level, to persist as a long-term complication of solid organ Tx [1, 54, 60, 223, 224, 242, 254, 476]. In our renal recipients, high LDL-C levels and low ratios of HDL-C to TC were less frequent than during ESRD and, like triglyceride values, also seemed less prevalent and less severe than in earlier reports on adult renal recipients. Earlier reports on pediatric renal recipients have revealed approximately similar or possibly somewhat higher serum LDL-C levels than was seen in our renal recipients. Markedly elevated LDL-C levels (> 95th percentile) were as rare in our renal recipients as in the controls. Our renal recipients, however, had a higher prevalence of slightly increased LDL-C levels (> 3.0 mmol/l) than our liver or heart recipients, or the controls. On the average, the solid organ recipients did not have low HDL-C levels. Indeed, the renal recipients had the highest HDL-C levels and their HDL/TC was comparable with the controls after the first post-Tx year. This was observed despite the high frequency of high triglyceride levels. This lipid pattern has also been reported for other solid organ recipients receiving glucocorticoids.

Infrequency of low HDL-C levels in our pediatric solid organ recipients might indicate that catabolism of triglyceride rich lipoproteins is efficient enough and formation of small-dense LDL is not seen [84, 377]. In fact, the LDL particle sizes were on average larger in our solid organ recipients than in the controls, although the absolute differences were small. HDL concentration and function may be altered via reduced CETP activity which supports the accumulation of lipid-loaded larger sized LDL particles. The rare occurrence of small-dense LDL may also be explained by age, as full penetration of small-dense LDL phenotype has been reported in males over 20 years of age [36]. Despite the possible abnormalities in lipoprotein kinetics, there was an inverse association between serum triglyceride concentration and serum HDL-C level and LDL particle size in our renal recipients. As triglyceride levels were similarly elevated in the majority of our patients, the HDL-C levels were stronger predictors of the LDL particle sizes than the triglyceride levels.

Lipid-loaded HDL pool has been assumed to function as atheroprotectively in reverse cholesterol transport [286]. If applicable to our renal recipients, the mildly elevated LDL-C level may not be a strong risk factor for future cardiovascular disease. Hypercholesterolemia in our liver and also in most of our heart recipients and controls was equally infrequent and less frequent than in other pediatric studies [300, 324]. After HTx, the increase in serum cholesterol levels was possibly due in part to improved nutritional status and heart function as has been suggested in adult studies. However, some of the rise seems to be a complication of HTx as the LDL-C levels reached the level of dyslipidemia in approximately 30 % of the heart recipients. In the absence of better knowledge, the mildly elevated LDL-C levels emphasize the need for secondary prevention of atherosclerosis in also pediatric solid organ recipients as in adults [228].

6.1.3. Low-density lipoprotein susceptibility to oxidation

Renal recipients receiving CsA have so far had a shorter lag time of LDL oxidation possibly due to the suggested pro-oxidant effect of CsA. This pro-oxidant effect of CsA was not seen in our patients. Our patients used CsA in micro-emulsion composition containing DL-alpha tocopherol, which may act as an anti-oxidative agent. Further, in vitro studies do not suggest that CsA is a direct pro-oxidant [121].

6.2. Absorption efficiency and synthesis of cholesterol after transplantation

According to the ALERT Study, statin therapy results in an approximately 30 % decrease in LDL-C levels in renal recipients and administration of ezetimibe (inhibitor of cholesterol absorption) and an approximately 20 % reduction in LDL-C levels in heart recipients [194, 354]. Our liver recipients had their serum LDL-C concentrations roughly 20 % lower than the renal recipients. This difference in the LDL-C levels could be explained by a difference in either cholesterol synthesis or in the absorption efficiency of cholesterol between the two patient categories according to the studies above. The renal recipients seemed to have somewhat more efficient absorption and lower synthesis of cholesterol than the liver and heart recipients, although this was not consistently seen in all variables studied. As higher synthesis of cholesterol was associated with lower absorption efficiency of cholesterol and vice versa among our patients, these figures did not seem to explain the difference in the lipid levels between our patient categories.

The pattern of low synthesis and high absorption of cholesterol has been associated with decreased clearance of LDL in healthy males [312]. Further, delayed clearance of LDL has been seen in ESRD and in heart recipients with triple immunosuppressive therapy (CsA, prednisone, AZA) [196, 369]. Our renal recipients with moderately lowered GFR may have delayed clearance of LDL, as was indirectly suggested by the pattern of low synthesis and high absorption of cholesterol. By contrast, our liver or heart recipients with triple therapy did not seem to have delayed clearance of cholesterol. A difference in kinetics of LDL could have been behind the different lipid levels between our solid organ recipient categories.

6.3. Obesity and distribution of body fat

Estimates of obesity, BMI SDS and lean body mass (calculated from weight and skin-fold measurements, data not shown) were similar between our patients and controls. However, our renal recipients had smaller average triceps skin-fold

values and higher waist-to hip-circumference ratios than the controls. This suggests a central accumulation of body fat in the solid organ recipients. Thinner triceps skin-folds in our patients were unlikely to be due to poor nutrition, as the mid-upper arm circumferences and BMI SDS were comparable between our patients and controls. Glucocorticoid therapy predisposes to central accumulation of body fat and may be behind the differences noted in the anthropometric measures.

6.4. Etiology of dyslipidemia after transplantation

The serum lipid levels unified from the organ and end-stage disease specific levels after Tx, although some differences persisted. Fernandez-Miranda and co-writers (1998) suggested that the higher serum lipid levels in their renal than liver recipients were due to more effective glucocorticoid therapy in the renal recipients [142]. By contrast, all our three solid organ recipient categories received similar immunosuppression on average. The difference between our patient categories could have been due to differences in the functioning of the grafted organs, as laboratory markers of graft function showed that GFR in the renal recipients and ALT in liver recipients were to some extent abnormal. Further, this altered graft function is likely to have influenced serum lipid levels in opposite directions in the renal and liver recipients. In addition, absolute elevations in serum triglyceride concentrations were most apparent in our renal recipients and thus provided most substrate for hepatic LDL synthesis. A renal transplantation summarizes the influence of some known risk factors of dyslipidemia, like possibly deteriorating graft function and proteinuria, especially in the presence of nephrotic range proteinuria. Dyslipidemia and GFR were not associated in this study on renal recipients with fairly good GFRs. Pronounced increases in serum cholesterol and triglyceride concentrations have been reported in patients with GFR less than 30 ml/min/1.73 m^2 [1, 58]. Significant proteinuria (> 200 mg/d) in the few renal recipients was associated with dyslipidemia (a low HDL/TC, a high serum triglyceride level). Proteinuria is associated with multiple defects in lipid metabolism, such as overproduction of lipoproteins by the liver and loss of several apolipoproteins and possibly loss of transfer proteins into the urine.

A high BMI SDS was a risk factor for dyslipidemia in our patients as has also been observed in earlier studies. In Study I, age was a risk factor of high triglyceride levels in the renal recipients and fasting insulin levels were higher in the pubertal than pre-pubertal solid organ recipients. Accordingly, age as a risk factor may have been a surrogate of metabolic alterations of puberty in the renal recipients. After pediatric transplantation, major gender differences in serum lipid levels have not been reported in the literature and were not seen in our study either.

The pre-Tx lipid values explained some of the variation in the post-Tx lipid values. This impact includes the inherited pattern of lipoprotein metabolism, but

also reflects the influence of acquired circumstances like family lifestyle, etiology of solid organ end-stage disease and exposure to cancer therapy agents before Tx. Apolipoprotein E phenotype polymorphisms as genetic risk factors of dyslipidemia did not seem to promote dyslipidemia in our study.

Liver tumor as a risk factor of post-LTx hypercholesterolemia has not previously been described. The number of the liver recipients was limited, but earlier reports on non-transplant patients give some support to the possible association. An increased prevalence of metabolic syndrome has been observed in young adults treated with cancer therapy agents due to their malignant disease in childhood. Cancer therapy agents may damage internal organs or hypothalamus, which may further result in deficient GH production and its metabolic consequences [107, 122, 334, 445]. Other indications for Tx, especially NPHS1, did not increase the risk of post-Tx dyslipidemia.

The reduction in serum triglyceride concentrations and improvement of HDL/TC during the first three years after Tx coincided with a reduction in the median dosage of CsA and MP, but we did not detect independent positive associations of actual or cumulative doses of MP with serum triglyceride levels. Our patients were on low dose steroids, given every other day. The use of low dose glucocorticoids may also maintain good graft function and acceptable serum lipid levels at least in a sub-population of renal recipients [381]. In the view of the literature, glucocorticoid therapy probably explained some of the similarly elevated serum triglyceride and insulin concentrations in the three patient categories, and possibly also some of the clustering of the risk factors of metabolic syndrome. As a possibly beneficial lipid effect of the use of glucocorticoids, our renal recipients had higher concentrations of HDL-C and ApoA-I than the controls. This trend was also seen in the liver and heart recipients and has also been seen in many other studies. The means of this increase in serum HDL-C levels due to glucocorticoids may be increased ApoA-I messenger RNA levels and reduced plasma cholesterol ester transfer protein activity [276, 431]. In our study, larger doses of MP were associated with more efficient absorption efficiency of cholesterol and with diminished synthesis of cholesterol. Prednisone therapy has increased jejunal uptake of cholesterol and also intestinal uptake of fatty acids in rats, though the mechanism remained unknown [456].

The use of CsA has been associated with unfavorable serum lipid profile and increases in serum LDL-C levels, though not in all patient populations. Our data supported the association with a high B-CsA trough concentration and an unfavorable HDL/TC especially in our renal recipients. On the other hand, either CsA doses or B-CsA trough concentrations were not associated with the serum LDL-C levels. In the heart recipients, B-CsA trough levels were inversely associated with LDL particle sizes. This result is in accordance with earlier reports, as therapy with CsA compared to AZA (both agents given with prednisone) has been associated with smaller LDL particle size after RTx [470]. CsA may mediate hypercholesterolemia through impaired LDL-receptor expression and further lower catabolic rate of LDL [10, 294]. In our patients, larger doses of CsA were associated with more efficient absorption efficiency and

lower synthesis of cholesterol, although not consistently, which may indirectly support delayed clearance of LDL.

The use of beta-blockers and diuretics, though rare in our patients, was associated with a low HDL/TC. These antihypertensives are known to promote dyslipidemia [60]. Our renal and liver recipients with short stature and RhGH therapy had a tendency to hypertriglyceridemia already before the initiation of RhGH. The RhGH therapy was not associated with additional risk of hypertriglyceridemia, which is in line with earlier observations [193].

6.5. Diet after transplantation

The intake of macronutrients in our solid organ recipients and controls was similar, and also comparable to the Finnish literature [259]. Thus, diet did not explain the difference in the prevalence of dyslipidemia between our patient categories. The number of the patients was limited and we were not able to assess the influence of diet on individual serum lipid profiles.

In general population, the large amount of ingested saturated fat and cholesterol, small amount of ingested polyunsaturated fat, and excess total calories which lead to obesity, are well-known risk factors for dyslipidemia. Dietary intervention with restrictions on the amount of ingested of saturated fat and cholesterol and abundance of polyunsaturated fat would result in approximately 0.6 mmol/l reduction in serum LDL-C levels [99]. In the renal recipients, the reported intake of saturated fat ranged 9E % (data not shown) and exceeded the recommended 10E % in all. Further, the intake of polyunsaturated fat was below the recommended 5E % in 60 % of the renal recipients. This was observed even though some of the renal recipients had received dietary counseling. Multiple analyses provided some support for the influence of diet on dyslipidemia in individuals. Relatively lower serum HDL-C levels were associated with small amounts of ingested of fat (and accordingly a large amount of carbohydrate) in the renal recipients. This association is also known in general population. In lean patients, the amount of fat may need not to be restricted, although a diet containing a large amount of fat is often also abundant in saturated fat. But, according to the literature, the type of fat ingested is important. Dietary recommendations for the type and the amount of fat ingested are especially important in renal recipients with proteinuria, as they often have significant dyslipidemia during the proteinuric phase and possibly also lose essential fatty acids into the urine.

We observed inverse correlations between serum LDL-C and TC levels and ingested total amount of fiber in the renal recipients. The recommendation for a larger amount of fiber in the diet applies especially to our female renal recipients as their cereal, fruit and berry consumption was lower than that of the female controls. The low fruit intake may have originated from restrictions on dietary potassium during the dialysis phase.

6.6. Metabolic syndrome after transplantation

High fasting insulin concentrations were more frequent in all our three patient categories than in our controls. The prevalence of insulin resistance seemed to be similar among all of our patient categories but higher than in the reference Canadian pediatric population. These figures were comparable to those seen in adult renal and heart recipients [133, 408]. After adult LTx, insulin resistance may be less prevalent than after RTx or HTx [245]. Glucocorticoid therapy causes hyperinsulinemia in general and transplant recipient populations and may have contributed to our patients' hyperinsulinemia as well. Accordingly, fasting insulin levels were higher in our solid organ recipients than in controls. Studies on patients with metabolic syndrome have revealed low cholesterol absorption and most often also increased synthesis [417]. All our three patient categories displayed features of metabolic syndrome but the anticipated pattern of high cholesterol synthesis was seen only in our liver and heart recipients. A thorough explanation for the difference between the patient categories is lacking.

Clustering of metabolic risk factors was seen in our solid organ recipients more often than in the controls. Approximately one quarter of our solid organ recipients had at least three risk factors for metabolic syndrome according to the modified EGIR definition. However, the definition of metabolic changes during childhood and adolescence with adequate prognostic value of future cardiovascular disease is lacking. In pediatric solid organ recipient population, this validation should be done, as this patient population has multiple risk factors for both metabolic syndrome and cardiovascular disease.

6.7. Strengths and limitations

Our study presents a geographically comprehensive sample of the three solid organ recipient categories treated according to the same post-operative protocol and an excellent participation rate of approximately 90 %. As a limitation, the geographical coverage of the controls was limited to the catchment areas of Tampere or Helsinki University Hospitals and the participation rate of the controls could have caused a significant selection bias.

Written instructions were given for blood sampling and processing, but the standard procedure with immediate processing was not always achieved. All laboratory methods were under quality control. Use of drugs was systematically recorded and CsA blood trough level records available, but as a limitation compliance of patients and bioavailability of the drugs used was not assessed.

The BMI SDS estimates may have been falsely low due to altered renal function and decelerated growth in many of our solid organ recipients [399]. Both BMI SDS and skin folds (and percentage of body fat) as estimates of a child's lean body mass assume normal water balance [183]. This may have been disturbed

due to renal, liver or heart dysfunction. Skewing of the BMI data and skin fold data may have caused additional inaccuracy in very obese patients.

Checking and analyzing the food records was done carefully with approved software. However, the food consumption and further the food records may have been influenced by the awareness of being studied. Patients may have chosen easy foods for the recording dates or simply eaten less. As an example, teen-age patients and controls did not report alcohol consumption in their food records, but in the personal interviews admitted to at least occasional alcohol consumption (data not shown).

6.8. Summary and conclusion

Atherosclerotic vascular disease is a major cause of death in long-term survivors of solid organ Tx with the onset of the disease in childhood. Dyslipidemia is a key risk factor in the progression of atherosclerosis in general population. It is frequently found and plays a part in the progression of atherosclerosis after Tx. We studied the prevalence of dyslipidemia in pediatric solid organ recipients and and searched for associations between various risk factors and dyslipidemia.

Tx improved lipid profiles in most patients, although some alterations persisted or emerged after Tx. Hypertriglyceridemia was frequent, with a prevalence of approximately 50 % after RTx and HTx and of approximately 30 % after LTx without a low serum HDL-C concentration. A mildly increased serum LDL-C concentration was observed in approximately 40 % of the renal recipients. Despite the alterations detected, dyslipidemia in our patients was less severe and less prevalent than in earlier, mostly adult studies presumably due to carefully monitored triple therapy and good graft function.

Apolipoprotein E4 isoform carriage was as prevalent in our solid organ recipients as in the background population and not associated with an increased risk of dyslipidemia. High Lp(a) concentrations, small-dense LDL or LDL susceptible to oxidation were not frequent.

The renal recipients had higher absorption efficiency and lower synthesis of cholesterol than the liver recipients, but these differences did not explain the difference in the prevalence of dyslipidemia between the patient categories.

Increased frequency of high fasting insulin and HOMA (compared to Canadian reference population) was seen in 30 %–50 % of the patients. Approximately one quarter of the solid organ recipients had a cluster of at least three metabolic risk factors for cardiovascular disease.

Diet did not explain the higher prevalence of dyslipidemia in the renal recipients than in the liver or heart recipients, or in the controls. However, all the solid organ recipients seemed to consume an excess amount of saturated fat and and most of them also a low amount polyunsaturated fat compared to the recommendations (NCEP III).

Post-Tx dyslipidemia was associated with RTx, presence of mild proteinuria (> 200 mg/d), obesity, increasing age and an underlying predisposition to

dyslipidemia (based on the significance of pre-Tx values to post-Tx dyslipidemia). Associations were also found with dyslipidemia and a high trough concentration of CsA, a high dose of MP and a childhood hepatic tumor as pre-LTx diagnosis. The combination of these risk factors explained a maximum of 60 % of the variation in serum lipid variables.

The significance of the dyslipidemia and metabolic alterations detected in the progression of atherosclerosis and other vascular changes in pediatric, and further young adult, solid organ recipients is a forthcoming challenge. Without further knowledge, maintenance of good graft function without urinary protein excretion by lowest possible doses of immunosuppressive agents, encouragement to active healthy lifestyle with adequate weight control and active motion habits, restriction of saturated fat and cholesterol intake and abundant intake of polyunsaturated fat should be encouraged and carefully followed as a part of post-operative care.

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APPENDIX 1. Definitions of metabolic syndrome

Factor	Defining level				
	WHO (1998) [12]	**EGIR (1999) [44]**	**NCEP III (2002) [457]**	**AACE (2003) [131]**	**IDF (2005) [13]**
	By 1 of the following				
Insulin resistance	Type 2 diabetes FPG ≥6.1 mmol/l IGT or lowered insulin sensitivity*	Insulin resistance (as hyperinsulinemia-top 25% of fasting insulin values among the non-diabetic population)			
	Plus any 2 of the following		**Any 3 of the following**	**FPG↑ or 2h PG↑ plus clinical decision by the following**	**By the following**
Obesity/ Abdominal obesity	BMI > 30 kg/m^2 and/or WHR: Men > 0.9 Women > 0.85	Waist circumference: Men ≥ 94cm Women ≥ 80cm	Waist circumference: Men > 102 cm Women > 88 cm	BMI ≥ 25 kg/ m^2	Waist circumference: European men ≥ 94cm, women ≥ 80cm
					Plus any 2 of the following
Serum lipids	TG ≥1.7 mmol/L and/or HDL-C: Men < 0.9 mmol/l Women <1.0 mmol/l	TG > 2.0 mmol/l and/or HDL-C < 1.0 mmol/l or treatment	TG ≥ 1.7 mmol/l or HDL-C: Men <1.03 mmol/l Women <1.29 mmol/l	TG ≥ 1.69 mmol/l and/or HDL-C: Men < 1.04 mmol/l Women < 1.29 mmol/l	TG ≥ 1.7 mmol/l or treatment or HDL-C treatment or Men < 1.03 mmol/l Women < 1.29 mmol/l
Blood pressure	BP ≥ 140/ ≥ 90 mmHg and/or treatment	BP > 140/90 mmHg or treatment	BP ≥ 130/≥ 85 mmHg	BP ≥ 130/85 mmHg	BP ≥ 130/≥ 85 mmHg or treatment
Blood/ Plasma glucose	FPG ≥ 6.1 mmol/l or type 2 diabetes or IGT	FPG > 6.1-7.0 mmol/l 2h PG >7.8-11.1 mmol/L	FPG ≥ 6.1 mmol/l	FPG 6.1-7.0 mmol/l 2h PG > 7.8 mmol/l	FBG ≥5.6 mmol/l or type 2 diabetes
Other	Microalbuminuria (see notes)			Other risk factors of insulin resistance **	

*ABBREVIATIONS: WHO=World Health Organization, EGIR=European Group for the Study of Insulin Resistance, ATP III= Adult Treatment Panel III , AACE=The American Association of Clinical Endocrinologists, IDF= The new International Diabetes Federation, BMI=body mass index, WHR=waist-hip ratio, TG=Serum/plasma triglyceride concentration, HDL-C= Serum/plasma high-density lipoprotein cholesterol concentration, BP=blood pressure, FPG=fasting plasma glucose, FBG=fasting blood glucose, IGT=impaired glucose tolerance. *For those with normal fasting glucose levels (<6.1 mmol/l), glucose uptake below the lowest quartile for reference population under investigation under hyperinsulinemic, euglycemic conditions below the lowest quartile for reference population under investigation under hyperinsulinemic, euglycemic conditions. ** Includes family history of type 2 diabetes mellitus, hypertension or cardiovascular disease, polycystic ovary syndrome, sedentary lifestyle, advancing age, and ethnic groups susceptible to type 2 diabetes mellitus. NOTES: Microalbuminuria defined as urinary albumin excretion rate ≥20 µg/min or albumin:creatinine ratio ≥30 mg/g*

APPENDIX 2. Definitions of fat-restricted diets

American Heart Association step I diet:

1. Dietary total fat <30 percent of total calories (E%)
2. Dietary saturated fat <10 E%
3. Dietary cholesterol <300 mg/day

American Heart Association step II diet:

1. Dietary total fat <30 percent of total calories (E%),
2. Dietary saturated fat <7 E%
3. Dietary cholesterol <200 mg/day

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Serum Lipids in Children Three to Five years after Kidney, Liver and Heart Transplantation

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Abbreviations

ALT	alanine aminotransferase
ANOVA	analysis of variance
AZA	azathioprine
B-CsA	blood cyclosporine concentration
BMI SDS	body mass index standard deviation score
CI	confidence interval
CsA	cyclosporine
dU-prot	daily urine protein excretion
GFR	glomerular filtration rate
GH	growth hormone
HC	hydrocortisone
HDL/TC	ratio of serum high-density lipoprotein cholesterol concentration to serum total cholesterol concentration
HDL-C	serum high-density lipoprotein cholesterol
HOMA	homeostasis model assessment of insulin resistance
HSDS	height standard deviation score
HTx	heart transplantation
KTx	kidney transplantation
LDL-C	serum low-density lipoprotein cholesterol
LTx	liver transplantation
MP	methylprednisolone
N	number of patients
NPHS1	congenital nephrotic syndrome
OR	odds ratio
Q_1	25^{th} percentile, lower quartile
Q_3	75^{th} percentile, upper quartile
SD	standard deviation
TC	serum total cholesterol
TT	thromboplastin time
Tx	transplantation

ABSTRACT

Background. Although, dyslipidemia is common after solid organ transplantation (Tx), there are few long-term studies in children. *Methods.* We investigated the prevalence of dyslipidemia up to 5 years after Tx in 125 children on triple immunosuppression with one of three different well-functioning grafts: kidney (KTx), liver (LTx) and heart (HTx) and 181 controls. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations were measured annually. Low-density lipoprotein cholesterol concentrations were calculated. Risk factors for dyslipidemia were determined at three years. *Results.* There was a high prevalence of hypertriglyceridemia in all three groups, 50% in the KTx and HTx and 30% in the LTx group. In addition, 50% of KTx patients had a high TC. In the Tx groups together, the following independent associations were observed: KTx and high pre-Tx TC were associated with high TC and high trough concentration of blood cyclosporine with low HDL-C, older age at Tx accounted for higher TG. *Conclusions.* Dyslipidemia, especially hypertriglyceridemia, was common 3 to 5 years after Tx. The etiology is multifactorial and depends on the transplanted organ.

KEYWORDS: transplantation, cholesterol, triglyceride, pediatric, cyclosporine, methylprednisolone

INTRODUCTION

In adults, atherosclerotic vascular disease is the most important cause of death and graft loss after kidney (KTx) (1) and heart transplantation (HTx) (2). The incidence of symptomatic arterial disease after KTx is three- to four-fold that of the background population (3, 4). In the general population, high total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and low high-density cholesterol (HDL-C) concentration are well-known risk factors for atherosclerosis (5) and they are also believed to promote atherosclerosis after Tx (3). The reported prevalence of hyperlipidemia in adults after KTx varies widely, from 16 to 70 % (6). After liver transplantation (LTx) 16 to 50 % of patients have had elevated serum TC and about 40 % have had hypertriglyceridemia (7). LDL-C and TG remains high at six months despite post-operative dietary recommendations in 64 % and 41 % of HTx patients, respectively (8). Similarly, dyslipidemia seems to complicate pediatric solid organ Tx (9, 10, 11), though there are few prospective long-term data regarding its permanence.

Dyslipidemias have been considered the most significant non-immunological risk factors for transplant vascular disease (12). Especially hypertriglyceridemia has been considered a strong predictor of graft loss and chronic rejection (13, 14). The role of dyslipidemia in promoting post-transplantation atherosclerotic vascular disease has also been doubted, as there is little data on the effect of lipid-lowering therapy in the prevention of cardiovascular disease and chronic allograft rejection in the long term. However, lipid-lowering therapy improves lipid values, without considerable adverse effects in the short term (15, 16). In November 2002, U. S. FDA approved atorvastatin and pravastatin for treatment of familial hypercholesterolemia in children older than eight years. Preliminary results of atorvastatin and pravastatin inreducing cholesterol levels in pediatric heart transplant recipients have also been published (17, 18)

Pretransplantation disease and lipid levels, weight gain after Tx, age, sex, the presence of diabetes, reduced kidney function, proteinuria, unfavorable diet and the use of cyclosporine A (CsA), corticosteroids and antihypertensives (ß-blockers and diuretics) have all been associated with dyslipidemia after Tx (9, 16, 19, 20). Though these risk factors are common their relative importance has been difficult to determine.

The aim of the present study was to characterize the serum lipid profile and to determine the prevalence and permanence of dyslipidemias in children one to five years after KTx, LTx or HTx, and to compare the values with controls. A further aim was to assess the determinants of dyslipidemia three years after Tx in children with stable graft function.

PATIENTS AND METHODS

Subjects

A total of 125 children, who had received a kidney, liver or heart transplant between October 1987 and October 1997 were followed for three to five years after Tx. There were 71, 34 and 20 children in the KTx, LTx and HTx groups, respectively. Correspondingly, 57, 31 and 13 of the children were followed for five years. The study group was selected from all 130 children, who survived at least three years after Tx. Four KTx patients were excluded because of lacking data and one HTx patient because lipid-lowering medication was started at one and a half years. The clinical characteristics are presented in Table 1. All the subjects volunteered for the study, which had been approved by Ethical Committee of Tampere and Helsinki University Hospital.

All children with end-stage kidney disease were on peritoneal dialysis before KTx. Pre-Tx diagnoses were: congenital nephrosis (n=38), urethral valve (n=10), nephronophthisis (n=5), polycystic kidney disease (n=3), prune-belly syndrome (n=2), vesico-ureteral reflux (n=2), glomerulonephritis (n=2), Alport´s syndrome (n=1), mega-ureter (n=1), neuroblastoma (n=1), dysplastic kidney (n=1), vaginal cancer (n=1), bilateral multicystic kidneys (n=1), Denys-Drash syndrome (n=1), renal insufficiency due to a complication of prematurity (n=1) and congenital nephropathy (juvenile nephronophthisis) (n=1). Bilateral nephrectomy was undertaken prior to Tx in all patients with congenital nephrosis (NPHS1) except one, and uni- or bilateral nephrectomy in 8 patients with other diagnoses.

Indications for LTx were biliary atresia (n=12), tyrosinemia (n=7), hepatitis (one neonatal) (n=5), hepatoblastoma (n=4), Wilson´s disease (n=2), hepatocellular carcinoma (n=1), homozygous familial hypercholesterolemia (n=1), hepatic adenoma (n=1) and α-1-antitrypsin deficiency (n=1). The high number of malignant liver disease is explained with our active policy of accepting children with hepatoblastoma, without extrahepatic disease, for liver transplantation after chemotherapy (21). Indications for HTx were a congenital heart defect (n=9) and restrictive or dilative cardiomyopathy (n=11).

Living related donor grafts were transplanted only in the KTx group in 25 (35 %) patients. Seven had a second graft, three in the KTx and four in the LTx group. None of the patients was hypothyroid or diabetic, and all were on a common Finnish diet. If there was marked weight gain after Tx, caloric restriction and a diet low in saturated fat was advised.

The control group was recruited between November 1997 and August 1999 and comprised 181 first-visit pediatric clinic outpatients or minor pediatric or oto-rhino-laryngologic surgery patients without regular medication, acute inflammation or metabolic disease.

Immunosuppression

The immunosuppressive protocols have been described (22, 23, 24); they included triple therapy with azathioprine (AZA), CsA and methylprednisolone (MP), in addition to anti-thymocyte globulin after HTx. In the KTx group, the CsA whole-blood through level (B-CsA) was maintained between 80-120 µg/l after the first year and between 100-200 µg/l in the LTx and HTx groups. The CsA dose was individually adjusted, according to through levels and renal function tests to maintain sufficient immunosuppression and to avoid nephrotoxicity. Preschool children took CsA in three daily doses, because of their faster metabolism, and older children in two. In 1994, CsA in a microemulsion composition was introduced. In the KTx group, one boy was on triple therapy with tacrolimus from two years on and two boys received cyclophosphamide instead of AZA at three years as treatment for renephrosis (25). MP was given on alternate days after the first 3 to 6 months after Tx. One girl was switched to tacrolimus and MP during the first year, as were two children during the second year after LTx. One especially steroid-sensitive boy was taken off MP during the first year after LTx. Antihypertensive therapy was instituted if ambulatory measured blood pressure exceeded age-specific reference values. Calcium-channel blockers were mostly used. Two children in the KTx group were on warfarin as thrombosis prophylaxis subsequent to Tx. In one warfarin was replaced with acetosalicylic acid (ASA) between one and two years. All HTx, 6 KTx and 2 LTx patients received ASA after Tx. Acute rejection during the first three months after Tx was treated with MP 1,5 mg/kg per orally, followed by 3 mg/kg/d, for five days or until the blast cell reaction in a fine needle aspirate subsided.

Methods

The following clinical data were collected: gender, age at Tx, preceding nephrectomy (KTx), indication for Tx, living related donor vs. cadaver graft, first or second graft, doses of AZA, CsA, MP and cumulative dose of CsA and MP at three years; the use of antihypertensives (nifedipin, ß-blocker or diuretics), antiepileptics, HC substitution and growth hormone (GH); the occurrence and number of acute rejections, height, weight, kidney function [glomerular filtration rate (GFR), creatinine, daily urinary protein excretion (dU-prot)], liver function [serum alanine aminotransferase (ALT), plasma thromboplastin time (TT), serum total bilirubin], fasting blood glucose and insulin, coronary narrowing in angiography (HTx) and heart function (clinical echocardiography). Data of dyslipidemia or early onset cardiovascular disease (men < 55 years of age, women < 65 years of age) in first or second degree relatives was collected by questionnaire in a subgroup of 80 patients and 180 controls.

Anthropometry

Height was measured using a Harpenden stadiometer (Holtain LTD., Crymych, Dyfed, United Kingdom) and weight on electronic scales at noon. Body mass index (BMI) was calculated according to the formula: weight (kg)/ height^2 (m^2). BMI standard deviation score (BMI SDS) was calculated according to the following equation: (individual BMI-mean BMI for age)/SD, and height standard deviation score (HSDS) according to the following equation: (observed height-mean height for age) / SD. SD represents the standard deviation for the normal Finnish population of the same chronological age and gender (26, 27). Growth velocity was defined as the rate of change in HSDS during follow-up (ΔHSDS). A positive value indicates catch-up growth a negative value deceleration of growth.

Laboratory analyses

Blood samples for lipid, B-CsA through levels, glucose and insulin were taken after overnight fasting, while blood samples for other laboratory tests were taken the previous day. A 24-hour urine sample was collected within a week from the fasting blood sample. B-CsA was determined by specific monoclonal radioimmunoassay. GFR was determined by ^{51}Cr-EDTA clearance (22). If the distribution space deviated more than 10 % from the expected extracellular fluid volume the result was ignored. DU-prot, determined from 24-hour urine output, creatinine, ALT, TT (expressed as percentage of normal mean), total bilirubin, insulin and glucose were determined by routine laboratory methods. A result above the age- and sex-specific reference value of the laboratory was considered abnormal. Insulin resistance index as homeostasis model assessment (HOMA) was calculated according to the equation: resistance=insulin/(22.5$e^{-\ln \text{glucose}}$) (28).

Lipid analyses were performed on fresh samples according to laboratory routine. TC, HDL-C and TG concentrations were analysed enzymatically (Reagena, Roche). LDL-C was calculated according to Friedewald´s formula though not if TG values exceeded 4.0 mmol/l (29). HDL-C was determined after precipitation of other lipoproteins by dextran sulphate and $MgCl_2$ between 1987 and February 1997 and directly, without precipitation of other lipoproteins, from February 1997 on. During follow-up, the calibrator for TC and TG analyses was changed. The effect of changes in calibrators and methods was corrected by a regression equation.

Statistical analysis

The figures are presented as mean and 95 % confidence interval (CI) and/or range or standard deviation (SD) or median and lower (Q_1=25th percentile) and upper quartile (Q_3=75th percentile) or number (N) and percentages of subjects. MP dose is presented as daily dose,

which equals the dose on alternate days divided by two. The normality of the distribution of the variables was tested by one-sample Kolmogorov-Smirnov goodness of fit test. If the distribution was skewed, parameters were tried to normalize by log transformation. As distribution of TG was skewed, the mean of TG distribution was displayed as geometric mean (the antilogarithm of the mean of the log transformed distribution) instead of arithmetic mean (Fig. 1). Arithmetic means are shown for TC and LDL-C distributions since the distribution was skewed only before Tx.

Differences in means between groups were tested with the analysis of variance (ANOVA) for normally distributed continuous variables or Kruskal-Wallis test for skewed or discrete variables. To evaluate, if changes in variables within time were statistically significant, ANOVA for repeated measures (normal distribution) or Wilcoxon test (skewed or discrete distribution) were used. When making group comparisons in each time-point separately, p-values were multiplied with the number of tests performed to avoid a multiple comparison problem. The significance of differences in the categorical variables was tested by χ^2-test. In cases where the frequencies in the cells were low, Fisher´s exact test was used instead. McNemar test was used to evaluate the significance of changes within time in categorical variables. The association with the pre-Tx and subsequent lipid values to third year values was evaluated by univariate linear regression and coefficients of determination are reported.

Determinants of dyslipidemia were analysed by univariate and multivariate forward stepwise logistic regression at three years as all patients were followed for at least three years. Altogether 120 children, who were treated with CsA and MP, were included in the logistic regression analysis at three years to determine independent significance of the variables associated with dyslipidemia. Lipid variables (TC, HDL-C, LDL-C, TG and HDL/TC) were

dichotomized using the most markedly dyslipidemic quartile as an indicator of dyslipidemia. Analyses were done for all groups together and separately for each Tx group. Possible risk factors are described in Table 2. Variables were divided in three blocks, each of the blocks including transplanted organ or pre-Tx diagnosis and respective pre-Tx lipid data (Table 2). Univariate association between lipid variable and each possible risk factor were first separately analysed by logistic regression. Then, multivariate logistic regression analysis was done using each block of variables separately in the model. Variables significant in the first multivariate blocks were included in the final model. The variables in the model did not correlate significantly (based on the correlation coefficient or cross-tabulation). A variable was included in the multivariate model if its significance was less than 0.05 and removed if the significance was >0.1. Otherwise a p value less than 0.05 was considered statistically significant. Computations were carried out using SPSS for Windows version 10.1 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Clinical characteristics

Table 1 shows some clinical characteristics of the three Tx groups, and the controls. Sex distribution was similar in the LTx and HTx groups, whereas 70 % of the KTx patients were boys (p=0.024). HTx patients were on average older (p=0.004). In average, children in all three patient groups delayed their growth velocity during the first year after Tx [KTx: ΔHSDS = –0.1 ± 0.6 SD (mean ± SD), LTx: ΔHSDS = –0.7 ± 0.5 SD and HTx: ΔHSDS = –0.3 ± 0.5 SD]. Patients in the KTx group showed an average catch-up growth of +0.3 ± 0.6 SD between the first and third year while patients in LTx and HTx groups did not show any catch-up growth (0.0 ± 0.7 SD and 0.0 ± 0.4 SD, respectively). Table 3 shows the medication used. The cumulative MP dose for the first year was highest for LTx patients (p=0.003) and decreased

significantly in all three Tx groups ($p<0.001$) from the first to the third year. KTx patients needed more antihypertensive therapy, but the difference between groups was not statistically significant. The frequency of antihypertensive therapy decreased from one to three years within KTx and LTx groups. GH therapy was introduced until three years to 17 (23.9%), 6 (17.6%) and 3 (15.0%) children in the KTx, LTx and HTx groups, respectively. There was no difference in the prevalence of dyslipidemia or early onset cardiovascular disease in first or second degree relatives between groups.

Graft function

At three years, 35.2% of the KTx patients had a GFR below 60 ml/min/1.73 m^2. The corresponding figures in the LTx and HTx groups were 8.8 % and 10.0 % (Table 4). Nephrotic syndrome (proteinuria >40 mg/ m^2/h with oedema and an albumin concentration <25 g/l) was diagnosed during the first three years in 8 KTx subjects (6 NPHS1, 1 urethral valve, 1 chronic glomerulonephritis). All except one of these 8 patients were in remission at the time of the lipid tests. In the KTx group, mild proteinuria (>200 mg/day) occurred at least once in 14 children (19.7 %), while in the LTx group the figure was four (11.8 %). At three years, a serum protein concentration below normal was seen in 4 (5.6%) children in KTx, 2 (5.9%) in LTx and 4 (20.0%) in HTx group. Reduced liver function (TT < 70 % of the normal mean) was rare in the KTx group (3 %), more common in the LTx (18 %) and HTx (20 %) groups. An abnormal ALT was most common in the LTx group, 21 % vs. 0% in the KTx and HTx groups ($p<0.001$). Increased total bilirubin was seen in 0 %, 9 % and 7 % in the KTx, LTx and HTx patients, respectively. Three children (15 %) in the HTx group had angiographically visible coronary narrowing at three years. In echocardiography, the ejection fraction and fractional shortening were within normal limits.

Serum lipids

TG and associations

Figure 1 shows the mean serum lipid concentrations in our patients and controls. All three patient groups had higher mean TG level than the controls ($p<0.001$). However, the mean TG concentration decreased in all three groups from one to three years ($p<0.001$, Fig. 1). The prevalence of high TG (TG >1.5 mmol/l) varied from 17 to 61 % in the three groups and was 3 to 7 times higher than in the controls ($p<0.001$, Fig. 2). After KTx, the prevalence of high TG decreased from a pre-Tx prevalence of 94 %, and affected only 61 % of the patients at one and 45 % at three years (prevalence prior to Tx vs. one year, $p<0.001$). Mean TG concentration was slightly lower in LTx than in KTx and HTx patients from one to three years (Fig. 1). Pre-Tx TG did not statistically significantly predict the lipid values after Tx, but after Tx, preceding TG values explained 19 to 33% of the variation in TG in all groups.

Statistically significant explanatory variables from univariate and multivariate models lipid variables are given in Tables 5 and 6, respectively. As GH seemed to be associated with high TG in the LTx group, lipid values at one and three years were compared between children without GH and children who were introduced to GH after one year. In the KTx group, children who were introduced to GH had higher mean TG concentration levels before and during GH therapy than children without GH. TG decreased in all children but no change in TG due to GH seemed to occur [TG in children without GH at one year after Tx: 2.17 mmol/l (geometric mean) vs.1.60 mmol/l, respectively, and at three years with and without GH: 1.76 mmol/l vs. 1.40 mmol/l; significance of change between time points: $p=0.007$, significance of difference between groups: $p=0.013$, and interaction between time and group: $p=0.51$, ANOVA for repeated measures]. In the LTx group, GH-treated children also had higher TG, though not statistically significantly, $p=0.082$. Kidney function was not independently

associated with high TG. Patients in the highest quartile of TG were significantly older when all groups were analysed together (7.2 vs. 3.3 y, median, p=0.003 and in the KTx group (6.3 vs. 2.7 y, median, p=0.011).

Cholesterol and associations

Mean TC and LDL-C concentrations were stable after Tx, but HDL-C concentration as well as HDL/TC increased in all groups, p<0.001 (Fig. 1). A difference was observed in the magnitude of improvement of HDL/TC, p=0.03, as this was most obvious in the LTx group. During follow-up, 47% to 56% of the KTx, 6% to 26% of the LTx and 19% to 40% of the HTx children were hypercholesterolemic (Fig. 2). At three years, 39 %, 59 % and 42 % of the children in the KTx, LTx and HTx groups, respectively, had HDL-C, LDL-C and TG within the normal range. The KTx patients had a high mean TC and LDL-C but not a low HDL-C. Pre-Tx TC and HDL-C were of moderate predictive value explaining a maximum of 10 % of the variation in TC and HDL-C at three years in the KTx group. After Tx, the lipid values mostly predicted subsequent lipid values, p<0.01, explaining from 23 % (TC in the LTx group) to 74 % (HDL/TC in the HTx group) of the variation. In the final multivariate model for all Tx groups, a kidney graft together with a high pre-Tx TC explained independently a high TC concentration at three years (Table 6). A high B-CsA concentration explained independently low HDL-C in all groups together, while a high MP dose explained a low HDL-C in the KTx and HTx groups.

As LTx without pre Tx liver failure (N=7) was an independent determinant of the highest quartile of TC (Table 6), the role of hepatic cancer (N=5) was tested against other indications for LTx in the multivariate model and was statistically significant, OR=29.3 (95% CI: 2.4-357.9), p=0.008. The patient with homozygous familial hypercholesterolemia and no liver

failure prior to Tx had normal TC of 4.2-4.9 mmol/l after LTx. Cumulative doses of CsA and MP did not add any new lipid associations.

DISCUSSION

This is the first prospective, long term study in which lipid profiles are followed in kidney, liver and heart transplanted children, all on triple immunosuppression and with acceptable graft function, to find determinants of dyslipidemia. High TG was common in all three Tx patient categories, and significantly more frequent than in controls, with a prevalence of 50 % in the KTx and HTx patients, and 30 % in the LTx ones. However, during the first three years, in every Tx group, mean TG concentration decreased. Before KTx, our patients had severe hypertriglyceridemia, typical of patients with end-stage kidney disease and on dialysis (30, 31). After KTx high TG was much less frequent. LTx patients had mild hypertriglyceridemia before and after LTx, while HTx patients had a more severe hypertriglyceridemia after HTx.
As risk factors for dyslipidemia analysed for all Tx patients together, the risk for high TG at three years after Tx seemed to increase with increasing age at Tx. A kidney graft and a high concentration of TC before Tx were independent risk factors for hypercholesterolemia after Tx. High CsA through level was associated with low HDL-C concentration and the use of β-blocker or diuretic agents with a low HDL/TC. Associations of the risk factors within separate Tx groups are discussed below.

Mean TG concentration was reduced, though not normalized, in every Tx group during the first three years. This change in TG concentration coincided with a reduction in the median dosage of MP and CsA, as well as antihypertensive medication, whilst GH therapy was introduced in one fifth of the children. Glucocorticoids may increase TG after Tx (32, 33, 34). They may decrease their elimination, and increase hepatic synthesis of TG containing

lipoproteins, through the action of lipoprotein lipase and fatty-acid synthase and acetyl-CoA carboxylase (35, 36). However, in our patients a high dose of MP did not show an independent association with high TG. Though, it has to be noted that our patients were on low dose steroids, given every other day. In the LTx patients, the use of GH was associated with high TG, but further analysis showed that GH-treated children had higher TG already before introduction of GH. Thus, GH therapy did not cause an increase in TG in our patients, in line with previous observations (37). In the literature, reduced kidney function and proteinuria have also been associated with high TG (32, 38). In our patients high TG was not independently associated with reduced GFR or urinary protein concentration. This might be explained by the fairly good GFR seen in most of our patients, even in the KTx group, the mean GFR was 68.6 ml/min/1.73 m^2 at three years after Tx.

After Tx, hypercholesterolemia under triple immunosuppression with CsA is a frequent finding and in adult patients mean TC, LDL-C and HDL-C concentrations remain stable from one year on (16, 19, 32, 38, 39, 40, 41, 42). However, our patients showed significant improvement of HDL/TC making HDL/TC comparable with the controls after one year. This reduces the significance of a high LDL-C, assuming that HDL-C would be functionally normal. Hypercholesterolemia was most prevalent in KTx patients but less prevalent and less severe, than in previous reports in adults (20, 42). Low HDL/TC was most prevalent at one and two years after KTx and seen in 17 and 13 % of the KTx patients, respectively. The prevalence of a reduced HDL/TC was much lower, than in adult KTx patients treated with CsA, where one fifth to one third have a significantly reduced HDL/TC (42, 43). Of our LTx patients 16 % had a high TC, similar to controls. This is excellent, as high TC values have been reported in up to 50 % after LTx in childhood (7, 9). Children with heart failure prior to HTx were hypocholesterolemic. Nutritional status and heart function improves after HTx leading to an

increase in TC, HDL-C and LDL-C (44, 45). In 30 % of our children, however, TC exceeded 5.0 mmol/l after HTx and in 20 % LDL-C exceeded 3.0 mmol/l. In adults, high TC is frequent in up to 40 % of patients, though, in children LDL-C has been within normal variation (11, 39, 46). To conclude, the KTx patients differed from those with a liver transplant through their high mean TC, and to a lesser extent LDL-C, but without a low HDL-C concentration. Possible explanation for this difference will be discussed below.

Kidney function, as well as nephrosis and proteinuria, have been related to high TC and low HDL/TC in kidney patients with or without Tx (19, 42, 47, 48). In end-stage kidney disease with a GFR from 30 to 60 ml/min/1.73 m^2, HDL-C values decline (47). KTx patients with a GFR below 54 ml/min/1.73 m^2 have had high TC values, but low HDL-C only with a GFR below 30 ml/min/1.73 m^2 (42). In our patients, low GFR was not independently associated with lipid values. In our KTx patients, proteinuria associated with a low HDL/TC. Thus, even slight proteinuria is a risk factor of dyslipidemia, though it might not be important in explaining the difference between the groups, as proteinuria and low serum protein were equally rare in all three groups. Liver dysfunction is accompanied by multiple changes in lipoprotein metabolism (49, 50). Even though, liver parameters in our patients not were related to the lipid values, the function of a transplanted liver might not be totally comparable to that of the native liver in KTx and HTx patients. As an indicator of slightly altered function, LTx patients had a higher mean ALT than the other patients. Despite of the inclusion of the kidney and liver function variables and pre-Tx lipid data in the multivariate model, a kidney graft was an independent risk factor for hypercholesterolemia.

In the present study, indications for LTx without liver failure were independently associated with high TC after LTx. Two of the five children transplanted because of cancer had TC above

the normal range and, when included in the multivariate model, hepatic cancer compared to other indications for LTx was also an indenpendent risk factor. This might reflect the consequences of cancer therapy agents on internal organs, or be a random association. Other indications for Tx, especially NPHS1, which is associated with severe pathology prior to Tx (51), did not increase the risk of post-transplantation dyslipidemia. Obesity after KTx and HTx has been considered a risk factor for dyslipidemia (42, 46). In the present study, BMI was associated with low HDL/TC in the KTx, possibly with high LDL-C in the LTx patients, and emphasizes adequate weight control after Tx.

The use of CsA has often (40, 52, 53), though not invariably (19, 42, 54), been associated with increased TC and LDL-C, and an unfavourable HDL/TC (53). In the present study, reduction of CsA and MP coincided with improvement of HDL/TC. However, in our KTx patients, B-CsA was independently associated with high TC at three years. Various mechanisms of CsA-induced hypercholesterolemia have been proposed, including diminished LDL-receptor expression (15, 54). Use of corticosteroids, in addition to CsA, has been associated with an increase in TC though not with a decline in HDL/TC (32, 33, 34, 38, 39, 41, 52). In our study, blood pressure therapy with β-blockers or diuretic agents was independently associated with a low HDL/TC within all groups. This might not be independent of the influence of blood pressure, though the association with β-blocker and diuretic use and dyslipidemia is previously reported (19). Finally, the lipid values observed in our patients could reflect individual genetically determined lipid patterns modified by the influence of end-stage disease.

In conclusion, the most important lipid pathology in our kidney, liver and heart transplanted children was an elevated TG concentration without low HDL-C, seen in 40 % of our patients, together with elevated TC and LDL-C, especially in KTx patients. Significant risk factors for

dyslipidemia were a kidney graft, high pre-Tx TC and a high CsA through concentration, increasing age at Tx and β-blocker or diuretic use. Still, within the KTx group, obesity, high dosage of MP, and proteinuria were significant risk factors. Severe dyslipidemia with an increased risk for atherosclerosis was less prevalent in our patients, than in previous mostly adult studies, presumably due to our carefully monitored triple therapy and good graft function. Still, some children are at an increased risk for future vascular complications, and we need to try to further eliminate even this, relatively mild dyslipidemia.

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FIGURE LEGENDS

Figure 1. Serial changes in lipid values of 125 children one to five years after kidney, liver and heart transplantation. Mean and CI 95 % for the mean are displayed.

Abbreviation for figure 1:

KTx: solid line; LTx: dashed line; HTx: spotted line; Control group: shadowed area.

Textboxes in the figure represent results of ANOVA for repeated measures.

Figure 2. Frequencies of dyslipidemias in 125 children one to five years after kidney, liver and heart transplantation and controls.

Abbreviation for figure 2:

KTx: filled column; LTx: hatched with vertical lines; HTx: hatched with oblique lines; Control group: horizontal line behind the columns. ¤ $p<0.05$, ¤¤ $p<0.01$, ¤¤¤ $p<0.001$: significance of difference in frequencies of dyslipidemia between the three Tx and control groups (cross-tabulation). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ significance of difference of dyslipidemia between the Tx groups (cross-tabulation).

Table 1. Clinical characteristics of 125 children studied for dyslipidemia after kidney, liver or heart transplantation and controls. P values of statistically significant differences are displayed in abbreviations.

	Kidney		**Liver**		**Heart**		**Controls**
Gender, Male/Female, N[a]	**50/21**		**16/18**		**9/11**		**112/69**
Median age at Tx, y[b] (range)	**3.8** (1.1 - 15.9)		**3.6** (0.4 -16.3)		**12.3** (1.0 -16.8)		**9.1** (3.2 - 18.7)
Patients with acute rejections, % (N)	**62.0** (44)		**73.5** (25)		**60.0** (12)		
Timepoints	**1 y**	**3 y**	**1 y**	**3 y**	**1 y**	**3 y**	
Height SDS, Mean[c] (95% CI)	**-1.7** (-2.0; -1.5)	**-1.5** (-1.7; -1.2)	**-2.0** (-2.6; -1.5)	**-2.0** (-2.5; -1.5)	**-1.4** (-2.2; -0.7)	**-1.5** (-2.1; -0.8)	**0.2** (0.0; 0.3)
BMI SDS, Mean (95% CI)	**0.5** (0.2; 0.8)	**0.5** (0.2; 0.8)	**0.4** (-0.3; 1.2)	**0.8** (0.3; 1.3)	**0.1** (-0.7; 0.9)	**0.0** (-0.8; 0.9)	**0.3** (0.1; 0.5)
HOMA, Median[d] (Q_1, Q_3)	**1.6** (0.7;2.7)	**1.8** (1.1;3.1)	**0.8** (0.6;1.8)	**1.7** (1.0;3.0)	**2.5** (1.5;3.4)	**1.8** (1.2;3.1)	

N=Number of patients. Q_1=25th percentile, Q_3=75th percentile. CI=confidence interval for mean. [a] Within Tx groups, sex distribution differed, p=0.024. [b]Difference between Tx groups, p=0.004 and all groups at three years, p=0.001. [c]Difference between groups at one and three years, p<0.001 (ANOVA). [d]HOMA=homeostasis model assessment of insulin resistance. Log transformation normalized the distribution. Approximation

of HOMA is based on assumption that normal-weight normal subjects aged < 35 years have an insulin resistance of 1 (28). Mean + 2 SDS for healthy adults is about 2.6 (56). Change in HOMA between time points: p=0.029, difference between groups: p=0.048, interaction between time and group: NS (ANOVA for repeated measures).

Table 2. Block division of the variables in multivariate logistic regression analysis[a].

	All (N=115)	**Kidney group** (N=66)	**Liver group** (N=33)	**Heart group** (N=19)
Block 1	Organ[b]	NPHS1 vs. other[c]	No pre-Tx liver failure vs. other[d]	Cardiomyopathy vs. other[e]
Organ	Respective pre-Tx lipid data (mmol/l)	Respective pre-Tx lipid data (mmol/l)	Respective pre-Tx lipid data (mmol/l)	Respective pre-Tx lipid data (mmol/l)
function	Low GFR[f]	Low GFR[f]	Low GFR[f]	Low GFR[f]
	DU-prot > 200 mg[g]	DU-prot > 200 mg[g]		
	High thromboplastin time[h]	High thromboplastin time[h]	High thromboplastin time[h]	High thromboplastin time[h]
Block 2	Organ[b]	NPHS1 vs. other[c]	No pre-Tx liver failure vs. other[d]	Cardiomyopathy vs. other[e]
Medication	Respective pre-Tx lipid data	Respective pre-Tx lipid data	Respective pre-Tx lipid data	Respective pre-Tx lipid data
	High methylpredinisolone[i]	High methylpredinisolone[i]	High methylpredinisolone[i]	High methylpredinisolone[i]
	B-CsA (µmol/l)	B-CsA (µmol/l)	B-CsA (µmol/l)	B-CsA (µmol/l)
	Growth hormone use[j]	Growth hormone use[j]	Growth hormone use[j]	Growth hormone use[j]
	β-blocker or diuretic use[k]	β-blocker or diuretic use[k]		
Block 3	Organ[b]	NPHS1 vs. other[c]	No pre-Tx liver failure vs. other[d]	Cardiomyopathy vs. other[e]
Clinical	Respective pre-Tx lipid data	Respective pre-Tx lipid data	Respective pre-Tx lipid data	Respective pre-Tx lipid data
characteristics	Age at Tx	Age at Tx	Age at Tx	Age at Tx
	BMI SDS	BMI SDS	BMI SDS	BMI SDS
	Gender[l]	Gender[l]	Gender[l]	Gender[l]

[a]Variables were included categorized or continuous. [b]Transplanted organ (kidney vs. liver vs. heart), [c]NPHS1=congenital nephrotic syndrome vs. other, [d]pre Tx liver failure (hepatic cancer, familial hypercholesterolemia, hepatic adenoma) vs. other, [e]cardiomyopathy vs. other, [f]GFR=glomerular filtration rate (three highest quartiles=0, lowest quartile=1), [g]dU-prot >200 mg (absent=0, present=1), [h]thromboplastin time (three highest

quartiles=0, lowest quartile=1), [i]methylprednisolone dose at three years (three lowest quartiles=0, highest quartile=1), [j]use of ß-blocker or diuretics (0=no, 1=yes), [k]use of GH (0=no, 1=yes), [l]gender (boy vs. girl).

Table 3. Medication at one and three years in 125 children studied for dyslipidemia after kidney, liver or heart transplantation. P values of statistically significant differences are displayed in abbreviations.

	Kidney		Liver		Heart	
	1 y	3 y	1 y	3y	1y	3 y
CsA, mg/kg/day, median (Q_1, Q_3)[a]	**8.0** (5.6;10.6)	**5.4** (4.2;6.8)	**8.7** (5.3;10.5)	**5.3** (3.4;6.6)	**6.9** (5.0;9.5)	**5.7** (4.2;6.6)
B-CsA, µg/l , median (Q_1, Q_3)[b]	**110** (83;147)	**93** (77;113)	**184** (109;225)	**130** (80;160)	**194** (150;244)	**164** (137;196)
MP, mg/kg/day, median (Q_1, Q_3)[a]	**0.2** (0.1;0.2)	**0.1** (0.1;0.1)	**0.2** (0.1;0.2)	**0.1** (0.1;0.1)	**0.1** (0.1;0.2)	**0.1** (0.1;0.1)
MP, mg/kg/year, median (Q_1, Q_3)[a, c]	**87** (78;101)	**46** (35; 52)	**99** (92; 115)	**40** (31;53)	**90** (82;97)	**33** (29;48)
AZA, mg/kg/day, median (Q_1, Q_3)	**1.2** (1.2;1.4)	**1.3** (1.2;1.4)	**1.3** (1.2;1.3)	**1.3** (1.1;1.4)	**1.3** (1.2;1.4)	**1.3** (1.2;1.5)
Antihypertensives, % (N)[d]	**49.3** (35)	**26.8** (19)	**32.4** (11)	**8.8** (3)	**30.0** (6)	**20.0** (4)
β-blockers or diuretics, % (N)	**19.7** (14)	**11.3** (8)	**8.8** (3)	**0** (0)	**15.0** (3)	**5.0** (1)
GH treatment, % (N)[e]	**0** (0)	**23.9** (17)	**0** (0)	**17.6** (6)	**5.0** (1)	**15.0** (3)
HC substitution, % (N)	**25.4** (18)	**15.5** (11)	**38.2** (13)	**47.1** (16)	**75** (15)	**50.0** (10)

Q_1=25th percentile, Q_3=75th percentile, N = number of patients. [a]Change between one and three years: p<0.001 for kidney, liver and heart transplantation group. [b]Change between one and three years: p<0.01 for kidney and liver patients and difference between groups at one and three years, p<0.001. [c]Difference between the groups at one year: p=0.003. [d]Difference between one and three years, kidney transplantation group: p<0.001, liver transplantation group: p=0.008. [e]Difference between one and three years all groups together, p<0.001.

Table 4. Graft function in 125 children 1 and 3 years after kidney, liver or heart transplantation. P values of statistically significant differences are displayed in abbreviations.

	Kidney		Liver		Heart	
	1 y	**3 y**	**1 y**	**3y**	**1y**	**3 y**
GFR, ml/min/1.73 m^2, mean (95% CI)[a] (range)	**76** (71; 82) (26 - 130)	**69** (63; 74) (26 - 144)	**104** (93; 115) (48 - 182)	**98** (88; 108) (38 - 154)	**104** (89; 120) (33 - 161)	**93** (81; 105) (52 - 155)
DU-prot >500 mg, % (N)	**4.2** (3)	**5.6** (4)	**0** (0)	**5.9** (2)	**0** (0)	**0** (0)
>200 mg, % (N)	**11.3** (8)	**11.3** (8)	**2.9** (1)	**8.8** (3)	**5.0** (1)	**0** (0)
ALT,U/l , median (Q_1, Q_3)[a]	**15** (13; 20)	**14** (10; 16)	**29** (19; 56)	**24** (18; 39)	**17** (12; 29)	**13** (11; 15)
Total bilirubin, µmol/l,median (Q_1, Q_3)[b]	**7** (5; 10)	**8** (5; 10)	**10** (6; 15)	**10** (8; 14)	**8** (6; 13)	**9** (6; 11)
TT, %, median (Q_1, Q_3)[c]	**107** (95; 131)	**109** (94; 124)	**87** (75; 102)	**89** (72; 108)	**87** (65; 102)	**81** (75; 106)

N = number of patients. CI = confidence interval for mean. GFR = glomerular filtration rate. GFR was determined by ^{51}Cr-EDTA clearance. If the distribution space deviated more than 10 % from the expected extracellular fluid volume the result was ignored. DU-prot=24-h urine protein excretion, ALT=alanine amino transferase, TT=thromboplastin time. Q_1=25th percentile, Q_3=75th percentile. [a]Time: $p<0.001$, group: $p<0.001$,

time*group: NS (ANOVA for repeated measures), [b]Change in total bilirubin between one and three years: p=0.042 for LTx group [c]Difference between groups: p<0.001 at one and three years.

Table 5. Statistically significant associations from univariate logistic regression analysis between categorized lipid variables [most markedly dyslipidemic lipid quartile (below 25th or above 75th percentile) vs. other quartiles] and independent variables displayed in Table 2.

Group	Lipid variable	Independent variable	OR	(CI 95 %)	Sig.
All (N=120)	High TC	TC before Tx (mmol/l)	**1.22**	(1.05-1.41)	0.009
		High thromboplastin time (s)	**0.26**	(0.07-0.92)	0.040
		Organ			0.005
		Liver vs. kidney	**0.06**	(0.01-0.43)	
		Heart vs. kidney	**0.28**	(0.08-1.05)	
	High LDL-C	Organ			0.015
		Liver vs. kidney	**0.06**	(0.01-0.45)	
		Heart vs. kidney	**0.45**	(0.14-1.51)	
		DU-prot > 200 mg	**4.04**	(1.01-16.16)	0.048
	Low HDL-C	B-CsA (μmol/l)	**1.02**	(1.01-1.02)	0.001
		Organ			0.007
		Liver vs. kidney	**1.63**	(0.56-4.73)	
		Heart vs. kidney	**5.96**	(1.97-18.02)	
	High TG	Age at Tx (years)	**1.12**	(1.03-1.21)	0.009
		High thromboplastin time (s)	**0.17**	(0.04-0.79)	0.023
		Growth hormone use	**2.67**	(1.04-6.86)	0.042
		DU-prot > 200 mg	**4.53**	(1.13-18.20)	0.033
	Low HDL-C/TC	β-blocker or diuretic use	**7.17**	(1.67-30.79)	0.008

Kidney Tx group N=70	High TC	B-CsA (µmol/l)	**1.01**	(1.00-1.02)	0.057
	Low HDL-C	B-CsA (µmol/l)	**1.01**	(1.00-1.02)	0.069
		High methylprednisolone	**5.63**	(1.71-18.51)	0.004
	High TG	Age at Tx (years)	**1.16**	(1.04-1.30)	0.011
	Low HDL-C/TC	BMI SDS	**2.24**	(1.26-3.96)	0.006
		DU-prot > 200 mg	**6.94**	(1.45-33.18)	0.015
		β-blocker or diuretic use	**10.29**	(1.87-56.72)	0.007
Liver Tx group N=30	High TC	No pre Tx liver failure	**8.89**	(1.29-61.06)	0.026
		Cancer vs. other	**29.33**	(2.40-357.85)	0.008
	Low HDL-C	Low GFR	**11.00**	(1.27-95.18)	0.029
	High TG	BMI SDS	**2.17**	(1.00-4.72)	0.051
		Growth hormone use	**17.25**	(1.73-172.00)	0.015
Heart Tx group N=20	Low HDL-C	High methylprednisolone	**19.50**	(1.30-292.67)	0.032

Abbreviations: OR = odds ratio, CI 95 % = 95% confidence interval of odds ratio, Sig. = significance, TC = serum total cholesterol concentration,

Tx = transplantation, LDL-C = serum low-density lipoprotein cholesterol concentration, dU-prot = diurnal urinary protein excretion, HDL-C =

serum high-density lipoprotein cholesterol concentration, B-CsA = blood cyclosporine A trough concentration, TG = serum triglyceride concentration, BMI SDS = body mass index standard deviation score.

Table 6. Results from multivariate logistic regression. Dependent variables were categorized lipid variables. Block division of the included independent variables is displayed in Table 2. Those variables significant in the first blocks were included in the final model.

			Significant in the first block			**Significant in the final block**		
Group	**Lipid variable**	**Independent variable**	**OR**	**(CI 95 %)**	**Sig.**	**OR**	**(CI 95 %)**	**Sig.**
All	High TC	TC before Tx[a]	**1.21**	(1.02-1.45)	0.034	**1.21**	(1.02-1.45)	0.034
		Organ[a]			0.027			0.027
		Liver vs. kidney	**0.05**	(0.01-0.52)		**0.05**	(0.01-0.52)	
		Heart vs. kidney	**0.54**	(0.13-2.25)		**0.54**	(0.13-2.25)	
	Low HDL-C	Organ[b]			0.026			
		Liver vs. kidney	**1.58**	(0.41-6.10)				
		Heart vs. kidney	**5.12**	(1.55-16.92)				
		B-CsA (μmol/l)	**1.02**	(1.004-1.03)	0.006	**1.02**	(1.006-1.023)	0.001
	High TG	DU-prot >200 mg	**4.88**	(1.20-19.84)	0.027			
		TG before Tx (mmo/l)	**1.15**	(0.99-1.35)	0.072	**1.15**	(0.99-1.35)	0.072
		Age at Tx	**1.16**	(1.06-1.28)	0.002	**1.16**	(1.06-1.28)	0.002
	Low HDL-C/TC	β-blocker or diuretic use	**5.33**	(1.08-26.26)	0.040	**7.17**	(1.67-30.79)	0.008
		Age at Tx	**1.10**	(1.00-1.21)	0.052			
Kidney Tx group	High TC	B-CsA (μmol/l)	**1.02**	(1.001-1.03)	0.031	**1.01**	(1.00-1.02)	0.057
	High LDL-C	B-CsA (μmol/l)	**1.03**	(1.001-1.061)	0.042			
	Low HDL-C	DU-prot >200 mg	**6.33**	(0.92-43.68)	0.061			
		High methylprednisolone	**9.25**	(2.00-42.77)	0.004	**6.33**	(1.88-21.30)	0.003
	High TG	Age at Tx (years)	**1.18**	(1.05-1.33)	0.007	**1.16**	(1.04-1.30)	0.011

	Low HDL-C/TC	BMI SDS	**2.03**	(1.15-3.59)	0.014	**2.25**	(1.25-4.05)	0.007
		β-blocker or diuretic use	**5.71**	(0.95-32.24)	0.056	**6.24**	(0.93-41.68)	0.059
		DU-prot > 200 mg	**8.79**	(1.24-62.41)	0.030	**9.73**	(1.51-62.69)	0.017
Liver Tx group	High TC	No pre Tx liver failure[c]	**10.22**	(1.50 -69.76)	0.018	**8.889**	(1.294-61.058)	0.026
	High LDL-C	BMI SDS	**2.17**	(0.99-4.72)	0.051			
	High TG	Growth hormone use	**15.00**	(1.50-150.39)	0.021	**17.25**	(1.730-172.00)	0.015
		Age at Tx	**1.21**	(1.00-1.46)	0.051			
		BMI SDS	**2.87**	(1.17-7.20)	0.022			
Heart Tx group	Low HDL-C	High methylprednisolone	**13.00**	(0.77-219.05)	0.075	**19.50**	(1.30-292.67)	0.032

Abbreviations: OR = odds ratio, CI 95 % =95 % confidence interval of odds ratio, Sig. = significance, TC = serum total cholesterol concentration, Tx = transplantation, HDL-C = serum high-density lipoprotein cholesterol concentration, B-CsA = blood cyclosporine A trough concentration, TG = serum triglyceride concentration, dU-prot = diurnal urinary protein excretion, LDL-C = serum low-density lipoprotein cholesterol concentration, BMI SDS = body mass index standard deviation score.

[a]Significant in all blocks. Results of the second and third block displayed in Table. First block: TC before Tx: OR=1.20, 95% CI (1.01-1.43), p=0.044; organ: liver vs. kidney OR=0.06, 95% CI (0.01-0.54) and heart vs. kidney OR=0.55, 95% CI (0.13-2.30); p=0.031.

[b]Significant in first and third block. Result of the first block displayed in Table. Third block: liver vs. kidney OR=1.73, 95% CI (0.45-6.67) and heart vs. kidney OR=5.62, 95% CI (1.71-18.51); p=0.017.

[c]Significant in all blocks. Result of the first and third block displayed. Second block: OR=8.44, 95% CI (1.22-58.16), p=0.030.

Figure 1

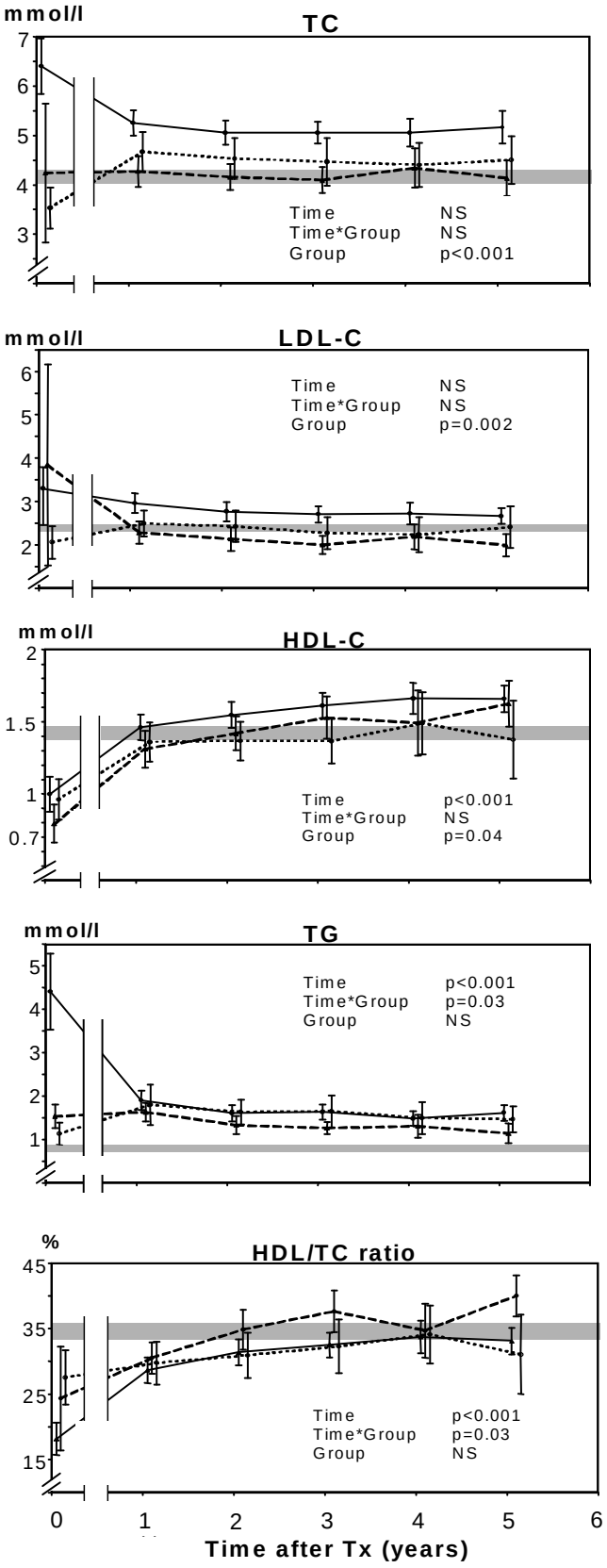
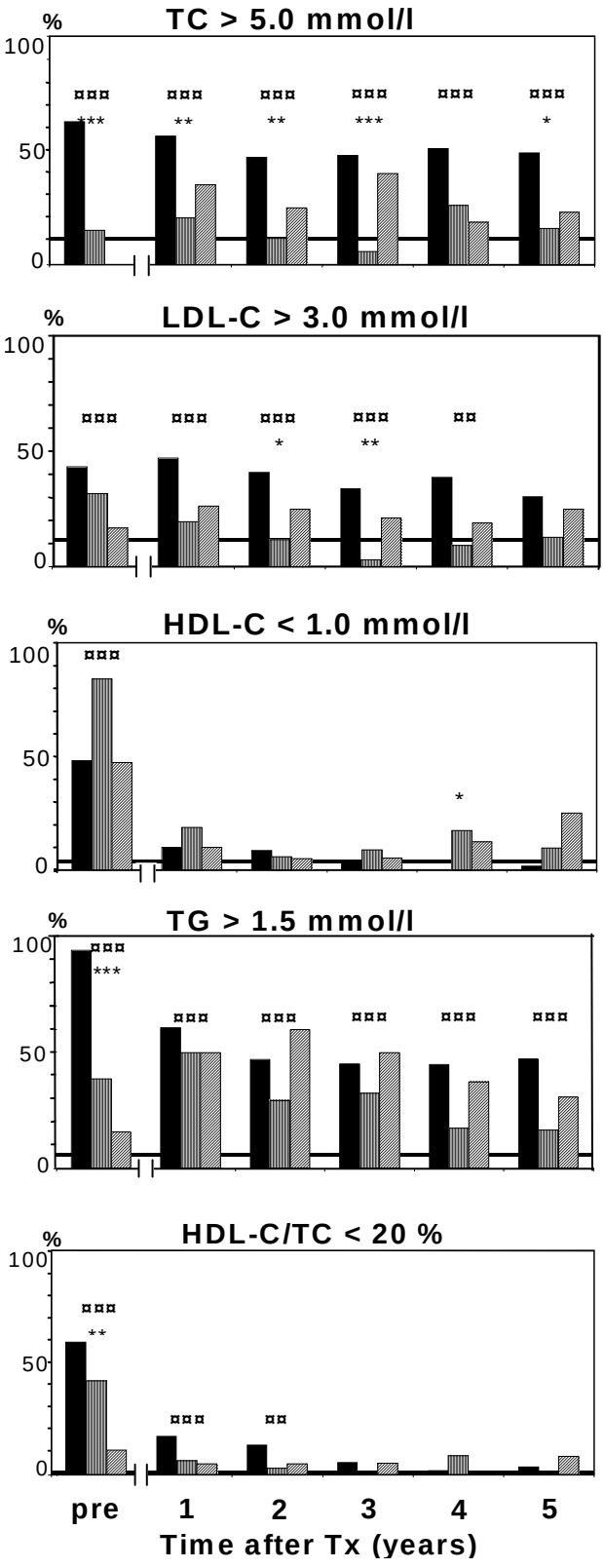

mmol/l
TC
Time NS
Time*Group NS
Group p<0.001
mmol/l
LDL-C
Time NS
Time*Group NS
Group p=0.002
mmol/l
HDL-C
Time p<0.001
Time*Group NS
Group p=0.04
mmol/l
TG
Time p<0.001
Time*Group p=0.03
Group NS
%
HDL/TC ratio
Time p<0.001
Time*Group p=0.03
Group NS
Time after Tx (years)

Figure 2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TC > 5.0 mmol/l
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HDL-C/TC < 20 %
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pre
1
2
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5
Time after Tx (years)

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Pediatric transplantation

Studies on LDL Particle Size and Susceptibility to Oxidation and their Association with Glucose Metabolism in Children after Heart Transplantation [1]

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Abbreviations:

ALT	alanine aminotransferase
ApoE	apolipoprotein E
AZA	azathioprine
B-CsA	blood cyclosporine through level
BMI (SDS)	body mass index standard deviation score
CAD	coronary artery disease
CsA	cyclosporine
GFR	glomerular filtration rate
GH	growth hormone
HC	hydrocortisone
HDL/TC	high-density lipoprotein to serum total cholesterol concentration ratio

HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment for insulin resistance
HSDS	height standard deviation score
HTx	heart transplantation
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
Lp(a)	lipoprotein (a)
MP	methylprednisolone
OGTT	oral glucose tolerance test
TC	total cholesterol
TG	triglyceride
TT	thromboplastin time
Tx	transplantation

ABSTRACT

Background. Elevated concentrations of serum triglyceride (TG) and low-density lipoprotein (LDL) cholesterol, are common after heart transplantation (HTx). These abnormalities might promote transplant vascular disease and atherosclerosis, especially, if LDL is small-dense and oxidized. There are no previous studies on LDL particle size and LDL susceptibility to oxidation in children after HTx. *Methods.* Twenty-three HTx children (aged 3 to 19 years) on triple immunosuppression after HTx and 181 control children within the same age range participated in the study. Total, high-density lipoprotein (HDL-C) and LDL-cholesterol (LDL-C), TG, as well as glucose and insulin concentrations during oral glucose tolerance tests were determined on an average 3 years after HTx (range 1 to 7 years). Moreover, serum lipoprotein (a) concentration, apolipoprotein E phenotype, LDL particle size and indices of LDL susceptibility to copper-induced oxidation were determined from 12 HTx children. *Results.* Hypertriglyceridemia was seen in 56.5 % and hyperinsulinemia in 30.4 % of patients. TG concentration and body mass index were significantly associated with insulin concentration ($p<0.008$ for both). LDL particle size, LDL susceptibility to in vitro oxidation and lipoprotein (a) concentrations did not significantly differ between HTx patients and controls. LDL particle size was inversely associated with cyclosporine (CsA) through level (Neoral®, $r = -0.59$, $p = 0.045$), while weight adjusted dosage of CsA correlated positively with longer lag time of LDL oxidation ($r = 0.69$, $p = 0.013$). *Conclusions.* Hypertriglyceridemia and hyperinsulinemia were common in children on triple immunosuppression after HTx. High CsA through concentration was associated with small LDL particle size but did not increase LDL susceptibility to oxidation.

Keywords

Heart transplantation, child, lipid, lipoprotein(a), LDL particle size, LDL oxidation, insulin, glucose

INTRODUCTION

Despite improved survival rates after heart transplantation (HTx), accelerated coronary artery disease (CAD) is the main cause for poor long-term survival. Transplant vascular disease probably has multiple etiologies from which immunological and infectious factors are thought to be most important. Hypercholesterolemia and hypertriglyceridemia are frequent findings after HTx. Especially, elevated triglyceride (TG) concentration together with insulin resistance and high low-density lipoprotein cholesterol (LDL-C) concentration, are considered significant predisposing factors for transplant vascular disease[1, 2].

LDL seems to promote atherosclerosis especially if the LDL particles are small, dense, and prone to oxidation[3]. Shortened lag time in vitro oxidation of LDL has been shown to be associated with CAD[4]. Oxidized LDL (ox-LDL) may promote atherosclerosis by increased affinity to macrophage scavenger receptors and consequent foam cell and fatty plague formation[5]. Lag time of oxidative modification of LDL in vitro reflects the total antioxidant status of LDL[5].

Valantine et al (2001) has shown in a prospective study that the degree of coronary arterial intimal thickening is correlated with insulin resistance, hyperinsulinemia, hyperglycemia, high TG and low high-density lipoprotein cholesterol (HDL-C) concentrations[6]. These metabolic derangements may be a consequence of usage of immunosuppressive agents, especially corticosteroids.

In addition to LDL, a high concentration of lipoprotein (a) [(Lp(a)], and apolipoprotein (apo) ε4 allele has been shown to be independent risk factors for atherosclerosis[3, 7, 8]. ApoE is a constituent of chylomicrons and very low-density lipoproteins, and their degradation products. ApoE acts as a ligand for LDL and chylomicron remnant receptors in the liver. ApoE is coded in the three codominant alleles ε2, ε3 and ε4 resulting in the respective isoforms (E2, E3, E4) and six different

phenotypes. The isoform E4 has been related to enhanced cholesterol absorption resulting in elevated serum total cholesterol (TC) and LDL-C concentrations while the apoE2 isoform is associated with lipoprotein remnant metabolism and is more frequent in patients with hypertriglyceridemia and Type III dyslipidemia[9, 10]. Also after HTx, the apoE2 isoform has been associated with hypertriglyceridemia[11].

The aim of our study was to assess the impact of HTx on serum lipid and apoprotein concentrations, LDL particle size and indices of LDL susceptibility to in vitro oxidation. Furthermore, we measured insulin and glucose concentrations at fasting state and during oral glucose tolerance test (OGTT) in order to determine whether a specific atherogenic alteration occurs in our HTx patients.

MATERIALS AND METHODS

Subjects

Patients. Twenty-three HTx patients (12 boys and 11 girls), aged 3 to 19 years (mean 13.1 years), with well functioning heart allografts took part in the study during their annual follow-up visits, between June 1996 and February 2000. Mean follow-up time after HTx was 3 years (range 1 - 7 years). At the end of the year 2002, 21 patients were alive with well functioning grafts. One of the patients died 21 months and another 7 years after HTx. HTx was performed due to a congenital heart defect (n=11), dilative cardiomyopathy (n=7) or restrictive cardiomyopathy (n=5). None of the children was either diabetic or hypothyroid. From these patients 13 children (7 boys and 6 girls), within an age-range of 3 to 17 years, were asked to participate in a more detailed cross-sectional study. One boy refused to participate and thus, a subgroup of 12 children were studied for LDL characteristics, Lp(a) and apoE phenotype.

Controls. The control group was recruited between November 1997 and August 1999 and was composed of 181 (112 boys and 69 girls) children. They were patients from the pediatric outpatient clinic and minor pediatric or oto-rhino-laryngologic surgery patients without regular medication, acute inflammation or metabolic disease. Table 1 gives clinical characteristics of HTx patients and controls.

Ethics. All patients and controls, and/or their parents, gave a written informed consent. Ethical Committees of Tampere and Helsinki University Hospital have approved the study.

Medication. Our immunosuppressive protocol has been previously described[12] and included triple therapy with cyclosporine A (CsA) in microemulsion composition, methylprednisolone (MP), azathioprine (AZA), and anti-thymocyte globulin. The CsA whole-blood through level (B-CsA) was maintained between 100-200 µg/l after the first year. The CsA dose was individually adjusted, according to through levels, to maintain sufficient immunosuppression and to avoid nephrotoxicity. Preschool children took CsA in three daily doses, because of their faster metabolism, and older children in two. Recommendations for MP dosage were 0.30 mg/kg/d for 1-4 weeks, 0.25 mg/kg/d for 1 to 6 months, and 0.37 mg/kg/d given on alternate days after the first 6 months. Four of the patients were on a high dose at the time of lipid tests (> 0.3 mg/kg/alternate days; in our patients 0.32 – 0.52 mg/kg/alternate days). No patients were weaned from steroids. Antihypertensive therapy was used if ambulatory measured blood pressure exceeded age-specific reference values. Calcium-channel blockers were mostly used as antihypertensive agents. Acute rejection was diagnosed according to the clinical picture, changes in ultrasound examination, and a cellular reaction in the endomyocardial biopsy (EMB). Rejection was treated with MP 1,5 mg/kg p.o. followed by 3mg/kg/d, for five days or until EMB was normalized. The medication used is displayed in Table 1.

Clinical data. Collected clinical data included patient characteristics, pubertal stage (Tanner´s classification, 1 = pre–pubertal, 2 – 4 = pubertal, 5 = post–pubertal), anthropometry, medication, kidney function [glomerular filtration rate (GFR)] and liver function [alanine amino transferase (ALT) and albumin].

Methods

Anthropometry. Height was measured by a Harpenden stadiometer (Holtain LTD., Crymych, Dyfed, United Kingdom) and weight by electronic scales at noon. Body mass index (BMI) was calculated according to the formula: weight (kg)/ height^2 (m^2). Height standard deviation score (HSDS) was calculated according to the equation: (observed height – mean height for age) / SD, as well as BMI standard deviation score [BMI (SDS)] according to the equation: (calculated individual BMI – mean BMI for age)/SD. SD represents the standard deviation for the Finnish normal population of the same chronological age and gender[13, 14].

Blood sample collection. Blood samples for lipid and B-CsA analyses were taken after an overnight fast and at least 10 min rest. Blood samples for other laboratory tests were taken in the previous day without fasting. Blood sample for LDL in vitro oxidation analysis and particle size were taken into EDTA containing tubes and cooled immediately. Serum and plasma were immediately separated by centrifugation and stored at –70°C. The maximal storage times for apolipoprotein E phenotype, in vitro oxidation of LDL and LDL particle size were 29, 30 and 45 months, respectively.

Laboratory analyses. Glomerular filtration rate (GFR) was determined by ^{51}Cr-EDTA clearance. If the distribution space deviated more than 10 % from the expected extracellular fluid volume the result was ignored. ALT and albumin concentrations were determined according to laboratory routine.

Lipid and apolipoprotein measurements. The fasting serum TG, TC and HDL-C concentrations as well as apolipoprotein B (apoB) and A-I (apoA-I) concentrations were analyzed using Cobas Integra 700 automatic analyser with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). LDL concentration was calculated using the Friedewald´s formula[15]. Serum TG did not exceed 4.0 mmol/l in any of the samples. The inter-assay coefficient of variation of the total cholesterol assessment was 1.4 %, of triglycerides 1.0 %, of HDL cholesterol 3.7 %, of apoA-I 3.8 %, and of apoB 3.5 %.

Lp(a) was determined radioimmunologically[16] from 12 HTx patients and 181 controls. ApoE phenotype was determined from 12 HTx patients by isoelectric focusing and immunoblotting as described previously[17]. The susceptibility of LDL to oxidation and LDL particle size was determined for 88 and 95 controls, respectively, representing the whole control group according to age and sex.

LDL particle size. For the estimation of LDL particle size EDTA-plasma samples were subjected to non-denaturing gradient gel electrophoresis as described by Krauss and Burke (1982)[18]. However, the 2-16% polyacrylamide gels were cast in-house according to the instructions given by Pharmacia (Uppsala, Sweden). To calibrate for particle hydrated diameter we used the High Molecular Weight calibration kit (Pharmacia) supplemented with LDL particles whose peak particle diameter was 25 nm. The LDL was isolated by ultracentrifugation as described[19] and kept at -70°C in 0.15M NaCl/1mM EDTA solution containing 0.6% saccharose. After the 24-hour electrophoresis the gels were first stained with Oil Red O for lipids and thereafter a filter paper soaked with Coomassie Brilliant Blue was placed on the standard lane to stain the proteins. The gels were scanned with a laser densitometer (LKB, Ultroscan 2202) connected to an integrator (Hewlett-Packard, 3309A).

LDL peak particle diameters were determined from a calibration curve constructed from the migration distances and log-transformed diameters of the standards. A control plasma sample (peak particle diameter 27.00 nm) stored at -70°C was included in every gel. The inter-assay coefficient of variation during this study was 1.0%.

Copper-induced oxidation of LDL. Plasma LDL was isolated by single-step nonequilibrium density gradient ultrasentrifugation at 100000 rpm for 30 min at 10ºC as described[20]. The isolated LDL fraction was desalted by passing it through a gel filtration column (Econo-Pac 10 DG, Bio Rad Laboratories, CA, USA). The protein concentration of the eluate was measured using bovine serum albumin as standard. The LDL preparations were diluted with phosphate buffered saline to contain 0.05 g/l protein (≈ 0.1 μmol/l LDL). Oxidation was started by adding 10 μl of freshly prepared 0.167 mmol/l $CuSO_4$ to 1.0 ml of LDL solution in a 1 cm quartz cuvette. Oxidation was determined as the production of hydroperoxides with conjugated double bonds (conjugated dienes) by continuously (at 1 min intervals for 5 h) monitoring the change in absorbance at 234 nm at 37ºC, as described[21]. We used a Perkin Elmer Lambda Bio 10 spectrophotometer (Überlingen, Germany) equipped with an 8-position automatic cell changer. Several indices were obtained from the absorbance versus time curves. The lag time (min) was determined from the intercept of lines drawn through the linear portions of the lag phase and propagation phase. The rate of propagation (Prorate, μmol/l of dienes/min) was obtained from the slope of the absorbance curve during the propagation phase using the molar absorptivity of $\varepsilon_{234\ nm}$ of 29500 l/mol/cm for conjugated dienes. The maximal concentration of dienes formed (MaxDC, nmol/mg LDL protein) was calculated from the difference in absorbance at zero time and at diene peak. In every oxidation run, one reference LDL isolated from a reference plasma stored at –70ºC, was used to control the whole procedure. The intra-assay CV of lag time measurements was 1.5% and the inter-assay was 3.2%.

Oral glucose tolerance test (OGTT) was performed after a 12 h overnight fast and an oral load of glucose 1.75 g/kg (maximum 75 g) was given. Samples for measurement of blood glucose and serum insulin were collected at 0, 30, 60, 90, 120, and 180 min. Blood glucose was analyzed by enzymatic method and serum insulin radioimmunologically. Fasting insulin was determined for healthy controls. Hyperinsulinemia was diagnosed as serum fasting insulin concentration exceeding 20 mU/l and or as a peak insulin concentration exceeding 150 mU/l[22]. Impaired glucose tolerance was diagnosed according to criteria: blood glucose 5.6 - 6.0 mmol/l after an overnight fast or 6.7 - 9.9 mmol/l at 120 min in the OGTT (WHO criteria). Insulin resistance was estimated by homeostasis model assessment [HOMA; insulin resistance=fasting insulin/($22.5e^{-\ln \text{ fasting glucose}}$)][23]. HOMA index is based on assumption that normal adults have a HOMA index of 1.

Statistical analysis

Normality of distributions in the control group was tested by one-sample Kolmogorov-Smirnov goodness of fit test. The figures are represented as mean and 95% confidence interval (CI) and/or range (normal distribution) or median and range and/or 25th and 75th percentile (skewed or discrete distribution). MP dose is presented as daily dose, which equals the dose on alternate days divided by two. Parameters of skewed distributions were tried to normalize by logarithmic transformation. Differences in means between groups were tested with Student´s t test for normally distributed continuous variables or Mann-Whitney test for skewed or discrete variables. The significance of difference in the frequency distribution of independent categorized variables was tested by χ^2-test. In the case the frequencies in the cells were low, Fisher´s exact test was used instead. Bivariate correlation coefficients were calculated by Spearman-Rank correlation. A p value less than 0.05 was considered statistically significant. Computations were carried out using SPSS for Windows version 10.1 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Clinical characteristics of study population. Characteristics of HTx and control patients are displayed in Table 1. Of the HTx children, 34.8 % were pre-pubertal, while in the subgroup of children studied for LDL size and susceptibility to oxidation, 58.3 % and 61.9 % of the HTx and the control children were pre-pubertal, respectively (p = NS). Pre-pubertal patients received significantly higher weight adjusted doses of MP (0.15 vs. 0.08 mg/kg/d, median , respectively, p = 0.002).

Serum lipid and apolipoprotein concentrations. Table 2 shows serum lipid concentrations, LDL characteristics and insulin values in patients and controls. Children in the HTx group had in average higher TG (p<0.001), lower apoA-I (p = 0.037), LDL-C (p = 0.045) concentrations and a higher HDL/TC (p = 0.023). A high Lp(a) concentration above 800 U/l was recorded in one child (8.3%) in the HTx and 4 (2.2%) children in the control group. Age at Tx, time after Tx or age of the graft did not show any statistically significant correlation with these lipid values.

Distribution of apoE phenotypes and associations with lipids. Only two apoE phenotypes were found in these HTx patients: E3/3 (N=7, 58.3 %) and E3/4 (N=5, 41.7 %) with the following frequencies of the apoE isoforms: E3=0.792 and E4=0.208. These do not differ from the frequencies in the Finnish background population[17]. LDL particle size was smaller in patients with the apoE4 isoform than in other patients with E3/3 phenotype (26.4 vs. 26.9 nm, p = 0.041) while lipoprotein concentrations did not differ between apoE phenotypes.

LDL particle size. The majority of our study subjects had one clearly discernible LDL subfraction in gel electrophoresis. One control had two major LDL bands of equal intensity; the larger band was used in analysis. In addition to the most intensively stained LDL band, three patients and 15

controls had an additional LDL band and one control had two additional bands. Small LDL (<25.5 nm) was not present among the major LDL bands, and patients and controls did not differ according to LDL particle size. An LDL diameter <26.5 nm was documented in 3 (25 %) patients and 48 (50.5 %) controls (p = NS). LDL particle size of the patients was positively but insignificantly associated with HDL-C and negatively with TG (HDL-C: r = 0.48, p = 0.115; TG: –0.44, p = 0.149) comparable to the similar associations in controls (HDL-C: r = 0.43, p<0.001; TG: –0.28, p = 0.006). In patients, LDL particle size correlated inversely with B-CsA through level (r = –0.59, p = 0.045), Fig. 1 and Table 3.

LDL susceptibility to in vitro oxidation. LDL susceptibility to in vitro oxidation did not differ between HTx and control groups. Lag time varied from 64 to 99 minutes in patients, and from 58 to 98 minutes in controls. In patients, lag time correlated with CsA dose (r = 0.69, p = 0.013), Table 3 and Fig 2. Impaired glucose tolerance or insulin resistance was not associated with LDL susceptibility to oxidation.

TG and insulin concentrations. High TG concentration (TG > 1.5 mmol/l) was seen in 56.5 % of the HTx patients compared to 7.2 % of the controls (p< 0.001). There was no correlation between TG and LDL-C or HDL-C concentrations in HTx patients (r = –0.11 and r = –0.11, respectively). TG concentration correlated positively with BMI (r = 0.49, p = 0.018). Fasting glucose was normal in all subjects, but impaired glucose tolerance in OGTT was seen in 26.1 % of the HTx patients (N=6). Patients with impaired glucose tolerance were younger and transplanted at an earlier age than those with a normal glucose tolerance (7.5 vs. 17.5 years at OGTT, median, p = 0.014 and 6.5 vs. 12.5 years at Tx, median, p = 0.021, respectively), but the time after transplantation was similar. They had a lower dosage of AZA (1.1 vs. 1.4 mg/kg, p = 0.036), a tendency for a higher TG concentration (2.0 vs. 1.5 mmol/l, median, p = 0.054), two of them also had hyperinsulinemia and

four were in the highest quartile of MP dosage (0.14 - 0.26 mg/kg/d). Hyperinsulinemia either during OGTT or at fasting state was seen in 30.4% (N=7) of the patients and a fasting insulin value above 20 mU/l occurred more often in HTx patients than in controls [21.7% (N=5) vs. 1.7% (N=3), respectively, p = 0.001]. All five patients with high fasting insulin also had higher fasting TG concentration than patients with fasting insulin values within normal range [1.45 vs. 2.14 mmol/l (median), p = 0.007]. Patients with hyperinsulinemia were more obese [BMI (SDS) –0.6 vs. 1.3 SD, p = 0.038]. Fasting insulin and insulin resistance or sensitivity indices correlated strongly with the fasting TG concentration (r = 0.73 for fasting insulin, r = 0.74 for HOMA, p<0.001 for both, Table 3). However, insulin resistance indices did not correlate with the actual dose or with the cumulative yearly dose of MP.

Pubertal development had an influence on the insulin resistance indexes, as pre-pubertal children were most insulin sensitive and the pubertal ones most insulin resistant. The difference in fasting insulin and HOMA insulin resistance index was statistically significant between patients in the pre-pubertal and pubertal or post-pubertal stages in HTx patients (4 vs. 13 mU/l, median, p=0.045 and 1.4 vs. 3.2, median, p = 0.033, respectively). The time from transplantation did not statistically significantly differ between pre-pubertal and pubertal children (1.5 vs. 4.0 years. p = 0.12, but the age of the graft was younger (3.5 vs. 15 years, respectively, p < 0.001). The difference between patients and controls in fasting insulin was seen in pubertal children (median: 17 vs. 7 mU/l, p< 0.001).

DISCUSSION

This is the first study in which in vitro characteristics of LDL are studied in paediatric HTx patients. LDL size and LDL susceptibility to in vitro oxidation did not differ between HTx patients and healthy controls though variability of oxidation time was more than 30 minutes in both groups.

Small LDL size was inversely associated with blood CsA (Neoral®) trough level while CsA trough level was not associated with shortened lag time for in vitro oxidation of LDL. The major abnormalities in our HTx patients were hypertriglyceridemia, (in 56.5 % of the patients) and hyperinsulinemia (in 30.4 %). High TG concentration and obesity were associated with insulin resistance.

Small–dense LDL (< 25.5 nm), which is regarded as a risk factor for atherosclerosis[3], was not found in the major LDL subfractions of our children, and was similarly infrequent in HTx patients and controls. However, a full penetration of small–dense LDL is described in males over 20 years of age [24]. LDL diameter has been associated positively with HDL-C and negatively with TG concentration in adults, with or without Tx[25, 26], and inversely with insulin resistance in Finnish normoglycemic men, though this relationship was modified with TG concentration[27]. In our study, HDL-C and TG were associated with LDL particle size in controls, but the same trend was not statistically significant in our patients, probably due to the lower number. Also, there was only an insignificant association between LDL diameter and fasting insulin concentration. In our patients, a high B-CsA through level seemed to be associated with a smaller LDL particle size. This result is in accordance with previous reports, as therapy with CsA compared to AZA (both agents given with prednisone) has been described to associate with smaller LDL particle size after kidney Tx[28].

In non-transplanted adults, apoE4 phenotype seemed to be associated with smaller LDL particle size, as previously described[29], though not consistently[26, 30]. In our study and in a study on adult HTx patients carried out by Gonzales-Amieva[11], apoE4 phenotype was not associated with high LDL-C as in non-transplanted healthy adults[9].

LDL of HTx patients and controls were equally susceptible to in vitro oxidation. Approximately 30 percent of CsA in plasma is bound to the LDL fraction[31] and might interfere with LDL oxidation. Previously, in kidney transplant patients receiving CsA a shorter lag time for LDL oxidation was seen[28, 32]. In our 12 patients, CsA dose and lag time correlated positively and no pro-oxidant effect was seen. However, our patients used CsA in microemulsion composition containing DL-alpha tocopherol, and thus an anti-oxidant effect is likely due to this drug component[33]. Still, in vitro studies do not suggest CsA to be a direct pro-oxidant[34]. All water-soluble molecules are filtered away from the LDL fraction before copper-induced oxidation of LDL. Thus, serum total bilirubin, urate, and albumin as potential antioxidants in vitro[35, 36], do not interfere with LDL oxidation in our study.

HOMA as an insulin resistance index, estimates hepatic insulin resistance[23] and, was in average higher in obese patients and strongly associated with serum TG concentration. These findings imply that markers of the dysmetabolic syndrome already exist in paediatric heart transplant recipients. HTx children undergoing pubertal development are more insulin resistant than normal children[37]. Further studies are necessary to see whether the dysmetabolic syndrome in children after HTx is associated with the development of transplant CAD as described by Valantine[6].

In conclusion, hypertriglyceridemia and hyperinsulinemia were common in children on triple immunosuppression after HTx. High CsA through concentration was associated with small LDL particle size but did not increase LDL susceptibility to oxidation.

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FIGURE LEGENDS

Figure 1

Association of blood cyclosporine through concentration with low-density lipoprotein particle size.

Figure 2

Association of weight adjusted cyclosporine dose with lag time to in vitro oxidation of low-density lipoprotein.

Table 1. Clinical characteristics and medication of 23 heart transplanted children and 181 controls.

VARIABLES	Heart Tx group (n=23)	Heart Tx subgroup (n=12)	Controls (n=181)
GENERAL CHARACTERISTICS			
Gender, Male/Female	12/11	6/6	112/69
Age, years, mean (range)	13.1 (3.0 - 19.4)[a]	9.6 (3.0 - 17.7)	9.6 (3.2 - 18.7)
Age at Tx, mean (range)	9.9 (1.0 - 16.8)	6.9 (1.0 - 12.7)	
Time after Tx, mean (range)	3.0 (1.0 - 7.0)	2.7 (1.0 - 7.0)	
Height SDS, mean (CI)	–1.3 (–1.8; –0.7)[b]	–1.0 (–1.8; –0.3)	0.2 (0.0; 0.3)
BMI (SDS), mean (CI)	–0.1 (–0.8; 0.7)	0.5 (–0.8; 1.7)	0.3 (0.1; 0.5)
Systolic BP, mmHg, mean (range)	111 (90 - 143)	106 (90 - 128)	109 (83 - 144)
Diastolic BP, mmHg, mean (range)	67 (40 - 97)	64 (40 - 80)	65 (40 - 90)
MEDICATION			
CsA, mg/kg, median (range)	4.7 (2.8 - 10.4)	5.8 (2.8 - 10.4)	
B-CsA, μmol/l, median (range)	159 (105 - 297)	157 (105 - 236)	
MP, mg/kg a.d., median (range)	0.1 (0.04 - 0.3)	0.1 (0.04 - 0.3)	
AZA, mg/kg, median (range)	1.4 (0.8 - 1.7)	1.2 (0.8 - 1.6)	
Use of antihypertensives, % (n)	39.1 (9)	41.7 (5)	
Use of β-blockers and diuretics, % (n)	21.7 (5)	25.0 (3)	
KIDNEY AND LIVER FUNCTION			
GFR ml/min/1.73m^2, mean (range)	91 (41 - 161)	87 (52 - 161)	
Albumin, g/l, mean (range)	41 (33 - 56)	41 (38 - 46)	
ALT, IU/l, median (range)	16 (8 - 103)	16 (8 - 32)	

ABBREVIATIONS for Table 1: CI = 95% confidence interval for mean, Tx = transplantation, SDS = standard deviation score, BMI= body mass index, BP = blood pressure, CsA = cyclosporine A, B-CsA = blood cyclosporine trough concentration, MP = methylprednisolone, AZA =

azathioprine, n = number of patients, GFR = glomerular filtration rate, ALT = alanine aminotransferase.

STATISTICS: [a]Significance for the difference between HTx and control groups, $p = 0.003$. [b]Significance for the difference between HTx and control groups, $p < 0.001$.

Table 2. Serum lipids, LDL particle size and susceptibility to in vitro oxidation, and insulin values in 23 heart transplanted children and 181 controls.

	Heart group (n=23)	HTx sample (n=12)	Control group (n=181)[e]
Lipids and apolipoproteins			
TC, mmol/l, mean (CI)	4.51 (4.13; 4.88)	4.10 (3.58; 4.62)	4.18 (4.07; 4.29)
TG, mmol/l, geometric mean (CI)	1.54 (1.30; 1.83)[a]	1.51 (1.12; 2.03)	0.72 (0.67; 0.77)
HDL-C, mmol/l, mean (CI)	1.36 (1.21; 1.52)	1.33 (1.13; 1.53)	1.42 (1.38; 1.47)
ApoA-I, g/l, mean (CI)		1.24 (1.10; 1.38)[b]	1.39 (1.35; 1.42)
ApoB, g/l, mean (CI)		0.72 (0.62; 0.82)	0.73 (0.70; 0.76)
LDL-C, mmol/l, mean (CI)	2.36 (2.05; 2.68)	2.02 (1.62; 2.42)[c]	2.39 (2.30; 2.48)
HDL/TC-ratio, %, mean (CI)	30.1 (27.7; 33.8)[d]	32.8 (28.4; 37.3)	34.6 (33.5; 35.7)
Lp(a), U/l, median (range)		29 (<17 - 832)	85 (<17 - 1157)
LDL characteristics			
Diameter of LDL, nm, mean (CI)		26.7 (26.4; 27.0)	26.6 (26.5; 26.7)
Lag time, min, mean (CI)		78 (72; 85)	77 (75; 78)
Oxidation rate, μM/min, mean (CI)		0.43 (0.40; 0.46)	0.43 (0.42; 0.44)
Max amount of dienes, nmol/mg, mean (CI)		557 (531; 583)	547 (538; 556)
Insulin			
Fasting insulin, mU/l, median (Q_1; Q_3)	13 (5; 20)[f]	13 (3; 20)	5 (3; 7)
Frequency of high fasting insulin, % (n)	17.4 (4)		1.7 (3)
Frequency of hyperinsulinemia during OGTT, % (n)	17.4 (4)		
Insulin resistance			
HOMA, median (Q_1; Q_3)	2.6 (1.4; 3.7)	2.4 (1.1; 3.5)	

ABBREVIATIONS for Table 2: CI = 95% confidence interval for mean, n = number of patients, Q_1 = lower quartile, 25th percentile; Q_3 = upper quartile, 75th percentile, TC = total cholesterol, TG = triglyceride, HDL-C = high-density lipoprotein cholestrol, apo A-I = apolipoprotein A-I, apo B = apolipoprotein B, LDL-C = low-density lipoprotein cholesterol, Lp(a) = lipoprotein (a), OGTT = oral glucose tolerance test, HOMA = insulin resistance index according to homeostasis model assessment

STATISTICS: [a]Significance for the difference between HTx group and controls: $p < 0.001$; [b]Significance for the difference between HTx subgroup and controls: $p = 0.037$; [c]Significance for the difference between HTx subgroup and controls: $p = 0.045$; [d]Significance for the difference between HTx group and controls: $p = 0.023$; [e]LDL particle size was analysed for 12 children in HTx group 95 children and LDL susceptibility for in vitro oxidation for 88 children in the control group; other analyses were done to 181 controls; [f]Significance for the difference between HTx group and controls: $p < 0.001$.

Table 3. Significant associations between serum lipids, LDL characteristics and insulin in 23 heart transplanted children.

		R*	p-value
Lipoproteins			
LDL-C concentration	Lag time for LDL oxidation (n=12)	0.629	0.028
	Oxidation rate for LDL oxidation (n=12)	0.740	0.006
TG concentration	BMI (SDS)	0.490	0.018
	Fasting insulin concentration	0.730	< 0.001
LDL particle			
Particle size	B-CyA through level (n=12)	– 0.590	0.045
	Apolipoprotein E4+ phenotype (n=5 vs. 7)		0.041
Lag time for oxidation	CyA dose (mg/kg/d) (n=12)	0.692	0.013
Oxidation rate	Lp(a) (n=12)	0.688	0.013
Maximum amount of dienes formed	LDL-C (n=12)	0.660	0.020
	Lp(a) (n=12)	0.741	0.006
Insulin			
Fasting insulin	BMI (SDS)	0.539	0.008
	TG	0.730	< 0.001
	Fasting glucose	0.595	0.003
OGTT insulin area under the curve	BMI (SDS)	0.594	0.003
	TG	0.702	< 0.001
	Fasting glucose	0.427	0.042
Insulin resistance			
HOMA index	BMI (SDS)	0.490	0.018
	TG	– 0.736	< 0.001
	Serum albumin concentration	0.474	0.030
	Serum total bilirubin concentration	0.646	0.003
	Systolic blood pressure	0.530	0.009

ABBREVIATIONS for Table 3: n = number patients, TG = triglyceride, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, B-CsA = blood cyclosporine trough concentration, BMI (SDS)= body mass index standard deviation score, CsA = cyclosporine A, Lp(a) = lipoprotein (a), OGTT = oral glucose tolerance test, HOMA = insulin resistance index according to homeostasis model assessment

**STATISTICS: ***Spearman –Rank correlation. The influence of apolipoprotein E4+ phenotype on LDL particle size was analyzed by Mann-Whitney test.

NOTES: Correlation coefficient was calculated for 23 children if not otherwise stated.

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TITLE PAGE

Insulin resistance, LDL particle size and LDL susceptibility to oxidation in pediatric kidney and liver transplant recipients

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SHORT TITLE PAGE

Title: Insulin resistance, LDL particle size and LDL susceptibility to oxidation in pediatric kidney or liver transplant recipients

Short title: Insulin resistance in kidney transplant recipient children

ABBREVIATIONS

ALT	alanine aminotransferase
ApoA-I	apolipoprotein A-I
ApoB	apolipoprotein B
ApoE	apolipoprotein E
AZA	azathioprine
B-CsA	blood cyclosporine trough level
BMI SDS	body mass index standard deviation score
CI	confidence interval
CsA	cyclosporine A
GFR	glomerular filtration rate
GH	growth hormone
HC	hydrocortisone
HDL/TC	high-density lipoprotein to serum total cholesterol concentration ratio
HDL-C	high-density lipoprotein cholesterol
HOMA	homeostasis model assessment for insulin resistance
HSDS	height standard deviation score
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
Lp (a)	lipoprotein (a)
MP	methylprednisolone
OGTT	oral glucose tolerance test
TC	total cholesterol
TG	triglyceride
TT	thromboplastin time
Tx	transplantation

ABSTRACT

Background. Dyslipidemia is common after solid organ transplantation. We have described hypertriglyceridemia in about 50% of our pediatric kidney and in about 30% of our liver recipients. The aim of the present study was to find out whether this post-transplantation hypertriglyceridemia after pediatric solid organ transplantation is associated with insulin resistance and the occurrence of small, dense low-density lipoprotein (LDL).

Methods. Fifty kidney and 25 liver recipients (aged 4 to 18 years) on triple immunosuppression and 181 controls participated in the study for an average of 5.3 and 6.4 years after kidney and liver transplantation (range 1 to 11 years) respectively. Homeostasis model assessments for insulin resistance (HOMA) were calculated and fasting lipoprotein lipid profile, apolipoprotein A-I and B concentrations, LDL particle diameter and indices of LDL susceptibility to copper-induced oxidation determined.

Results. Kidney patients had significantly higher serum total, high-density (HDL-C) and low-density lipoprotein (LDL) cholesterol, triglyceride, apolipoprotein A-I and B concentrations than liver patients or controls ($p<0.003$ for all). HOMA indices higher than the 95th percentile of Canadian normal children were seen in 50.0% of kidney (of liver 41.2%) recipients younger than 11 years and in 27.3% of older recipients (of liver 37.5%). Smaller sized LDL or LDL of increased oxidizability was not more frequent in patients than in controls.

Conclusions. Pediatric kidney recipients had significantly higher lipid and insulin concentrations than healthy control children. Combined hyperlipidemia and features of the dysmetabolic syndrome were common in children after kidney and liver transplantation. However, no small, dense LDL or LDL prone to oxidation was seen in either group.

Index words: kidney, liver, transplantation, child, lipid, triglyceride, LDL particle size, LDL oxidation, insulin, glucose

Insulin resistance, LDL particle size and susceptibility to oxidation in pediatric kidney or liver transplant recipients (title)

INTRODUCTION

Atherosclerosis is the most important cause of death and late graft loss after kidney transplantation (KTx) (1). Adult liver recipients have a 2.5-fold relative risk for ischemic cardiovascular events and a three-fold risk for death (2). Dyslipidemia is frequent after transplantation (Tx) affecting up to 70% of kidney (3) and 50% of liver recipients (4). High triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) concentrations, predominance of small low-density lipoprotein (LDL) particles and elevated insulin and glucose concentrations characterize insulin resistance syndrome (5). When combined with abdominal obesity, impaired glucose tolerance or type 2 diabetes mellitus and hypertension, they form a cluster of risk factors with high atherogenic potential (6). Increased prevalence of insulin resistance (7, 8) and dysmetabolic syndrome have been described in adult KTx patients (9). After a liver transplantation (LTx) due to cirrhosis, increased first-phase insulin secretion in response to high glucose levels persisted despite LTx normalized pre-Tx insulin resistance (10). In a large meta-analysis, new onset insulin dependent diabetes was reported in 13.4% of patients on calcineurin inhibitors (cyclosporine and tacrolimus) after solid organ transplantation (11).

Elevated LDL cholesterol (LDL-C) concentration and small, dense LDL particles prone to oxidation promote atherosclerosis (12, 13, 14). Variation in TG concentration predicted 62% of LDL diameter variation in healthy adults (15). In addition to environmental factors, the diameter of LDL is genetically determined (16). Lag time before oxidative modification of LDL *in vitro* is thought to reflect the total antioxidant status of LDL (17). HDL provides

multiple mechanisms in protection against atherosclerosis, e.g., through reverse cholesterol transport and the ability of HDL to decrease the peroxidation of LDL (18).

We have reported elevated TG concentrations in 50% and 30% of pediatric kidney and liver recipients on similar triple immunosuppression respectively, without increased frequency of low HDL-C concentrations. Half of the kidney recipients but only one sixth of the liver recipients and controls had elevated total cholesterol (TC) concentration (>5.0 mmol/l). Kidney graft and pre-transplantation TC concentration, independent of glomerular filtration rate (GFR) and proteinuria were risk factors for this hypercholesterolemia (18). In the kidney recipients, obesity, a high dose of MP, and proteinuria were significant risk factors for dyslipidemia (18). These findings raised hypotheses that 1) children with solid organ transplantation have increased prevalence of small, dense and easily oxidative LDL, and hyperinsulinemia, 2) size and oxidizability of LDL are associated with lipoprotein, apolipoprotein B and insulin concentrations, as well as apolipoprotein E phenotype, 3) the prevalence of small, dense LDL is higher in kidney than in liver recipients.

PATIENTS AND METHODS

Patients

Patients. 50 children in the KTx group and 25 children in the LTx group with acceptably functioning grafts took part in a cross-sectional study between September 1997 and April 1999. The inclusion criteria were: age between 3 and 17 years, follow-up time at least one year after Tx and glomerular filtration rate (GFR) >40 ml/min/1.73 m^2. Mean follow-up time after Tx was 5.3 years (range 1-11 years) and 6.4 (range 1-11 years) in the KTx and LTx groups respectively. The participation rate was 88.0% in the KTx and 92.6% in the LTx group. Pre-Tx diagnoses in the KTx group were the following: congenital nephrosis (N=31), urethral valve (N=5), nephronophthisis (N=2), polycystic kidney disease (N=3), glomerulonephritis

(N=2), prune-belly syndrome (N=1), Alport´s syndrome (N=1), mega-ureter (N=1), dysplastic kidney (N=1), bilateral multicystic kidneys (N=1), Denys-Drash syndrome (N=1) and renal insufficiency due to a complication of prematurity (N=1). Pre-Tx diagnoses in the liver group were: tyrosinemia (N=7), hepatitis (N=1), biliary atresia (N=9), hepatoblastoma (N=4), hepatocellular carcinoma (N=1), homozygous familial hypercholesterolemia (N=1), Wilson´s disease (N=1) and α-1-antitrypsin deficiency (N=1). None of the patients was hypothyroid, and all were on their usual diet. If there was marked weight gain after Tx, energy restriction and a diet low in saturated fatty acids was advised. The control group consisted 181 (112 boys and 69 girls) children without regular medication, acute infection or inflammatory or metabolic disease (19).

Medication. The immunosuppressive protocols have been described (19); they included triple therapy with cyclosporine A (CsA) in microemulsion composition, methylprednisolone (MP) given on alternate days, and azathioprine (AZA). Two boys were on triple therapy with tacrolimus instead of CsA in both the KTx and LTx groups. In the LTx group, one girl was on tacrolimus and MP.

Ethics. Parents and children gave written informed consent. The ethical Committees of Tampere and Helsinki University Hospital approved the study.

Methods

Collection of clinical data (kidney function as GFR, creatinine and diurnal urinary protein; liver function as serum alanine aminotransferase, albumin, total bilirubin and thromboplastin time; pubertal stage according to Tanner's classification), anthropometric measurements, blood sampling and analyses were done as described (20). In short, we expressed measured height, and body mass index (BMI) calculated from measured height and weight, as standard deviation

score (SDS) calculated according to the equation: (observation – mean observation for age) / SD. SD represents the standard deviation for the normal Finnish population of the same numerical age and gender (21, 22). We measured blood pressure from the right upper arm of the sitting patient with an electronic sphygmomanometer and used the mean of the three measurements in the analysis.

Lipid and apolipoprotein assays. Blood samples for lipid, cyclosporine trough concentration, insulin, and glucose analyses were taken after a 12 h overnight fast. Serum and plasma for lipid analyses were immediately separated by centrifugation and stored at –70°C. The serum TG, TC and HDL-C concentrations as well as apolipoprotein B (apoB) and A-I (apoA-I) concentrations were analyzed using Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). We calculated LDL-C concentration by Friedewald´s formula (23). Serum TG did not exceed 4.0 mmol/l in any of the subjects studied. Lp (a) was determined by a radioimmunological method and expressed in units per liter (U/l; 1 mg/l = 0.7 U/l). Sensitivity of the assay was 17 U/l. Concentrations >651 U/l (95th percentile of our control children) were considered to be elevated. ApoE phenotype was determined from Tx patients by isoelectric focusing and immunoblotting (20).

Glucose and insulin assays. Blood glucose was analyzed by enzymatic and serum insulin by radioimmunological methods. We estimated insulin resistance by homeostasis model assessment [insulin resistance=fasting insulin/($22.5e^{-\ln \text{fasting glucose}}$)] (24). The HOMA index is based on the assumption that normal adults have a HOMA index of 1. We compared the HOMA indices of 9 and 13 year old Canadian healthy children (25) to our patients divided to sub-groups according to gender and age (the median between the two cohorts of 9 and 13 year

old Canadians, i.e., <11 years vs. older). Oral glucose tolerance tests (OGTT) were performed after a 12 h fast and an oral dose of glucose 1.75 g/kg (maximum 75 g) was given. Samples for blood glucose and serum insulin measurements were collected at 0, 30, 60, 90, 120, and 180 min. Criteria for hyperinsulinemia were diagnosed as serum fasting insulin exceeding 20 mU/l and or a peak insulin concentration exceeding 150 mU/l (26). Impaired glucose tolerance was determined according to WHO criteria: blood glucose 5.6 - 6.0 mmol/l after an overnight fast or 6.7 - 9.9 mmol/l at 120 min in the OGTT.

LDL characteristics. The estimation of LDL particle size EDTA plasma samples was done by non-denaturing gradient gel electrophoresis. *In vitro* oxidation of LDL was done by copper-induced oxidation of LDL (20). The maximal storage times for *in vitro* oxidation of LDL and LDL particle size were 30 and 45 months respectively.

Food records. Forty-seven of the kidney and 24 of the liver patients completed a 6-day food record, which included two weekend days. The patients or their parents were asked to record the type and quantity of the all foods and drinks consumed. The record was checked in an interview with the patient or parent by one of the authors (A.S.). Complementary data from another parent or caretaker was in some cases elicited by telephone (e.g., in cases when the type of fat used in preparing the dish was not reported). Data on day-care meals and school lunches was checked by telephone from the kitchen personnel of the day-care centers and schools. Simple models of foods (e.g., different-sized potatoes), volume and household measures and pictures of food amounts and pictures of dietary fat packages on the market at the time were used in checking the food record data. Of the controls, 178 completed a 3-day food record, which included one weekend day. Food record data were analyzed with a Finnish nutrient software program (Nutrica 3.1 Fin, The Social Insurance Institution of Finland, Turku, Finland).

Statistical analysis. Normality of distributions was tested by one-sample Kolmogorov-Smirnov goodness of fit test. If the distribution was skewed, we used a logarithmic scale in appropriate cases, but described the data as untransformed values. When the distribution of TG was skewed, we displayed the mean of TG distribution as a geometric mean (the antilogarithm of the mean of the log transformed distribution). We presented normally distributed continuous variables as mean, range and confidence intervals and discrete or skewed distributed variables as median, lower (Q_1) and upper quartile (Q_3). We presented MP dose as daily dose, which equals the dose on alternate days divided by two. We tested differences in means between groups with t-test or analysis of variance (ANOVA, Bonferroni correction for multiple comparisons) for normally distributed continuous variables or Mann-Whitney or Kruskal-Wallis test for skewed or discrete variables. The significance of difference in the frequency distribution was tested by chi-square test. In cases where the frequencies in the cells were low, Fisher´s exact test was used instead. We calculated Pearson correlation coefficients for normally distributed variables and Spearman correlation coefficients for variables of skewed or discrete distributions. We presented a maximum of four most significant univariate correlation coefficients. In the tables, we tested all differences between the groups and presented those which were statistically significant. We considered two-sided p values less than to be 0.05 statistically significant.

We evaluated the determinants of TG concentration, LDL susceptibility to in vitro oxidation (lag time and oxidation rate) and LDL particle size by multivariate linear regression analysis and used forward stepwise method. Normally distributed variables were included as continuous and variables of skewed or discrete distributions as dichotomized. We did analyses separately for both Tx groups. We included a maximum of seven possible risk factors first in four different blocks (Table 1). In all first blocks for LDL characteristics, HDL-C and TG

concentrations were included. Similarly, in all first blocks for TG, HDL-C concentration and HOMA index were included. Significant variables from the first blocks were then included in the final block. The variables in the model did not show a strong correlation ($r<0.4$). A variable was included in the multivariate model if its significance was <0.05 and removed if the significance was >0.1. Computations were carried out using SPSS for Windows version 10.1 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

The patient and control groups were similar in age, relative weight, and distributions of pubertal stage, Table 2. The kidney recipients had higher systolic blood pressure than the controls ($p<0.001$). There were differences in the intake of carbohydrates between the groups; 41.7% (N=10) of the liver recipients reported high carbohydrate intake (> 55 % of energy) compared to 10.6% (N=5) of the kidney recipients and 22.5% (N=40) of the controls ($p=0.011$), Table 2. Medications are given in Table 3. The kidney function of kidney recipients was inferior to that of liver recipients (GFR: $p<0.001$; creatinine: $p<0.001$), Table 4. Liver recipients had higher serum alanine aminotransferase concentration ($p<0.001$) and lower serum thromboplastin time ($p=0.019$) than kidney recipients. Kidney recipients had higher serum TC ($p<0.001$) and LDL-C concentrations ($p=0.002$), and also higher HDL-C ($p<0.001$) and apoA-I concentrations ($p=0.001$), than liver recipients or controls, Table 5.

Insulin, glucose and TG. An increased TG concentration (>1.5 mmol/l) was seen in 40.0% (N=20) of the KTx, in 16.0% (N=4) of the LTx patients, and in 7.2% (N=13) of controls ($p<0.001$). Hyperinsulinemia, according to either high fasting or peak insulin concentration >150 mU/l during OGTT, was seen in 10.0 % (N=5) of the kidney and in 16.0 % (N=4) of the liver recipients, Table 6. In the KTx group, HOMA indices higher than the 95th percentile of Canadian normal population were observed in 50.0% (N=14) of the younger and in 27.3%

(N=6) of older children respectively, indicating a high prevalence of insulin resistance. In the LTx group, the corresponding figures were 41.2% (N=7) and 37.5% (N=3). Both an increased TG concentration and a high HOMA were present in 22.0 % (N=11) of KTx and 4.0 % (N=1) of LTx patients. Impaired glucose tolerance was seen in 20.0 % (N=10) and 32.0 % (N=8) of patients in KTx and LTx groups respectively. One girl in the LTx group was classified as pre-diabetic with blood glucose of 10.4 mmol/l at 2 h. In KTx patients, TG concentration correlated with HDL-C (r=– 0.381, p=0.006) and BMI SDS (r=0.369, p=0.008), while no such correlations were seen in the LTx group. In both Tx groups, fasting insulin correlated with systolic blood pressure (kidney: r=0.379, p=0.007; liver: r=0.565, p=0.003). In the final multivariate regression model, variation in HDL-C, BMI SDS and urinary protein explained 30.8 % of variation in TG in the KTx group, Table 8.

LDL particle size. KTx patients had larger LDL particle size than controls (p<0.001), Table 5. In the KTx group, variation in HDL-C concentration explained 40.7% of variation in LDL particle size, whereas that in TG concentration explained 12.8%, Table 7. In the LTx and control groups, variation in HDL-C concentration explained 36.1% and 17.9% of variation in LDL diameter respectively. Univariate correlation was seen between LDL particle size and HOMA index for insulin resistance (r=0.321, p=0.023) in the KTx group and in the LTx group between LDL particle size and BMI SDS (r=0.510, p=0.011). In the final multivariate regression model, variation in HDL-C concentration and pubertal stage explained 46.5 % of variation in the LDL particle size in the KTx group, Table 8, and variation in HDL-C concentration and BMI SDS explained 59.8 % of variation in the LDL particle size in the LTx group.

LDL susceptibility to oxidation. LDL oxidation rate and maximum amount of formed dienes were highest in the KTx group and lowest in the LTx group (p=0.001, Table 5). In kidney

patients, lag time correlated positively with HDL-C (r=0.290, p=0.05), and negatively in the liver patients (r=–0.539, p=0.005), Table 7. In the liver group, oxidation rate correlated with TC concentration (r=0.724, p<0.001). Oxidation rate was also associated with maximum amount of dienes formed in both groups (kidney group: r=0.748, p<0.001; liver group: r=0.864, p<0.001).

DISCUSSION

Risk for cardiovascular diseases is significantly elevated in kidney and liver recipients (1,2). This risk is a combination of pre-existing factors, such as the underlying kidney disease leading to specific metabolic abnormalities and transplantation, and the factors associated with transplantation, especially graft function and immunosuppressive therapy (1, 27). In our study, kidney transplant recipients had significantly higher TC, LDL-C, and apoB concentrations than liver recipients and controls. Both kidney and liver recipients had higher serum triglyceride concentrations than the controls. Despite this dyslipidemia, small, dense and easily oxidative LDL was not seen in either the kidney or in the liver Tx group. Signs of dysmetabolic syndrome were seen in both solid organ recipient groups since a high HOMA index indicating insulin resistance was found in 50.0% of the young kidney recipients (<11 years of age) and in 41.2% of the liver recipients. Impaired glucose tolerance was found in 20.0% and 32.0% of kidney and liver recipients, respectively.

Hyperlipidemia, hypertension, dysmetabolic syndrome, and new-onset insulin dependent diabetes are well-known risk factors for cardiovascular diseases after transplantation, but also risk factors for graft failure, because they may promote vascular changes in transplanted organs and thereby enhance the development of chronic rejection (1, 9, 28, 29). Insulin resistant individuals usually have an elevated concentration of TG, a decreased concentration of HDL-C and preponderance of small, dense LDL (30). Forty percent of the kidney recipients had an

elevated TG concentration. As expected, variation in TG concentration showed an association with variation in HDL-C concentration, obesity and proteinuria, and also a univariate association with LDL diameter. Thus, the lipid associations typical of dysmetabolic syndrome were seen in our kidney recipients. The underlying causes of hypertriglyceridemia can be treated, at least to some extent, by adequate weight control and by minimizing factors leading to proteinuria.

Corticosteroids are associated with combined hyperlipidemia, hyperinsulinemia, and hypertension in non-transplant patients (31) and transplant recipients (32). Lemieux et al. showed that kidney recipients on prednisone had higher serum insulin and apoB concentrations and female kidney recipients also more weight than the same patients after cessation of prednisone (8). According to Ekstrand (7), the most important mechanism associated with steroid induced insulin resistance is decreased activity of glycogen synthase. This, together with decreased insulin secretion, predisposes to glucose intolerance (7). Glucocorticoids might increase lipid production and impair lipid catabolism in the liver (8, 31). In our study, both kidney and liver recipients were on similar low-dose, every-other-day corticosteroid regimen, the average dosage being slightly higher in kidney than in liver recipients. Though no dose dependent influence was seen, the features of insulin resistance found in both of our Tx groups could have been due to corticosteroid therapy.

Small, dense LDL is associated with increased risk of coronary heart disease (14). Hypertriglyceridemia, insulin resistance and hypertension have been reported to be associated with increased prevalence of small, dense LDL (33). Our kidney recipients especially had risk factors for increased prevalence of small, dense LDL. Thus, it was unexpected that neither small, dense LDL nor LDL prone to in vitro oxidative modification existed more frequently in our patients than controls. In fact, kidney recipients seemed to have rather increased frequency

of large LDL diameters. The rare occurrence of small, dense LDL in our patients may be explained by age, as full penetration has been reported in only subjects over 20 years of age or genetic predisposition to larger-sized LDL (16). Nevertheless, small, dense LDL was rare in our patients, we wanted to model these lipoproteins in order to see whether the risk factors usually reported to influence the prevalence of small, dense LDL controlled the variation of LDL diameters in our patients. In our KTx patients, LDL diameter showed an independent positive association with HDL-C and an inverse univariate association with TG concentration, as previously reported in adults, with or without Tx (14, 34). In previous reports, TG concentration has typically been the strongest determinant of LDL diameter, but in our study HDL-C concentration was most important univariate lipid determinant explaining up to 40.7% of variation of LDL diameter in our kidney patients. Due to the metabolic relationship between a high TG and a low HDL-C concentration (increased catabolic rate of triglyceride-rich HDL particles resulting to low HDL-C concentration (35), the association of LDL diameter with HDL-C concentration probably reflected some persisting influence of the hypertriglyceridemia. Our kidney recipients had higher concentrations of HDL-C and apoA-I than our controls, probably because glucocorticoid therapy increases HDL-C concentration and apoA-I messenger RNA levels (36) and reduces plasma cholesterol ester transfer protein activity (8). This might indicate efficient reverse cholesterol transport, which supports the catabolism of triglyceride-rich lipoproteins decreasing the formation of small, dense LDL (37, 38). Contrary to our initial hypothesis and a previous report on Japanese children (39), insulin resistance, seen especially in our patients with ongoing puberty, and obesity were not associated with small, dense but rather with large LDL diameters in our patients. Thus, in pediatric kidney or liver recipients, the cardiovascular risk did not seem to be mediated through small, dense and easily oxidative LDL.

According to the 6-day food records, the amount of fat in the diet (percent of energy) did not differ statistically significantly between the groups, but high relative carbohydrate intake was more common in liver than in kidney patients. Previously, high carbohydrate diet has been shown to be associated with increased concentration of TG and prevalence of small, dense LDL (40). These associations of high carbohydrate diet were not seen in our patients, probably due to the complex influence of diet on lipoprotein values and also due to the multifarious nature of dyslipidemia in solid organ recipients.

Previously, in kidney transplant patients receiving CsA a shorter lag time for LDL oxidation has been seen (34, 41, 42). In our patients, we saw no pro-oxidant effect of CsA. Our patients used CsA in micro-emulsion composition containing DL-alpha tocopherol, which might act as an anti-oxidative agent (43). Still, *in vitro* studies do not suggest CsA to be a direct pro-oxidant (44). *In vivo,* HDL protects LDL against lipid peroxidation via various mechanisms, as acting as a reservoir for lipid peroxides and retarding LDL oxidation by HDL-associated enzymes (18). Our kidney and liver patients and controls were different in associations of cholesterol with lag time. However, patients with GH treatment had a longer lag time in both Tx groups. These findings remain to be explained. With regard to lipid metabolism, kidney and liver are metabolically different organs since the liver plays an essential, well-known role in the synthesis and storage of lipids, while deteriorating kidney function leads to alterations in concentrations and compositions of lipids, but the role of kidney in the regulation of lipid homeostasis is not clearly known.

In our study, multivariate associations were assessed first in blocks that let us include more independent variables in analysis, Table 1. We tried to group possible related risk factors in a block, e.g., kidney and liver function. However, in the block of patient characteristics, the variables were only weakly related. This setting did not allow all interactions between the

variables to be studied, and the final model may not show the best possible subset of all variables in first blocks.

In conclusion, higher TG and insulin concentrations were found in kidney and liver transplant recipients compared to controls. Also, features of dysmetabolic syndrome, except increased prevalence of small, dense and easily oxidative LDL, were seen in both organ recipient groups. HDL-C concentration was the most important determinant for LDL particle size in all groups. These preliminary findings in a limited number of pediatric kidney and liver recipients should be followed up with a larger patient group in the future. As dyslipidemia, hypertension, dysmetabolic syndrome, and new-onset insulin dependent diabetes are risk factors for future cardiovascular disease, the occurrence of these risk factors should be minimized and be a prime consideration in any management strategy to reduce the mortality and morbidity of cardiovascular diseases in pediatric transplant recipients.

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Table 1. Block division of multivariate linear regression models for triglyceride, LDL particle size and variables of LDL oxidation

A. Characteristics of patients
Gender (0=boy, 1=girl)
Time since transplantation (years)
Body mass index standard deviation score
Pubertal stage (0=pre-pubertal, 1=pubertal or post-pubertal)
Indication for transplantation (kidney: 0=NPHS1 vs. 1=other; liver: 0=liver failure vs. 1=other)
HDL-C (mmol/l)
Triglyceride (mmol/l, logarithmic scale, in the model for triglyceride HOMA included)
B. Medication and blood pressure
Blood cyclosporine trough level (μmol/l)
Methylprednisolone dose (0=two lowest tertiles, 1=the highest tertile)
Use of growth hormone (0=no, 1=yes)
Use of antihypertensives (*in the model for kidney recipients only*; 0=no, 1=yes)
Systolic blood pressure (mmHg)
HDL-C (mmol/l)
Triglyceride (mmol/l, logarithmic scale, in the model for triglyceride HOMA included)
C. Kidney and liver function
Graft (*in the model for kidney recipients only*, 0=cadaver, 1=living related donor)
Glomerular filtration rate [0=two highest tertiles, 1=the lowest tertile (in the kidney group <48.6 ml/min/1.73m^2, liver group <65.4 ml/min/1.73m^2)]
Thromboplastin time (0=two highest tertiles, 1=the lowest tertile)
Urinary protein (*in the model for kidney recipients only*; 0="≤200 mg/day", 1=">200 mg/day")
HDL-C (mmol/l)
Triglyceride (mmol/l, logarithmic scale, in the model for triglyceride HOMA included)
D. Lipids, insulin resistance and diet
Low-density lipoprotein particle size (not included the model for triglyceride)
Homeostasis model assessment index for insulin resistance (ref. 24) (0=two lowest tertiles, 1=the highest tertile)
Apolipoprotein E phenotype (E3/E4 or E4/E4, 0=no, 1=yes)
Intake of carbohydrate (percentage of total energy; 0 = "<55 E%, 1 = " = 55 E%")
Intake of saturated fat (percentage of total energy)
HDL-C (mmol/l)
Triglyceride (mmol/l, logarithmic scale, in the model for triglyceride HOMA included)

ABBREVIATIONS: NPHS1=congenital nephrotic syndrome, HDL-C=serum high-density lipoprotein cholesterol concentration

NOTES: Dependent variables were not included in the model as independent variables.

Table 2. Clinical characteristics of 75 kidney or liver transplant recipient children and 181 controls

	Kidney (N=50)	Liver (N=25)	Control (N=181)
Gender, Male/Female, N	33/17	15/10	112/69
Age, years, median (range)	10.8 (4.3 - 17.2)	9.3 (3.9 -17.9)	9.1 (3.2 - 18.7)
Median age at Tx, years, median (range)	3.2 (1.1 - 15.3) [a]	2.0 (0.4 -16.3)	
Height SDS, mean (CI)	-1.3 (-1.6; -1.0) [b]	-1.7 (-2.1; -1.3)	0.2 (0.0; 0.3)
BMI SDS, mean (CI)	0.3 (-0.1; 0.6)	0.5 (-0.1; 1.1)	0.3 (0.1; 0.5)
Pubertal stage (Tanner's classification)			
Pre-pubertal % (N)	48.0 (24)	60.0 (15)	61.8 (112)
Pubertal % (N)	42.0 (21)	32.0 (8)	29.8 (54)
Post-pubertal % (N)	10.0 (5)	8.0 (2)	8.3 (15)
Systolic blood pressure, mmHg, mean (CI)	119 (115; 122) [c]	115 (109; 120)	109 (107; 110)
Diastolic blood pressure, mmHg, mean (CI)	65 (61; 68)	69 (69; 74)	65 (63; 66)
Diet (N=47/24/178)			
Energy intake, kJ, median (Q_1; Q_3)	7.2 (5.6; 8.8)	7.0 (6.1; 7.6)	7.5 (6.4; 8.8)
Carbohydrates, E%, mean (CI)	49.0 (47.6; 50.5) [d]	53.2 (51.0; 55.4)	50.7 (49.8; 51.5)
Fat, E%, mean (CI)	34.1 (32.8; 35.4)	31.2 (29.1; 33.3)	33.2 (32.5; 33.9)
Saturated fat, E%, mean (CI)	14.5 (13.8; 15.2)	13.2 (11.8; 14.6)	14.1 (13.7; 14.5)
Dietary cholesterol, mg, median (Q_1; Q_3)	195 (157; 261)	207 (157; 254)	221 (174; 278)

[a]p=0.014

[b]p<0.001; kidney group vs. controls and liver group vs. controls, p<0.001

[c]p<0.001; kidney group vs. controls, p<0.001 (liver group vs. controls, p=0.074)

[d]p=0.012; kidney vs. liver p=0.01

ABBREVIATIONS: N=number of patients, Tx=transplantation, SDS=standard deviation score, CI=95 % confidence interval, BMI=body mass index

STATISTICS: chi square test, Kruskal-Wallis test, ANOVA (Bonferroni correction for multiple comparisons), E%=percent of energy

NOTES: Part of the control data has been published (19, 20).

Table 3. Medication of 75 kidney or liver transplant recipient children

	Kidney (N=50)	Liver (N=25)
Cyclosporine A, mg/kg/day, median (Q_1; Q_3)	5.4 (3.7; 6.8)	4.6 (3.6; 5.3)
Blood cyclosporine trough concentration, µg/l, median (Q_1; Q_3)	94 (78; 119)	102 (73; 134)
Methylprednisolone, mg/kg/day, median (Q_1; Q_3)	0.09 (0.07; 0.12) [a]	0.07 (0.06; 0.10)
Azathioprine, mg/kg/day, median (Q_1; Q_3)	1.3 (1.2; 1.4) [b]	1.2 (1.1; 1.3)
Antihypertensives, % (N)	28.0 (14) [c]	8.0 (2)
β-blockers or diuretics, % (N)	6.0 (3)	4.0 (1)
Growth hormone treatment, % (N)	16.0 (8)	24.0 (6)
Hydrocortisone substitution, % (N)	20.0 (10)	40.0 (10)

[a] p=0.014

[b] p=0.001

[c] p=0.046

ABBREVIATIONS: N=number of patients, Q_1=lower quartile, Q_3=upper quartile

STATISTICS: Mann-Whitney test, chi square test

Table 4. Graft function in 75 kidney or liver transplant recipient children studied for dyslipidemia

	Kidney (N=50)	Liver (N=25)
GFR, ml/min/1.73 m^2, mean (CI) (range)	60 (56; 65) [a] (41 - 106)	84 (71; 96) (41 - 165)
Creatinine, µmol/l, median (Q_1; Q_3)	96 (80; 109) [a]	58 (48; 75)
Alanine aminotransferase, U/l, median (Q_1; Q_3)	13 (12; 19) [a]	32 (14; 61)
Albumin, g/l, mean (range)	37 (24 – 45) [b]	39 (31 – 45)
Total bilirubin, µmol/l, median (Q_1; Q_3)	Nd	10 (7; 15)
Thromboplastin time, %, mean (CI)	102 (95; 109) [c]	88 (80; 96)

[a] $p<0.001$

[b] Serum albumin concentration of 9 kidney patients

[c] $p=0.019$

ABBREVIATIONS: N=number of patients, GFR=glomerular filtration rate, CI=95 % confidence interval for mean, Q_1=lower quartile, Q_3=upper quartile, nd =not determined

STATISTICS: t-test, Mann-Whitney test

Table 5. Serum lipids and lipoproteins and characteristics of LDL particles in 50 kidney and 25 liver transplant recipient children and 181 controls

	Kidney (N=50)	Liver (N=25)	Control (N=181)	P-value
Lipoprotein values				
TC, mmol/l, mean (CI)	4.99 (4.71; 5.28)	4.25 (3.83; 4.68)	4.18 (4.07; 4.29)	<0.001 [a,b]
LDL-C, mmol/l, mean (CI)	2.70 (2.48; 2.92)	2.16 (1.84; 2.48)	2.39 (2.30; 2.48)	0.002 [a,b]
HDL-C, mmol/l, mean (CI)	1.62 (1.52; 1.72)	1.56 (1.37; 1.74)	1.42 (1.38; 1.47)	<0.001 [a]
TG, mmol/l, geometric mean (CI)	1.34 (1.17; 1.52)	1.09 (0.93; 1.28)	0.72 (0.67; 0.77)	<0.001 [a,c]
HDL-C/TC, %, mean (CI)	33.2 (31.1; 35.2)	36.8 (33.4; 40.2)	34.6 (33.5; 35.7)	NS
ApoA-I, g/l, mean (CI)	1.53 (1.45; 1.60)	1.46 (1.34; 1.59)	1.39 (1.35; 1.42)	0.001 [a]
ApoB, g/l, mean (CI)	0.83 (0.77; 0.89)	0.70 (0.62; 0.77)	0.73 (0.71; 0.76)	0.001 [a,b]
Lp (a), U/l, median (Q_1; Q_3) (range)	65 (21; 143) (<17; 673)	67 (32; 161) (<17; 535)	85 (27; 238) (<17; 1157)	NS
Apolipoprotein E3/E4 or E4/E4 phenotype, % (N)	28.0 (14)	24.0 (6)	nd	NS
LDL characteristics				
Diameter of LDL, nm, mean (CI) [d]	26.9 (26.8; 27.1)	26.8 (26.6; 26.9)	26.6 (26.5; 26.7)	<0.001 [a]
Diameter of LDL<25.6 nm, % (N)	2.0 (1)	0.0 (0)	1.1 (1)	<0.001
Diameter of LDL 25.6<x<26.5 nm, % (N)	18.0 (9)	16.7 (4)	49.5 (47)	
Diameter of LDL 26.5<x<27.2 nm, % (N)	42.0 (21)	70.8 (17)	38.9 (37)	
Diameter of LDL>27.2 nm, % (N)	38.0 (19)	12.5 (3)	10.5 (10)	
Lag time, min, mean (CI) [e]	78 (76; 81)	76 (71; 80)	77 (75; 78)	NS
Oxidation rate, μM/min, mean (CI) [e]	0.44 (0.43; 0.45)	0.40 (0.37; 0.43)	0.43 (0.42; 0.44)	0.001 [b,c]
Maximum amount of dienes formed, nmol/mg, mean (CI) [e]	568 (553; 584)	522 (496; 549)	547 (538; 556)	0.001 [a,b]

[a]Kidney group vs. controls, p<0.05

[b]Kidney vs. liver group, p<0.05

[c]Liver group vs. controls, p<0.05

[d] LDL particle size was analyzed for 24 children in the liver recipient and for 95 children in the control group

[e] LDL susceptibility to in vitro oxidation was analyzed for 46 children in the kidney recipient and for 88 children in the control group

ABBREVIATIONS: N=number of patients, TC=serum total cholesterol concentration, CI=95% confidence interval for mean, LDL-C=serum low density lipoprotein concentration, HDL-C=serum high-density lipoprotein cholesterol concentration, TG=serum triglyceride concentration, ApoA-I=serum apolipoprotein A-I concentration, ApoB=serum apolipoprotein B concentration, Lp (a)=serum lipoprotein (a) concentration, NS=not significant, Q_1=lower quartile, Q_3=upper quartile, LDL=low density lipoprotein, nd=not determined

STATISTICS: ANOVA (Bonferroni correction for multiple comparisons), Kruskal-Wallis test, chi square test

NOTES: Part of the control data has been published (19, 20).

Table 6. Serum insulin and homeostasis model assessment index for insulin resistance in 50 kidney and 25 liver transplanted children and 181 controls

	Kidney (N=50)	Liver (N=25)	Control (N=181)	P-value
Insulin				
Insulin, mU/l, median (Q_1; Q_3)	10 (7; 16)	9 (5; 15)	5 (3; 7)	<0.001
Pre-pubertal, median (Q_1; Q_3) (N=24 / 15 / 112)	8 (4; 11)	6 (4; 11)	4 (3; 5)	<0.001
Pubertal, median (Q_1; Q_3) (N=21/ 8 / 54)	14 (10; 19)	13 (5; 36)	7 (5; 10)	<0.001
Post-pubertal, median (Q_1; Q_3) (N=5 / 2 / 15)	9 (8; 14)	(9; 18)	6 (5; 14)	NS
Frequency of high fasting insulin, % (N)	10.0 (5)	12.0 (3)	1.7 (3)	NS
Frequency of hyperinsulinemia during OGTT, % (N)	3.9 (2)	8.0 (2)		NS
Insulin resistance				
HOMA insulin resistance, median (Q_1; Q_3)	2.2 (1.4; 3.3)	1.8 (0.9; 2.9)		NS
Pre-pubertal, median (Q_1; Q_3) (N=24 / 15)	1.6 (0.8; 2.4)	1.2 (0.9; 2.2)		NS
Pubertal, median (Q_1; Q_3) (N=21/ 8)	2.9 (2.2; 4.4)	2.9 (2.0; 7.0)		NS
Post-pubertal, median (Q_1; Q_3) (N=5 / 2)	1.7 (1.6; 2.9)	2.7 (1.7; 3.7)		NS

ABBREVIATIONS: N=number of patients, Q_1=lower quartile, Q_3=upper quartile, NS=not significant, OGTT=oral glucose tolerance test, HOMA=insulin resistance index according to homeostasis model assessment (24)

STATISTICS: Kruskal-Wallis test, chi square test

NOTES: Part of the control data has been published (19, 20).

Table 7. Univariate linear regression models for characteristics of LDL with lipids, lipoproteins and apolipoproteins as independent variables in kidney and liver transplantation groups and controls

Dependent variable	Independent variable	Kidney transplant group				Liver transplant group				Control group			
		B	Std. error	P	R^2	B	Std. error	P	R^2	B	Std. error	P	R^2
LDL particle size (N=50/24/95)	HDL-C, mmol/l	1.01	0.18	<0.001	0.407	0.50	0.14	0.002	0.361	0.66	0.15	<0.001	0.179
	TG, mmol/l	–0.41	0.16	0.011	0.128					–0.29	0.10	0.006	0.077
	ApoA-I, g/l	1.02	0.28	0.001	0.223	0.62	0.22	0.010	0.263	0.61	0.21	0.005	0.082
	ApoB, g/l	–0.77	0.38	0.047	0.081					–0.63	0.28	0.030	0.050
Lag time for LDL oxidation (N=46/25/88)	TC, mmol/l					–5.28	1.85	0.009	0.261				
	LDL-C, mmol/l					–6.29	2.55	0.021	0.210				
	HDL-C, mmol/l	6.99	3.47	0.050	0.084	–12.54	4.09	0.005	0.291				
	TG, mmol/l												
	ApoA-I, g/l	9.84	4.74	0.044	0.091	–14.82	6.39	0.030	0.189				
	ApoB, g/l					–26.14	11.04	0.027	0.196				

ABBREVIATIONS: B=Beta i.e., the unstandardized coefficients of the estimated regression model, std. error=standard error of the unstandardized coefficients of the estimated regression model, p=p value of the significance, R^2=the proportion of variation in the dependent variable explained by the regression model, LDL=low density lipoprotein, HDL-C=serum high-density lipoprotein cholesterol concentration, TG=serum triglyceride concentration, ApoA-I=serum apolipoprotein A-I concentration, ApoB=serum apolipoprotein B concentration, TC= serum total cholesterol concentration

STATISTICS: Linear regression

Table 8. Multivariate linear regression models for 50 kidney and 25 liver transplant recipients

Organ group	Dependent variable	Block	Independent variable	First blocks				Final block			
				B	Std. error	P	R^2	B	Std. error	P	R^2
Kidney	TG	A.	HDL-C[a]	–0.44	0.18	0.019	0.234	–0.45	0.17	0.013	0.308
			BMI SDS	0.11	0.05	0.024		0.11	0.05	0.031	
		C.	HDL-C	–0.53	0.17	0.004	0.246				
			Urinary protein	0.56	0.24	0.026		0.53	0.24	0.031	
	LDL particle size	A.	HDL-C[a]	1.07	0.17	<0.001	0.465	1.07	0.17	<0.001	0.465
			Pubertal stage	0.26	0.11	0.028		0.26	0.11	0.028	
	Lag time for LDL oxidation (N=46)	B.	HDL-C[b]	8.57	3.35	0.014	0.210	7.15	3.32	0.037	0.179
			Use of growth hormone	6.33	2.92	0.036		6.66	2.98	0.031	
Liver	LDL particle size (N=24)	A.	HDL-C[a]	0.48	0.11	<0.001	0.598	0.48	0.11	<0.001	0.598
			BMI SDS	0.13	0.04	0.002		0.13	0.04	0.002	
	Lag time for LDL oxidation	A.	HDL-C[c]	–16.93	3.72	<0.001	0.542	–7.53	3.42	0.038	0.590
			Time since transplantation	1.96	0.63	0.005					
			Indication for LTx: failure vs. other	8.19	3.88	0.047					

	B.	Use of growth hormone	13.60	3.93	0.002	0.363	14.24	3.56	0.001	
Oxidation rate	B.	Methylprednisolone dose	–0.06	0.02	0.018	0.441				
		HDL-C [a]	0.06	0.02	0.023		0.08	0.03	0.005	0.302
	D.	HDL-C	0.09	0.02	0.001	0.462				
		Intake of saturated fat	–0.01	0.003	0.037					

ABBREVIATIONS: B=Beta i.e., the unstandardized coefficients of the estimated regression model, std. error=standard error of the unstandardized coefficients of the estimated regression model, p=p value of the significance, R^2=the proportion of variation in the dependent variable explained by the regression model TG=serum triglyceride concentration, HDL-C=serum high-density lipoprotein cholesterol concentration, BMI SDS=body mass index standard deviation score, LDL=low-density lipoprotein, LTx=liver transplantation

[a] HDL-C was significant in all first blocks. Blocks with HDL-C as the only significant variable are not presented.

[b] HDL-C was also significant in block D.

[c] HDL-C was also significant in blocks C and D.

STATISTICS: Linear regression

NOTES: The setting and block division of variables are presented in Table 1.