LONG-TERM OUTCOME AFTER PEDIATRIC RENAL TRANSPLANTATION

ENDOCRINOLOGIC AND METABOLIC EFFECTS

Juuso Tainio

ACADEMIC DISSERTATION

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Helsinki 2015
To my family
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Abstract

Renal transplantation (RTx) has become an established treatment modality for end-stage renal disease (ESRD). Along with the improvements in pre- and post-transplant (Tx) care, the patient and graft outcomes have improved significantly during the past two decades. This attracts more attention to avoiding secondary complications and long-term side effects of the post-Tx immunosuppressive medication. Several risk factors cast a shadow over patients’ normal physical and mental development, and quality of life, but detailed reports on long-term outcome after pediatric RTx are scarce.

This thesis was designed to investigate pubertal development and subsequent male fertility and semen quality with special emphasis on the effects of immunosuppressive medication on reproductive function. The study also aimed to scrutinize metabolic risk factors and their consequences in patients after pediatric RTx. Analyses of the prevalence of metabolic syndrome and its components, as well as the effects of metabolic factors and blood pressure (BP) on kidney graft function were conducted.

The study population included all 218 patients having undergone a pediatric RTx in Finland between 1979 and 2011. Background data were collected retrospectively from patient records. Cross-sectional data on bone age, testicular volume, and reproductive hormone levels were also gathered. Twenty-nine heart Tx (HTx) and 13 liver Tx (LTx) patients, and 56 healthy men served as controls in the BP and fertility studies, respectively.

Data on 109 RTx recipients (72 males) were included in a two-part study consisting analyses on pubertal development and puberty-related reproductive hormone levels. The onset of pubertal development occurred at the mean age of 12.7 years in 55 boys with 22% considered delayed. In 29 girls, however, no delayed development occurred, with the age at onset of puberty and menarche averaging 10.7 years and 12.5 years, respectively. Pubertal growth continued relatively long resulting in acceptable final height (on average -1.7 height standard deviation score in boys and -1.2 in girls). The serum levels of reproductive hormones assessed at the age of 8 to 21 years were normal in a great majority of the patients.

The reproductive hormone levels and semen samples of 24 men were examined at a median of 18.6 years after RTx and the results were compared to those of 56 age-matched healthy men. The RTx recipients’ free testosterone levels were lower and LH levels were higher in comparison with their healthy peers (322 vs. 399 pmol/L, p = 0.001 and 7.6 vs. 3.3 IU/L, p < 0.001, respectively). The RTx patients had smaller testicular volumes and total sperm counts than the controls (11.4 vs. 33.9 mL, p <0.001 and 1.3 vs. 135.5 million, p <0.001, respectively). Four men could not provide a semen sample and two refused. Only 4 out of 18 (22%) RTx men who provided a semen sample had normospermia. Patients with a history of cyclophosphamide therapy showed even worse outcome than those without.
Data on 210 RTx patients transplanted at a median age of 4.5 years were collected at several time points during a 13-year follow-up post-RTx. Serum lipid and glucose levels, weight, and BP results were correlated to the measured glomerular filtration rate (GFR). The mean decline of GFR after the first year of follow-up was 2.4 mL/min/1.73 m²/year. Hypertriglyceridemia associated with a lower GFR at 1.5 (p = 0.008) and 5 years post-RTx (p = 0.017) and it predicted the subsequent GFR decline rate after 1.5 years post-RTx. Beyond the first postoperative year, metabolic risk factors, except for triglycerides, associated modestly with the long-term kidney graft function in pediatric RTx patients.

The ambulatory BP monitoring (ABPM) data on 111 renal, 29 heart, and 13 liver Tx recipients were retrospectively analyzed 5 to 10 years post-transplantation. The BP data were compared within the Tx groups and the BP profiles were found to be similar. The BP index and load were abnormal especially at nighttime and the nocturnal BP dipping was often blunted. The BP variables were equally valued when assessing hypertension. The use of antihypertensive medication did not notably change the ABPM profile in renal Tx recipients. BP load of 50% instead of 25% seems to be a more adequate cut-off value. The BP variables correlated poorly with the metabolic parameters or kidney graft function.

In conclusion, our study shows that pubertal development was normal in all female and most of the male RTx patients. Testicular function was often impaired even years after RTx, and poor semen quality decreases the prospect of fertility in men after pediatric RTx. Metabolic risk factors had relatively little impact on the long-term kidney graft function. Hypertension is common, with emphasis on nocturnal prevalence, in Tx patients underlining the importance of the use of ABPM in diagnosing hypertension and in the follow-up.
List of original publications

This thesis is based on the following articles, referred to in the text by their Roman numerals:


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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABPM</td>
<td>ambulatory blood pressure monitoring</td>
</tr>
<tr>
<td>ALG</td>
<td>anti-lymphocyte globulin</td>
</tr>
<tr>
<td>AR</td>
<td>acute rejection</td>
</tr>
<tr>
<td>ATG</td>
<td>anti-thymocyte globulin</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CAN</td>
<td>chronic allograft nephropathy</td>
</tr>
<tr>
<td>CNI</td>
<td>calcineurin inhibitor</td>
</tr>
<tr>
<td>CsA</td>
<td>cyclosporine A</td>
</tr>
<tr>
<td>CNF</td>
<td>congenital nephrotic syndrome of the Finnish type</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>EBV</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>ESRD</td>
<td>end-stage renal disease</td>
</tr>
<tr>
<td>FH</td>
<td>final height</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
</tr>
<tr>
<td>GFR</td>
<td>glomerular filtration rate</td>
</tr>
<tr>
<td>GH</td>
<td>growth hormone</td>
</tr>
<tr>
<td>GHbA1c</td>
<td>glycosylated hemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HRQOL</td>
<td>health-related quality of life</td>
</tr>
<tr>
<td>hSDS</td>
<td>height standard deviation score</td>
</tr>
<tr>
<td>HTx</td>
<td>heart transplantation</td>
</tr>
<tr>
<td>IGT</td>
<td>impaired glucose tolerance</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LH</td>
<td>luteinizing hormone</td>
</tr>
<tr>
<td>LTx</td>
<td>liver transplantation</td>
</tr>
<tr>
<td>MP</td>
<td>methylprednisolone</td>
</tr>
<tr>
<td>MS</td>
<td>metabolic syndrome</td>
</tr>
<tr>
<td>OGS</td>
<td>onset of growth spurt</td>
</tr>
<tr>
<td>OGTT</td>
<td>oral glucose tolerance test</td>
</tr>
<tr>
<td>PHV</td>
<td>peak height velocity</td>
</tr>
<tr>
<td>PTLD</td>
<td>post-transplant lymphoproliferative disorder</td>
</tr>
<tr>
<td>RRT</td>
<td>renal replacement therapy</td>
</tr>
<tr>
<td>RTx</td>
<td>renal transplantation</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>Tx</td>
<td>transplantation</td>
</tr>
</tbody>
</table>
1 Introduction

Renal transplantation (RTx) restores the ability of the body of a patient with end-stage renal disease (ESRD) to maintain water and acid-base balance, regulate electrolytes, excrete soluble wastes, and produce several hormones and enzymes. Since the beginning of RTx in the 1960s, the survival of ESRD patients had theoretically potential to increase, but the results during the early decades were suboptimal, mainly because of the lack of appropriate immunosuppression. The introduction of cyclosporine A (CsA) in the early 1980s finally changed the setting from coping with the post-RTx complications to preservation of health by protecting the kidney graft from rejections (Starzl et al. 1982, Merion et al. 1984, Brodehl, Offner & Hoyer 1987). Along with the wider repertoire of immunosuppressive medication, the perioperative care improved and diagnosis and treatment of rejections and infections advanced, establishing RTx as a treatment of choice for ESRD patients.

The first renal transplantations for adolescent patients in Finland were performed in the late 1960s, but the systematic pediatric RTx program started in 1986 at Children’s Hospital, Helsinki University Central Hospital. By March 2011, a total of 218 children or adolescents had received 245 kidney grafts in Finland. The number of pediatric renal transplantations has been on average 10 operations/year over the past decade. The incidence of renal replacement therapy (RRT) in children aged 0–14 years is 4.4 per million age-related population in Europe and 6.8 per million age-related population in Finland (ESPN/ERA-EDTA Registry 2013). According to this report, the prevalence of pediatric patients on RRT was 84.4 per million age-related population in Finland, being the highest in Europe.

The function of the kidney graft may be impaired by both immunological and non-immunological risk factors, such as acute and chronic rejections, infections, calcineurin inhibitor (CNI) toxicity, and metabolic complications. Cardiovascular disease (CVD) is known to be the leading cause of mortality in RTx patients (NAPRTCS 2010). Pediatric patients are bound to receive immunosuppressive therapy for decades, emphasizing the importance of good graft function and optimal drug therapy.

Previous data suggest that, despite the catch-up growth occurring after pediatric RTx, the patients remain stunted and their pubertal development may be delayed. Due to the inevitable decrease in kidney graft function and life-long medication, especially CNIs and glucocorticoids, the patients are prone to diverse metabolic disorders. To date, long-term data on children transplanted in early childhood are scarce. This study was therefore carried out to study the endocrinologic and metabolic effects in the long run in pediatric RTx recipients.
2 Review of the literature

2.1 Renal transplantation in children

2.1.1 Indications

The indications for pediatric RTx differ from those of adult patients, with emphasis on congenital and structural causes. Furthermore, the most common disease leading to pediatric RTx in Finland, the congenital nephrotic syndrome of the Finnish type (CNF), differentiates the age distribution of children undergoing RTx in Finland from all other international registry reports. Possibly due to a limited gene pool in the Nordic countries in comparison with the US for instance, the possibility of inherited disorders is increased; and the age at RTx is lower (Tyden and Berg 1998). Half of the Finnish patients are aged 5 years or less at the time of index RTx, while the proportion of such young children in the US is currently 19% (Laine et al. 1994, NAPRTCS 2010).

CNF is caused by a mutation in the NPHS1 gene, the gene product of which is called nephrin, a protein responsible for the connection of podocyte foot process to the glomerular capillary wall (Ruotsalainen et al. 1999). Two founder mutations occur, Fin-major and Fin-minor, both leading to massive proteinuria during the first months after birth, with secondary consequences comprising hypoproteinemia, edema, oliguria, hyperlipidemia, hypothyreosis, and increased risk for infections and thrombotic complications (Holmberg et al. 2004).

The second most common indication for pediatric RTx in Finland is posterior urethral valve. It is a congenital disorder in which urinary flow is obstructed by tissue membranes in male patients leading to bladder dysfunction and, on average, later to ESRD in 10% of the patients (Hennus et al. 2012). In order to avoid adverse effects on graft survival, the lower urinary tract should be reconstructed prior to RTx (Reinberg et al. 1988). The other indications for pediatric RTx in the Finnish and US populations are listed in Table 1.

RRT is considered when renal insufficiency prevents the maintenance of body homeostasis and waste excretion. The actual indications for RRT depend on a combination of several biochemical and clinical characteristics, some of which may be managed with medications or dietary consulting (Greenbaum and Schaefer 2012, Warady, Morgenstern & Alexander 2004). Renal function assessment is obviously a critical part of the process, and the current consensus in the field of pediatric nephrology for the initiation of dialysis is when the glomerular filtration rate (GFR) falls below 10–15 mL/min/1.73 m² (Greenbaum and Schaefer 2012).
RRT is instituted in most children either by peritoneal or hemodialysis, or RTx. According to the European and North American registry reports, a fifth to a quarter of the patients undergo RTx pre-emptively, i.e., without prior dialysis (ESPN/ERA-EDTA Registry 2013, NAPRTCS 2010). About half of the kidney grafts in the US are from living related donors, whereas grafts from deceased donors have historically been used in Europe (Benfield et al. 1999). At our center, a sixth of the grafts during the last decade were of living related donor origin. This is a relatively higher proportion than in adult RTx but lower than in the pediatric RTx during the 1980’s and 1990’s in Finland (Mäkelä et al. 2013, Tyden and Berg 1998). Even though RTx is accepted as the optimal treatment for ESRD it has some limitations. In case of active and progressing disease (such as hemolytic uremic syndrome or malignancy) RTx should be postponed until the underlying condition is stabilized.

2.1.2 Histocompatibility and surgical aspects

The ABO blood group compatibility is required in the Finnish RTx protocol. Human leukocyte antigen (HLA) typing is made for the donor and recipient candidates and the alleles used at the histocompatibility matching are HLA-A, -B, and -DR. The best, thus the least, mismatched pairs are chosen for the subsequent leukocyte cross-matching test in which a negative result is further required before proceeding to operation. Among the pediatric RTx in Finland, the mismatching results have been very good and 91% of the donors are 2/1 or less mismatched with their donor. Although the beneficial effect of minimizing the mismatches has been shown, even the zero-mismatched grafts such as any graft, may fail for several reasons (Duquesnoy 2007).

The surgical procedure of RTx in children is performed in a similar manner as in adults, thus preferring the standard pelvic extraperitoneal placement (Vukcevic et al. 2007). In smaller children weighing less than 30 kg, individualized approach is advised, emphasizing the matching of blood vessel size and requirements of circulatory volume issues. Traditionally, intraperitoneal placement of the graft is used for children weighing less than 20 kg. According to our center’s experience an adult kidney graft can safely be placed extraperitoneally in children weighing over 10 kg (Laine et al. 1994).
Table 1. Indications for pediatric renal transplantation in Finland and North America.

<table>
<thead>
<tr>
<th>Primary disease</th>
<th>Finland (n = 218)</th>
<th>North America (n = 9969)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hereditary disorders</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary disorders</td>
<td>54% (n = 117)</td>
<td>12% (n = 1163)</td>
</tr>
<tr>
<td>Congenital nephrotic syndrome</td>
<td>54%</td>
<td>12%</td>
</tr>
<tr>
<td>Nephronophthisis</td>
<td>8%</td>
<td>3%</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Other</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>Structural disorders</strong></td>
<td>24% (n = 53)</td>
<td>41% (n = 4128)</td>
</tr>
<tr>
<td>Posterior urethral valve</td>
<td>12%</td>
<td>16%</td>
</tr>
<tr>
<td>Renal aplasia/hypoplasia/dysplasia</td>
<td>7%</td>
<td>17%</td>
</tr>
<tr>
<td>Reflux nephropathy</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>Prune belly syndrome</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td><strong>Other conditions</strong></td>
<td>22% (n = 48)</td>
<td>47% (n = 4678)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>6%</td>
<td>10%</td>
</tr>
<tr>
<td>Vasculitides</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>Hemolytic uremic syndrome</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>Interstitial nephritis</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Miscellaneous*</td>
<td>11%</td>
<td>29%</td>
</tr>
</tbody>
</table>

The Finnish and US data are between 1979–2011 and 1987–2010, respectively. US data were adapted from the NAPRTCS annual report (2010) after excluding patients with unknown diagnosis (n = 663). * Including focal segmental glomerulosclerosis (FSGS), which is the 3rd most common indication in the US.
2.1.3 Immunosuppression

The recipient’s immune system becomes aware of the presence of an organ graft instantly after implantation and launches a response to destroy it as an alien intracorporal agent, unless being blocked. Suppression of lymphocyte activity against the graft is thus an indispensable element of RTx and needs to be started perioperatively. Immunosuppressive agents cause immunodeficiency with the benefit of rejection suppression but the disbenefits of undesired effects, such as infections, cancer, and nonimmunologic toxicity. The basic goal of immunosuppressive drug therapy is, on one hand, to achieve the lowest level of immunosuppression to prevent rejection, and on the other hand, to avoid the side effects caused by overimmunosuppression, e.g. opportunistic infections and malignancies. This endless balancing with the optimal dosage remains the hallmark of immunosuppressive medication.

In general, immunosuppression is attained by blocking pathways of lymphocyte response, diverting lymphocyte traffic, or depleting lymphocytes (Halloran 2004). At present, a multidrug combination therapy, most commonly by triple medication, is administered, targeting different steps of T-cell activation (Figure 1). Each drug has different modes of action, which are preferably utilized in a synergistic manner. This is helpful in maintaining the balance between rejection and overimmunosuppression; in other words, the use of multiple drugs allows the reduction of individual agents to a minimum without losing the efficacy, together with improving the means to tolerate dose-dependent drug toxicities (Denton, Magee & Sayegh 1999). Still, all the immunosuppressants have their own dose-dependent adverse effects, which should be carefully followed and avoided. The favourable and the most common and clinically significant effects of immunosuppressants are listed in Table 2.

Children metabolize medications at different rates from adults; tailored schedules and special formulations are thus characteristic for treating pediatric patients (Hoppu et al. 1991, Bunchman et al. 2001, Seikku et al. 2006). The half-life of CsA, for example, has been shown to be shorter in children than in adults, thus requiring dosing three times daily rather than twice-daily which is common in adult recipients (Cooney, Habucky & Hoppu 1997).
Figure 1. Targets for immunosuppressive agents in relation to the three-signal model of T-cell activation. Stimulation of T-cell receptor (TCR) with the major histocompatibility complex (MHC) class II molecule (signal 1) leads to the activation of the calcineurin pathway, a process inhibited by cyclosporine A and tacrolimus. Calcineurin pathway activation results in the induction of a number of cytokine genes, including interleukin-2 (IL-2). Glucocorticoids inhibit cytokine gene transcription in lymphocytes and antigen-presenting cells by several mechanisms. Costimulatory signals (signal 2) are necessary to T-cell IL-2 gene transcription, prevention of T-cell anergy, and T-cell apoptosis inhibition. IL-2 receptor stimulation of the target lymphocyte (signal 3) induces the cell to enter cell cycle and proliferate, a process that may be blocked by anti-IL-2 receptor antibodies or by rapamycin. Following progression into cell cycle, azathioprine (Aza) and mycophenolate mofetil (MMF) interrupt DNA replication by inhibiting de novo purine synthesis. (Adapted from Lui 2001).
<table>
<thead>
<tr>
<th>Immunosuppressant</th>
<th>Mechanism of action</th>
<th>Favorable effect</th>
<th>Adverse effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclosporine A</td>
<td>Inhibition of IL-2 gene transcription</td>
<td>Decreased T-cell activation</td>
<td>Nephrotoxicity, hirsutism, neurotoxicity, hypertension, dyslipidemia, gingival hyperplasia</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Inhibition of IL-2 gene transcription</td>
<td>Decreased T-cell activation</td>
<td>Nephrotoxicity, neurotoxicity, diabetes, hypertension, gastrointestinal toxicity</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>Inhibition of de novo purine synthesis</td>
<td>Inhibited T- and B-lymphocyte proliferation</td>
<td>Bone marrow suppression, hepatotoxicity, skin cancer</td>
</tr>
<tr>
<td>Mycophenolate mofetil/enterocoated mycophenolate sodium</td>
<td>Inhibition of de novo purine synthesis</td>
<td>Inhibited T- and B-lymphocyte proliferation, cell adhesion, migration, and antibody formation</td>
<td>Gastrointestinal toxicity, bone marrow suppression</td>
</tr>
<tr>
<td>Sirolimus/everolimus</td>
<td>Blocking of signals from cell surface receptors</td>
<td>Inhibited differentiation and proliferation of lymphocytes, and stimulation of T-cell apoptosis</td>
<td>Impaired wound healing, dyslipidemia, infertility, myalgia/arthralgia, oral ulcerations, acne</td>
</tr>
<tr>
<td>Glucocorticoids</td>
<td>Complex interaction with non-signaling and signaling proteins and receptors, inside and outside cells</td>
<td>Reduced T-lymphocyte activation and proliferation as well as suppressed antibody and complex binding</td>
<td>Diabetes, hypertension, dyslipidemia, osteoporosis, impaired growth, weight gain, gastrointestinal toxicity, acne, adrenal dysfunction</td>
</tr>
<tr>
<td>Anti-thymocyte globulin/anti-lymphocyte globulin</td>
<td>Polyclonal cytotoxic antibodies against T-cell surface antigens</td>
<td>Depleted count of circulating lymphocytes and inhibition of lymphocyte function</td>
<td>Cytokine release syndrome, anaphylaxis, infections</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Interleukin 2 receptor antibody</td>
<td>Depleted count of T-lymphocytes</td>
<td>No substantial adverse effects</td>
</tr>
<tr>
<td>OKT3 (muromonab-CD3)</td>
<td>CD3 antibody</td>
<td>Interrupted antigen recognition, T-cell signaling and proliferation</td>
<td>Cytokine release syndrome</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of immunosuppressive agents commonly used in organ transplantation.
Calcineurin inhibitors (CNIs) CsA and tacrolimus inhibit T-lymphocyte activation and are the cornerstone of immunosuppression in the field of transplantation (Andreoni et al. 2007). In brief, they bind to their specific cytoplasmic receptors (cyclophilin and FK-binding protein 12, respectively) and the resulting complexes inhibit and inactivate calcineurin, a pivotal enzyme in T-cell receptor signaling (Clipstone, Crabtree 1992). Without the effect of calcineurin, translocation of nuclear factor of activated T lymphocytes (NFAT) from cytoplasm into the nucleus is impossible. NFAT, in turn, is needed for induction of interleukin-2 and other cytokine genes necessary for T-cell growth and differentiation (Kahan 1989).

CNIs are known to be nephrotoxic (Kaplan, Schold & Meier-Kriesche 2003, Webster et al. 2005) and the cumulative effect of CsA and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers causing glomerular hypoperfusion may further exacerbate the deleterious effects (Kengne-Wafo et al. 2009). At first, the effect was suggested to be reversible, characterized by arteriolar vasoconstriction leading to decreased renal function (Klintmalm, Iwatsuki & Starzl 1981, Morris et al. 1983). Today, this effect is known as “acute CNI nephrotoxicity”, but a greater problem is the irreversible renal dysfunction, “chronic CNI nephrotoxicity” discovered later, characterized by glomerulosclerosis, interstitial fibrosis, and tubular vacuolization (Myers et al. 1984, Starzl et al. 1990). The CNI-related nephrotoxicity is one of the predominant non-immunological factors for chronic kidney dysfunction, but some changes attributed to chronic CNI toxicity may, however, be a consequence of immunologic injury (Gaston et al. 2010, Issa, Kukla & Ibrahim 2013). The spectrum of the adverse effects of CsA and tacrolimus resemble each other due to the similar immunosuppressive activity but, remarkably, the structural disparity paves the way for differences.

Antiproliferative agents (antimetabolites) azathioprine, and mycophenolic acid (MPA; comprising mycophenolate mofetil, MMF and enterocoated mycophenolate sodium, EC-MPS) interfere with de novo purine nucleotide synthesis and metabolism. As a consequence, DNA replication is prevented, blocking the differentiation and proliferation of alloactivated T- and B-lymphocyte clones. Azathioprine, a prodrug metabolized to 6-mercaptopurine, was the first immunosuppressive agent approved for Tx use, but despite the wide experience, its mechanism of action remains ambiguous (Tiede et al. 2003). MMF and EC-MPS, in turn, are precursors of MPA, which acts by inhibiting inosine monophosphate dehydrogenase (IMPD), a key enzyme in de novo synthesis of guanosine mono phosphate (Eugui, Allison 1993).

Antimetabolites have a relatively wide therapeutic window and rather well tolerated side effect profiles (Salvadori et al. 2004, Budde et al. 2004). MPA has thus largely replaced azathioprine worldwide (Denton, Magee & Sayegh 1999). The most common side effects of antimetabolites are bone marrow suppression and various gastrointestinal symptoms (Table 2).
Glucocorticoids are potent unspecific anti-inflammatory drugs with a substantial list of indications alongside the field of transplantation. The glucocorticoid receptor-mediated effects primarily target transcription factors, such as nuclear factor kappa beta (NF-κB) (Heck et al. 1994, McKay and Cidlowski 1999). Consequently, this inhibits synthesis of multiple cytokines by T cells and macrophages essential for T lymphocyte activation and tissue injury (Scheinman et al. 1995). The immunosuppressive mode of action is, however, immensely diverse due to additional glucocorticoid receptor-independent effects occurring at higher doses (Buckbinder, Robinson 2002).

The downside of the polymorphic mode of action is the association with myriad deleterious effects, especially in long-term use. The list goes beyond those in Table 2: weight gain, cushinoid appearance, acne, skin fragility, sodium and fluid retention, aseptic bone necrosis, myopathy, cataracts, glaucoma, increased infection risk, impaired wound healing, and neuropsychiatric symptoms (such as depression, mania, psychosis, and insomnia) (Denton, Magee & Sayegh 1999, Bergmann et al. 2012). Not surprisingly, a long history of glucocorticoid withdrawal or avoidance exists in transplantation (Hricik et al. 1993). Despite the successful results of several studies (Rike et al. 2008, Pascual et al. 2004), other reports have been concerned with increased risk for acute rejection (AR) (Chao et al. 1994, Ahsan et al. 1999). Probably due to the short-term nature of these reports and the lack of long-term prospective studies for confirming the conclusions, most centers worldwide still consider glucocorticoids as a fundamental adjunct to immunosuppression (Pascual 2011).

The mammalian target of rapamycin (mTOR) inhibitors sirolimus (rapamycin) and its derivate everolimus are among the new antiproliferative immunosuppressive agents (Schuler et al. 1997). Rapamycin binds the same FK-binding protein 12 as tacrolimus, but the complex does not inhibit calcineurin. Instead, it inhibits the mTOR pathway by blocking IL-2 receptor-mediated signals from the cell surface, further inhibiting the proliferation of lymphocytes, mesenchymal cells, and tumor cells (Sehgal 2003).

The adverse effect profile is wide: bone marrow toxicity (anemia, leukocytopenia, and thrombocytopenia), aggravated nephrotoxicity when combined with CNIs, hyperlipidemia, reduced testosterone levels, infertility, pneumonitis, and wound-healing problems (Meier-Kriesche et al. 2005, Fritsche et al. 2004, Gonwa et al. 2003, Boobes et al. 2010).

Intravenously administered polyclonal antibodies, anti-lymphocyte or anti-thymocyte globulin (ALG/ATG), can be utilized against numerous surface antigens of T-cells resulting in depletion of circulating lymphocytes and inhibition of lymphocyte functions (Shield et al. 1997, Bonnefoy-Berard, Vincent & Revillard 1991). The major monoclonal antibodies in clinical use, basiliximab and daclizumab, exert their effect by targeting interleukin-2 receptor modulating T-cell functions and depleting T-lymphocytes from peripheral blood (Nashan et al. 1997, Ortho Multicenter Transplant Study Group 1985, Kahan, Rajagopalan & Hall 1999). OKT3 (muromonab-CD3) is rarely used today, mainly
for anti-rejection therapy with MP. It has a significant adverse effect, cytokine release syndrome, which may occur especially after the initial dose (Gaston et al. 1991). Pretreatment with antipyretics, antihistamines, and corticosteroids can be used to prevent these flu-like symptoms (fever, headache, diarrhea, nausea, bronchospasm, and fluctuations of blood pressure (BP)) (Goldman et al. 1989).

The clinical protocols and regimens consist of three major therapeutic phases: induction, maintenance, and antirejection therapies (the last of which is addressed in detail in the rejection section below). The rejection risk after RTx is not spread evenly over time; in fact, the risk is significantly higher during the first six months post-Tx with a decreasing trend thereafter (Nankivell et al. 2003). The immunosuppressive therapy during the induction and early maintenance phases emphasizes the need for greater doses followed by reduction over time. During the continuous life-long maintenance treatment doses may be stable for long, but in case of an established rejection multiplied doses may be needed as a short-course therapy.

Induction therapy is currently executed for the majority of pediatric RTx recipients by ALG, ATG, or anti-interleukin-2-receptor antibody basiliximab or daclizumab (NAPRTCS 2010). At our center basiliximab has been used since the year 2000 at 2 doses, intraoperatively and on the 4th post-RTx day. Children under 35 kg receive 10-mg doses while others receive 20-mg doses. The maintenance immunosuppressive is started perioperatively, the early maintenance therapy hence overlapping with the induction therapy. On the first postoperative day the CsA dose is 4.5 mg/kg and azathioprine dose is 2 mg/kg, both divided into three doses, and methylprednisolone (MP) dose is 1.5 mg/kg, divided into two doses.

The maintenance therapy protocol used at the study center is presented in Table 3. In brief, the first weeks post-RTx require attentive follow-up of CsA trough blood concentrations and adjustments of CsA dosage (usually 2–4(–6) mg/kg/day), respectively. During the first 12 months post-RTx CsA dose is gradually tapered to reflect a target trough blood concentration of 150 µg/L. When MP is switched to every-other-day dosing at 3–6 months post-RTx, the azathioprine dose is increased slightly. After the first post-RTx year, CsA is individually targeted to a level of 80–120 µg/L and azathioprine dose to 1.0–1.4 mg/kg/day. The MP daily dose is on average 0.06 mg/kg with no increase along patient’s growth.

Tacrolimus is used instead of CsA after retransplantation or later in case of recurrent rejections, gradually increasing creatinine, or major cosmetic problems (hypertrichosis, gum hyperplasia). The typical maintenance therapy trough concentration is 5-7 mg/L. Azathioprine is replaced by a mycophenolate in case of recurrent rejections or gradually increasing creatinine. The usual maintenance dose is 500–1000 mg/day. Also, if CsA or tacrolimus toxicity is suspected, azathioprine can be switched to mycophenolate and the dosing of CsA or tacrolimus reduced.
Table 3. Initial protocol for immunosuppressive therapy used at the study center.

<table>
<thead>
<tr>
<th>Post-RTx time</th>
<th>Cyclosporine A (trough concentration goal)</th>
<th>Azathioprine (mg/kg/day)</th>
<th>Methylprednisolone (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First month</td>
<td>300 µg/L</td>
<td>1.0–2.0</td>
<td>0.25–1.0</td>
</tr>
<tr>
<td>1–3 months</td>
<td>250–300 µg/L</td>
<td>1.0</td>
<td>0.25</td>
</tr>
<tr>
<td>3–6 months</td>
<td>200–250 µg/L</td>
<td>1.0</td>
<td>0.25</td>
</tr>
<tr>
<td>6–12 months</td>
<td>150–200 µg/L</td>
<td>1.4</td>
<td>0.3 e.o.d.</td>
</tr>
<tr>
<td>&gt;12 months</td>
<td>80–120 µg/L</td>
<td>1.0–1.4</td>
<td>0.1–0.2 e.o.d.</td>
</tr>
</tbody>
</table>

E.o.d. every other day. Cyclosporine A is switched to tacrolimus and/or azathioprine is replaced by mycophenolate in case of recurrent rejections or gradually increasing creatinine. If calcineurin inhibitor toxicity is suspected, azathioprine is switched to mycophenolate and the dosing of cyclosporine A or tacrolimus is reduced.

2.2 Complications

2.2.1 Rejections

Core needle biopsy is the gold standard for diagnosing varied rejectional and non-rejectional lesions related to the allograft. The immunopathologic mechanisms of rejection are cell-mediated (caused by T-cells) and humoral (caused by antibodies), either alone or together (Cohen 2007). Cell-mediated AR occurs typically within the first 2 months post-RTx but may rarely appear even years after engraftment. T-cells infiltrate the tubulo-interstitium and arteries separately or together and the lesions may be patchy. Antibody-mediated rejection (ABMR) is the result of donor-specific antibodies and can appear as hyperacute, acute humoral, and chronic rejection. Hyperacute rejection can be avoided by ensuring a negative result in crossmatch test screening for the recipient’s preformed antibodies against the donor’s HLA. ABMR is diagnosed by: 1. Identification of histological evidence of specific tissue injury, 2. Evidence of antibody interaction with vascular endothelium, and 3. Serologic evidence of donor-specific antibodies (HLA or other) (Haas et al. 2014).

Historically, more than half of deceased organ recipients experienced an AR during the first post-RTx weeks. The majority of patients now experience an AR-free year and less than half of patients experience an AR episode at all (NAPRTCS 2010). Treatment of the cell-mediated AR is initiated by intravenous corticosteroid pulses, typically with 10–30 mg/kg doses lasting for 3–5 consecutive days. In addition, augmentation of
Immunosuppressive therapy is usually performed with a slow tapering of oral corticosteroids. Severe, recurrent, or steroid-resistant rejections are typically treated with ALG/ATG. Treatment of an ABMR may require use of plasmapheresis, intravenous immunoglobulins, cyclophosphamide, or rituximab (anti-CD-20 antibody) in addition to the aforementioned therapies (Montgomery et al. 2000).

Chronic allograft nephropathy (CAN) in a kidney graft may occur as early as 3 months post-RTx and it typically develops over months to years. Clinically CAN is designated as gradual loss of renal function and the morphological changes occur in all components to varying degrees (Fletcher, Nankivell & Alexander 2009). Interstitial fibrosis and tubular atrophy (IF/TA), and prominent arterial and glomerular lesions may be seen in core needle biopsy samples (Sibley 1994). CAN accounts for a third of the graft failures in the NAPRTCS data, being the most common cause for graft loss (NAPRTCS 2010). On the other hand, Qvist et al. (2000) reported that two thirds of the patients having undergone an RTx under the age of 5 years had no signs of CAN in biopsy at 7 years post-RTx.

2.2.2 Infections

Advancements in immunosuppressive drugs have reduced the incidence of ARs but at the same time may have exacerbated the risk of infections post-RTx (Dharnidharka, Stablein & Harmon 2004). Within the first month post-RTx, infections are generally associated with donor- or recipient-derived pre-existing conditions or complications of surgery (Fishman 2007). The latter include bacterial wound infections, sepsis, urinary tract infections, and pneumonia. Between 1 and 6 months post-RTx, the nature of infections changes dramatically and viral infections are most common, either as a primary infection or a reactivation of viruses, paving way for other opportunistic infections as well (Green, Michaels 2007, Rubin 1993). Still, fungal infections are infrequent after RTx. Later on, in the 6- to 24-month post-RTx period, the percentage of hospitalization owing to viral infections has increased over time (Dharnidharka, Stablein & Harmon 2004). On the other hand, according to a Finnish study, upper respiratory tract infections are the most common problem in pediatric RTx recipients with a rate and severity similar to age-matched healthy children (Their et al. 2000). The study also reported severe bacterial infections being rare, but urinary tract infections were found in 39% of pediatric RTx patients.

Immunomodulating viruses, especially cytomegalovirus and Epstein-Barr virus (EBV), are a major threat for RTx patients without prophylactic medication (Fishman 2007, Korn et al. 1992). Cytomegalovirus is a common and important cause for viral infection post-RTx and without prophylaxis, a symptomatic disease typically manifests 1–3 months after transplantation (Green, Michaels 2007). A characteristic constellation of fever and hematological abnormalities (including leukopenia, atypical lymphocytosis and thrombocytopenia) is associated with cytomegalovirus disease and a disseminated disease may manifest by involvement of the gastrointestinal tract, liver, or lungs. The use of ganciclovir prophylaxis has, however, decreased the incidence and severity of
cytomegalovirus disease. The significance of EBV infections, especially in pediatric Tx where primary EBV infections are emphasized, has grown in parallel with recognition of mortality and morbidity related to EBV disease (Green et al. 1999). Careful diagnosing of EBV disease (by clinical, laboratory, and histopathologic examination) is important due to the ominous post-transplant lymphoproliferative disorders (PTLD) (see below).

Polyomaviruses BK and JC have been identified in association with nephropathy in RTx recipients (Fishman 2007). BK virus-associated nephropathy affects 1–10% of kidney Tx recipients and is primarily due to BK virus reactivation and replication in urothelial cells (Kumar 2010). Diagnosis of polyomaviruses can be confirmed by polymerase chain reaction (PCR) from plasma or urine, and with SV40 staining of a core needle biopsy sample. No effective antiviral medication for polyomaviruses exists and judicious reduction of immunosuppression with rejection surveillance is advised (Fishman 2007, Green, Michaels 2007, Egli et al. 2009).

Currently at our center, RTx patients receive two types of prophylactic therapy. Valganciclovir is used for six months in case of recipient or donor cytomegalovirus-seropositivity. Co-trimoxazole is administered for twelve months post-RTx to prevent pneumocystis jirovecii pneumonia.

2.2.3 Malignancies

Immunosuppression-induced malignancies are more commonly diagnosed in parallel with improved graft and patient survival rates. However, the pattern of malignancies in pediatric Tx patients differs from that of general childhood population and adult organ recipients. The reported probability of developing de novo malignancy was estimated by Coutinho et al. (2001) at 17% 25 years after the first pediatric RTx, a 10-fold incidence rate compared to the general age-matched population. In another study, over a 10-year period the risk of malignant lymphoma in Tx patients was 12-fold higher than in a matched nontransplant population (Opelz, Dohler 2004).

PTLD comprises a family of conditions occurring in organ Tx patients having evidence of EBV and lymphoid growths, thus, straddling the borderline of infection and neoplasia (Nalesnik, Starzl 1994). The majority (85–90%) of PTLD cases in children are EBV-driven, arising almost without exception in the first 3 post-RTx years (Webber, Green 2007). Furthermore, PTLD is more frequent in patients who are seronegative for EBV at the time of RTx and subsequently develop primary EBV infection (Ho et al. 1988). Adults are usually EBV-seropositive, explaining the difference reported by Penn et al. (1998) that in pediatric Tx patients PTLD accounts for 52% of all the malignancies compared to 15% in adult Tx patients. Furthermore, along with the improved patient and graft survival and advanced immunosuppression, the PTLD incidence has increased during the recent years (Dharnidharka et al. 2002).
2.3 Long-term outcome

2.3.1 Growth

Notable growth retardation, a significant concern for the patients and their families, is common in children with ESRD and is of multifactorial origin (Schaefer 2004). According to the most recent NAPRTCS data (2001), children with worse height deficit at the start of dialysis improve slightly, but those with less deficit at baseline experience worse deficit after 2 years of dialysis. Uremia causes nausea, vomiting, and lack of appetite. Metabolic acidosis inherent in uremia also disturbs the somatotrophic hormone axis and at the same time, results in excessive catabolic protein wasting state that could contribute to growth retardation (Schaefer 2004, Boirie et al. 2000). Moreover, in CNF, the most common underlying disease preceding RTx in Finland, severe loss of protein leads not only to malnutrition but also to endocrine alterations possibly distorting growth (Holmberg et al. 2004). Growth in children with ESRD is thus characterized by a continuous gradual deviation from the normal growth, a detrimental status that can be decelerated before RTx by nutritional management, growth hormone (GH) therapy, and dialysis (Kari et al. 2000, Johansson et al. 1990, Laakkonen et al. 2010).

RTx corrects the effect of preceding uremia and provides better conditions for growth, which is seen as accelerated growth after transplantation (Ingelfinger et al. 1981). Even though the growth velocities after RTx are greater than those of similarly aged children in dialysis, the restoration of kidney function does not fully restore the growth potential, and adult height is commonly blunted in pediatric RTx patients (Turrenne et al. 1997, Aschendorff et al. 1990, Harambat et al. 2014). Children having received a kidney graft from a living related donor show better growth post-Tx than those having received a deceased donor graft (Pape et al. 2005). During the past quarter century, the mean height standard deviation (SD) score (hSDS) deficit at the time of RTx has improved over the years from -2.43 SD in 1987 to -1.23 in 2009, with an overall average of -1.75 SD (NAPRTCS 2010). The major factors affecting growth, in addition to age and height at the time of RTx, are kidney graft function and medication post-RTx.

Catch-up growth is reportedly more apparent in patients transplanted in early childhood in comparison with those aged older than five years at RTx (Figure 2) (NAPRTCS 2010). Regardless of the age at RTx, however, the growth plateaus after the initial 2 years following transplantation. Also, an inverse correlation between the height at RTx and the rate of catch-up growth has been reported, thus the more stunted the patient is at RTx, the greater catch-up growth is observed (Bosque et al. 1983, Tejani, Cortes & Sullivan 1996, Qvist et al. 2002a). In this respect, age at RTx and duration of uremia may also distort the pubertal growth spurt (see below).
Figure 2. Mean height scores and growth patterns by age at transplant. Younger recipients experience better improvement in mean growth deficit after transplantation than the older ones. (Adapted from NAPRTCS 2010).

Growth is sensitive to deteriorated renal function in a similar manner in chronic failure of native kidneys or renal graft. A significant negative correlation between serum creatinine and decrease in height Z score was already reported by Tejani et al. in 1993. Soon afterwards, the growth-suppressive effect of poor renal function was confirmed to be independent of corticosteroid dosage (Hokken-Koelega et al. 1994b, Jabs et al. 1996).

The role of glucocorticoids affecting growth is obvious (Schaefer 2007). Results from studies analyzing the effect of steroid therapy modification, withdrawal, or primary avoidance have shown that catch-up growth can be promoted by adjusting glucocorticoid treatment. Broyer et al. (1992) reported that the mean change in hSDS was better in patients with alternate-day dosing than in those with daily regimen even though the cumulative dose was the same between the groups. Similarly, Höcker et al. reported in 2004 that patients with discontinuation of steroids in the second post-RTx year had height increased from -1.60 SD to -1.0 SD at 4 years post-RTx in comparison with unchanged hSDS in those with continued daily steroids, while Sarwal et al. (2012) reported that recipients under 5 years of age showed improved linear growth with steroid-free compared with steroid-based regimen. In the latter study, the results anticipated that, especially among older children receiving kidney grafts, there are other factors than steroids affecting catch-up growth. One explanation was presented by Kapila et al. (2001) reporting that reduced bioactivity of insulin-like growth factor-1, an important growth
promoter produced by stimulation of GH, occurs in RTx patients independent of steroid treatment.

GH therapy is effective in children with chronic renal failure and after RTx (Fine et al. 1996, Maxwell, Rees 1998). It can be initiated already during the first year of life in order to preventing growth retardation (Mencarelli et al. 2009). Despite an adequate GH therapy, the final height (FH) in RTx recipients remains blunted (Rodriguez-Soriano et al. 2000). GH therapy has also raised controversial questions on safety issues (Benfield, Kohaut 1997, Friedman 1997). The worries have been related to findings that GH augments immune function in several ways, such as promoting antibody synthesis, activating cytotoxic T- and natural killer cells, and increasing the production of tumor necrosis factor alpha (Kelley 1990). The possible immunoactivation-promoted worse graft survival or increased rejection rates raised concerns at first, but opinions have since been for and against such effects (Chavers et al. 1995, Laine et al. 1996, Fine et al. 2002). The present general opinion suggests that GH therapy does not threaten the allograft function, but patients with a history of at least one AR may have increased risk of AR during GH therapy (Broyer 1996, Guest et al. 1998, Fine, Stablein 2005).

2.3.2 Pubertal development

Puberty is a complex developmental process occurring in late childhood. It consists of rapid physiological alterations, such as maturation of secondary sexual characteristics, attainment of adult height, and changes in body composition (Diamanti-Kandarakis, Gore 2012). Pubertal development is thus the transitional phase from sexual immaturity to gained reproductive capacity. Puberty is initiated by the awakening of the dormant hypothalamic-pituitary-gonadal axis, the primary mechanism of which, however, remains unclear (Parent et al. 2003). The gonadotropin release hormone (GnRH) pulse generator in the hypothalamus activates secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, resulting in secretion of testosterone from the testes and estrogen from the ovaries in boys and girls, respectively (Figure 3). The onset and progress of puberty is assessed by the 5-stage scale of external primary and secondary sexual characteristics, the appearance of breasts in girls and genitalia in boys, and pubic hair in both genders, as reported by Marshall and Tanner (1969 and 1970). The first sign of puberty is an increase in testes volume in boys and an advance in breast development in girls, but the average schedule varies individually as presented in Figure 4.
Figure 3. Gonadotropin-releasing hormone (GnRH) is secreted from the hypothalamus and stimulates secretion of gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. LH and FSH in turn stimulate the sex steroid (testosterone, estrogen, and progesterone) secretion from the gonads. As a result of the negative feedback loop, the sex steroids inhibit the secretion of GnRH and the gonadotropins.
Figure 4. Diagram of the sequence of events at puberty in girls and boys. An average girl and boy are represented in relation to the scale of ages and the range within which some of the changes occur is indicated below. (Adapted from Marshall and Tanner 1970). G. rating refers to stages for genitalia.

Data on pubertal onset and tempo in pediatric RTx patients are scarce in comparison with studies of somatic growth. The available data, however, suggest that delayed puberty is common. In the two studies from the 1980s, Rees et al. (1988) found delay in the appearance of secondary sexual characteristics in both genders, and Van Diemen-Steenvoorde and Donckerwoncke (1988) reported similarly of delayed puberty. In 2004, Nissel and co-workers reported that onset of puberty is markedly delayed in RTx patients transplanted prepubertally. In the most recent study on pubertal onset, delayed sexual maturation was found in 22% of girls and 19% of boys (Ghanem et al. 2010). In keeping with the general pubertal delay, female pediatric RTx recipients experience delayed menarche. Van Diemen-Steenvoorde et al. reported in 1987 that menarche occurred in
girls transplanted prepubertally at the mean age of 15.3 years, but the concomitant mean bone age was 12.9 years, the typical age at which menarche occurs.

Pubertal growth is driven by sex steroids (Karlberg 1989). The loss of FH in pediatric RTx patients, however, is mainly determined by infancy and childhood growth, which are dependent on nutrition and GH (Schaefer 2004). ESRD and RTx may have a deleterious effect on the pubertal growth spurt, the apparent peak in growth velocity during adolescence. Turenne and co-workers (1997) reported of absent substantial pubertal growth spurt in a large study of more than 700 pediatric RTx patients. Two other studies have also reported a lack of pubertal growth spurt, emphasizing its contribution to the low adult height in concert with the preexisting height deficit at the time of RTx (Nissel et al. 2004, van Diemen-Steenvoorde et al. 1987). As mentioned above, GH therapy has indeed paved the way for height gain after RTx. It significantly improves growth and corrects the existing height deficit without interfering with growth and bone health. Haffner et al. (2000) reported that GH therapy was associated with accelerated prepubertal bone maturation, but not with shortening of the pubertal growth spurt, however. In contrast, three studies reported that GH therapy does not accelerate skeletal maturation or advance pubertal development (Hokken-Koene et al. 1996, Van Dop et al. 1992, Hokken-Koene et al. 1994a).

### 2.3.3 Fertility

ESRD disturbs the normal sexual function by various mechanisms, such as uremic milieu, neuropathy, vascular disease, pharmacological therapy, and psychological stress. The deterioration of the hypothalamic-pituitary-gonadal axis leads to impaired fertility in both genders. RTx is reportedly the most effective means to recover the hormonal function and restore the reproductive capacity (Palmer 1999, Lim, Fang 1975, Holdsworth, de Kretser & Atkins 1978, Prem et al. 1996). Kim et al. (1998) reported of resolved menstrual cycle dysfunction subsequent to RTx. Quite recently, Akbari et al. (2003) reported of almost normal steroidogenic function and recovery of spermatogenic function. However, conflicting results with continuation of hormonal, seminal, and ovarian disturbances after RTx have also been reported (Bozzini et al. 2013, Tauchmanova et al. 2004). The data are still scarce, especially in detailed long-term follow-up after pediatric RTx during childhood or adolescence. In male recipients, Inci et al. reported in 2006 that spermatogenesis does not improve after pediatric RTx and Koyun et al. (2009) showed that earlier onset and longer duration of RRT emphasize the impairment of reproductive function.

The gonadotoxic effect of some immunosuppressive medication, especially cyclophosphamide and sirolimus, is known (Boobes et al. 2010, Watson, Rance & Bain 1985, Bogdanovic, Banicevic & Cvoric 1990, Tondolo et al. 2005, Skrzypek, Krause 2007, Zuber et al. 2008). Germinal cell aplasia has been documented in cyclophosphamide-treated patients with intact Leydig cell appearance in testicular biopsy
(Etteldorf et al. 1976). Sirolimus interferes with the stem cell factor/c-Kit system, thus blocking spermatogenesis (Feng, Ravindranath & Dym 2000). Furthermore, deterioration of rat germ cell function by the CNIs has also been reported (Masuda et al. 2003, Hisatomi et al. 1996, Chen et al. 2013), but the results from studies in humans have been contradictory (Handelsman et al. 1984, Haberman et al. 1991, Samojlik et al. 1992).

2.3.4 Cardiovascular and metabolic outcome

CVD, accounting for 40% of late mortality in adult RTx recipients with functioning graft, is the most important long-term risk factor limiting the success of RTx (Ojo et al. 2000). The short-term complications and their incidences, risk factors, and treatment options are better known, in contrast to the long-term problems of which data have been recently started to emerge.

The same traditional risk factors for CVD (such as age, cigarette smoking, obesity, hypertension, dyslipidemia, diabetes) as in the general population are also predictive among RTx recipients (Kasiske 2001). In addition, the RTx patients have non-traditional risk factors (such as recurrent rejections and ESRD combined with diabetes) that show independent contribution to ischemic heart disease (Kasiske, Chakkera & Roel 2000). Native kidney nephrectomy has been indicated by severe hypertension before RTx but the results on long-term cardiovascular outcome are reportedly modest (Cavallini et al. 2010). Even though the death rate from CVD is considerably lower for adult RTx recipients than for dialysis patients, it still is double the rate in general population (Foley, Parfrey & Sarnak 1998). RTx children and adolescents have also severely impaired cardiorespiratory fitness in comparison with their healthy peers (Tangeraas 2010). The etiology of CVD as well as its risk factors is multifactorial. For example, immunosuppressive agents directly contribute to the risk for CVD but also predispose the patients to other risk factors, such as hypertension, dyslipidemia, and diabetes mellitus.

Hypertension is a common and serious complication in RTx recipients (Baluarte et al. 1994, Sorof et al. 1999). It associates with impaired graft survival and increased CVD morbidity and mortality (Tutone et al. 2005, Mange et al. 2000, Mitsnefes, Khoury & McEnery 2003). BP monitoring is therefore crucial in the follow-up of RTx patients. The reported prevalence of hypertension after RTx varies, however, mainly because of the different methods of measurement and definitions of hypertension used in various studies.

Ambulatory BP monitoring (ABPM) provides data on daytime, nighttime, and 24-hour BP levels and it has been shown to be superior to single office BP measurements, especially since it can reveal white-coat and nocturnal hypertension (Calzolari et al. 1998, Ferraris et al. 2007). Another advantage of the method is the ability to analyze the physiological decrease of BP during the night (nocturnal dipping). In a study by Lipkin et al. (1993), nocturnal dipping was found to associate with greater left ventricular mass in adult patients. In a pediatric study by Seeman et al. (2006), no such relation could be confirmed,
however. The main findings of several pediatric studies using 24-hour ambulatory BP monitoring (ABPM) on hypertension are summarized in Table 4. Several studies have indeed reported of predominance of nocturnal hypertension in RTx recipients (Seeman et al. 2006, Giordano et al. 2000, Morgan et al. 2001, McGlothian et al. 2006). Furthermore, ABPM has been reported to have better correlation with left ventricular hypertension and renal function than office BP measurements (Mitsnefes et al. 2001, Jacobi et al. 2000).

**Table 4.** Studies reporting ambulatory hypertension in pediatric renal transplantation patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Definition of hypertension</th>
<th>Prevalence of hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lingens et al. 1997</td>
<td>27</td>
<td>BP &gt;95\textsuperscript{th} percentile or medication</td>
<td>70%</td>
</tr>
<tr>
<td>Giordano et al. 2000</td>
<td>37</td>
<td>BP &gt;95\textsuperscript{th} percentile</td>
<td>62%</td>
</tr>
<tr>
<td>Sorof et al. 2000</td>
<td>42</td>
<td>BP load &gt;25% (BP &gt;95\textsuperscript{th} percentile)</td>
<td>83%</td>
</tr>
<tr>
<td>Morgan et al. 2001</td>
<td>45</td>
<td>BP &gt;95\textsuperscript{th} percentile and BP load &gt;30%</td>
<td>62%</td>
</tr>
<tr>
<td>Serdaroglu et al. 2005</td>
<td>26</td>
<td>BP &gt;95\textsuperscript{th} percentile and BP load &gt;30%</td>
<td>73%</td>
</tr>
<tr>
<td>Seeman et al. 2006</td>
<td>36</td>
<td>BP &gt;95\textsuperscript{th} percentile or medication</td>
<td>89%</td>
</tr>
<tr>
<td>Gülhan et al. 2014</td>
<td>29</td>
<td>BP &gt;95\textsuperscript{th} percentile</td>
<td>76%</td>
</tr>
</tbody>
</table>

BP, blood pressure.

The abovementioned studies emphasize the importance of the use of ABPM in the follow-up of Tx patients but the method still has significant limitations. ABPM provides excessive data on 24-hour BP, but reference values for indexing the results with regard to healthy children and adolescents are limited to Caucasian subjects (Wühl et al. 2002). Furthermore, for several years the relative importance of the parameters provided by ABPM (BP index, load, and dipping) was unclear until the American Heart Association (AHA) provided guidelines for interpretation of ABPM in 2008 (Urbina et al.). This classification uses a combination of systolic and diastolic office and mean ambulatory BP values, and BP loads in the staging of ABPM.

CNIs can contribute to hypertension by several mechanisms, the sum effect being vasoconstriction. The most important factors are renal or peripheral vasoconstriction, increased sympathetic and renin-angiotensin system activity, impaired nitric oxide-induced vasodilatation, sodium and water retention, and excess release of several vasoconstrictors (endothelin, thromboxane, and prostaglandins) (Buscher et al. 2004, Curtis 1994). Glucocorticoids, in turn, have the potential to alter both circulating volume
and vascular resistance (Brem 2001). The newer immunosuppressives, such as mycophenolate mofetil and sirolimus, seem not to predispose patients to hypertension (Buscher et al. 2004).

**Overweight and obesity** are a major concern in the long-term follow-up of RTx patients (Smith, McDonald 2007). Most children gain weight rapidly in the early post-Tx period with an average increase of 0.81 SD during the first year after the operation (NAPRTCS 2010). In a large pediatric study by Hanevold et al. in 2005, obese children aged 6 to 12 years had a higher risk for death than those of normal weight, and death was more likely as a result of cardiopulmonary disease. Other studies have also reported an association between body mass index (BMI) and worse survival rate after RTx (Aalten et al. 2006, Meier-Kriesche, Arndorfer and Kaplan 2002, Hoogeveen et al. 2011).

**Dyslipidemia** occurs in more than half of the pediatric RTx recipients in Europe (Bonthuis et al. 2014) and similar results have been reported by studies in the US (Wilson et al. 2010, Saland et al. 2010). In concert with a link to obesity and immunosuppressive medication, dyslipidemia may serve to aggravate renal injury (Weinberg 2006).

**Glucose metabolism** alters notably during postoperative follow-up. Hyperglycemia is common during the initial and early maintenance therapy due to postoperative stress and higher doses of immunosuppressive medication, especially glucocorticoid and CNI. Even though the diabetogenicity of immunosuppressants is reportedly dose-dependent, hyperglycemia in the early postoperative phase is associated with later incidence of diabetes (Kuypers et al. 2008). The predominant cause for corticosteroid-induced diabetes post-RTx seems to be insulin resistance, but also stimulation of gluconeogenesis and impaired insulin secretion have been reported to promote diabetes (Penfornis, Kury-Paulin 2006, Hjelmesaeth et al. 2005). CNIs induce diabetes post-RTx by a number of mechanisms, including insulin resistance, pancreatic beta cell toxicity, and decreased insulin secretion (Penfornis, Kury-Paulin 2006). The deleterious effects, which are more prominent with tacrolimus than with CsA, are in part dose-dependent and, diabetes may thus reverse after dose reduction (Heisel et al. 2004, Prokai et al. 2008, Rodrigo et al. 2005, Zielinska et al. 2003).

**Metabolic syndrome** (MS) is a constellation of CVD-promoting interrelated metabolic risk factors including obesity, hypertension, dyslipidemia, and impaired glucose metabolism (Hanevold et al. 2005, Zimmet et al. 2007). The current diagnostic criteria of the American Heart Association are based on the preceding Adult Treatment Panel III (ATP III) criteria and MS is diagnosed in the presence of abnormal results in a minimum of three out of five risk factors (overweight, hypertension, reduced high-density lipoprotein (HDL), elevated triglycerides, and elevated fasting glucose) (Grundy et al. 2005, National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002). The MS has been reported to associate with decreased renal allograft function, but the risk factors of MS may not contribute equally to long-term allograft function (de
Vries et al. 2004). In that study de Vries et al. found that only systolic BP and hypertriglyceridemia were independently associated with impaired renal function.

### 2.3.5 Psychological and cognitive outcome

Many of the children undergoing RTx have suffered from chronic illness affecting their cognitive development (Cadman et al. 1987). Failure of a major organ such as kidney often leads to neuropsychological sequelae with cognitive defects (Farmer 1994). Additional disabilities may increase the risk, especially in the case of early onset of the underlying disease, long-term end-stage organ failure and treatments, which can affect the developing brain. Although RTx restores the kidney function and removes the toxic effects of uremia, some of the perturbations and complications may be irreversible.

In school-aged RTx children, intellectual functioning is normal in the majority of patients (Falger et al. 2008). According to a study using siblings as matched controls, the results showed significantly worse intelligence scores for RTx recipients (Brouhard et al. 2000). According to a Finnish study, the majority (79%) of children having received a RTx before the age of 5 years attend normal school and 76% have normal motor performance (Qvist et al. 2002b). More recently, however, the same group reported of lower intelligent quotient in RTx patients with respect to test norms (Haavisto et al. 2012). In addition, they found that on neuropsychological assessment the RTx recipients scored worse than the control group in verbal and visuospatial domains.

Successful pediatric RTx permits the child to develop and live quite normally. This could mean the capability to attend school in parallel with their healthy peers. Still, re-entry to school and normal life can be limited in several ways. The changed body image in respect to that before a chronic illness and routine hospital visits, for instance, can be a significant burden for youngsters. Thus, health-related quality of life (HRQOL) issues have become more important. HRQOL can be evaluated, especially considering small children, from the perspective of a close proxy such as a parent. As a child grows older, more focus should be given to the patient’s own impression of HRQOL by assessing HRQOL from the recipient’s perspective. Parents can, however, underestimate distress and depression in children who had a Tx (Shemesh et al. 2005).

In a study by Sundaram et al. (2007), adolescent RTx recipients saw their quality of life as similar to healthy school-aged population. Recently, Haavisto et al. (2013) conducted a study on renal, heart (HTx), and liver (LTx) Tx recipients, and the results showed poorer HRQOL in preadolescents (8- to 11-year-old) compared with age-matched healthy peers. Lower HRQOL was associated with shorter follow-up after Tx, congenital disease, and psychiatric/neurological comorbidity (Haavisto et al. 2013). Similarly, Diseth et al. (2011) reported of higher levels of mental health problems and lower HRQOL in RTx children compared to both healthy children and those with a history of acute lymphoblastic leukemia. In line with the improved survival rates after pediatric RTx and the constantly
increasing number of adolescents surviving into adulthood, the issue of their quality of life is of paramount importance.

2.3.6 Survival

The **graft survival** rates in Finland are acceptable, with the 7-year survivals even for children transplanted under 5 years of age exceeding 80% (Qvist et al. 1999). Overall, living donor pediatric kidney recipients have better unadjusted 1-, 3- and 5-year graft survival than deceased donor recipients (Magee et al. 2008). According to Akkina et al. (2008), graft survival after a preemptive RTx is not necessarily positively correlated with the pretransplant estimated GFR. During the last two decades, the half-life of first kidney grafts has not improved (Meier-Kriesche, Schold & Kaplan 2004) and, accordingly, the attrition rate after the first year post-RTx has remained stable despite the better rejection prophylaxis (Lamb, Lodhi & Meier-Kriesche 2011). A single episode of cellular rejection with no components of vascular rejection and good response to rejection therapy allowed a similar graft survival as in those without rejection (McDonald et al. 2007). Chronic and acute rejections are, however, the most common reasons for graft failure in the US data (NAPRTCS 2010).

Graft thrombosis remains one of the major risk factors for graft failure, especially in children under two years of age, accounting for 12% of index graft loss (Singh, Stablein & Tejani 1997). Accordingly, patients with congenital nephrotic syndrome have graft failure due to thrombosis more frequently than patients with other primary diseases (8.3% vs. 2.9%, p = 0.002) (Kim, Stablein & Harmon 1998). Death with functioning graft, recurrence of original kidney disease, and discontinued medication are also reasons for graft loss worth noticing (NAPRTCS 2010).

The **patient survival** rate in Finland is world-class, with the 7-year survivals even for children transplanted under 5 years of age at 98% (Qvist et al. 1999). The patient survival rates, especially during the first post-Tx year, have improved over time and currently the 10-year patient survival rate in the US has exceeded 90% (Van Arendonk et al. 2014). Furthermore, the short-term outcomes of adult kidney grafts in small children are comparable to those of size-matched grafts (Goldsmith et al. 2010). CVD has kept its position as the most common cause of death with a functioning graft followed by infection and malignancy, but the overall death rates have been decreasing for the time being (Ojo et al. 2000, Pilmore et al. 2010, NAPRTCS 2010).
3 Aims of the study

The goal of this thesis was to examine the long-term endocrinologic and metabolic development and outcome during childhood, adolescence, and young adulthood. By the earliest possible identification of the patients at greatest risk for endocrinologic and metabolic problems their long-term prognosis and outcome can further be improved by means of enhanced therapy and follow-up.

The aims of the current study were:

1. To examine pubertal development and factors associating with it, with emphasis on reproductive hormone levels and growth.

2. To assess the reproductive hormone levels, testicular volumes, and semen quality in young men after RTx and to investigate the prevalence of testicular insufficiency and subsequent risk for infertility.

3. To study lipid and glucose metabolism, and MS in pediatric RTx recipients and to evaluate their possible impact on long-term graft function.

4. To analyze the ABPM profiles of children and adolescents with a renal, heart, or liver transplants and to study in RTx recipients the correlation between the ABPM variables and metabolic factors and concurrent and long-term graft function.
4 Subjects and methods

4.1 Patients and controls

This thesis comprises all 218 patients having undergone RTx during childhood or adolescence by March 2011 in Finland. Since 1986 all pediatric RTx operations in Finland have been performed at the study center (Children’s Hospital, Helsinki University Central Hospital). All the recipients visited for postoperative follow-up at 3, 6, 12, 18, and 24 months post-RTx and annually thereafter until the age of 18–20, after which they are transferred to adult care. All studies included a retrospective part, in which hospital charts were reviewed (Table 5).

Study I. The overall study cohort consisted of 109 RTx recipients. In the first part of the study we analyzed retrospectively clinical data on puberty in 98 RTx recipients. In the second cross-sectional part, data on reproductive hormones related to puberty were assessed in 87 patients, consisting of 50 consecutive patients who had their annual visit between February 2009 and January 2010 and of an additional 37 RTx patients who were older than 15 years at the time of sampling.

Study II. Fertility was studied in 24 male RTx recipients. The patient selection is presented in detail in Figure 5. The 56 controls were recruited from occupational health services in the Helsinki municipality area and Helsinki University Central Hospital. None of the subjects were on testosterone supplementation at the time of the study.

Study III. The data on metabolic characteristics in 210 RTx patients were collected at 1.5, 5, 9, and 13 years post-RTx. Seventeen patients had lost the first graft and 10 of them were included as new study patients. The remaining seven patients were included only once (after retransplantation). Eleven patients had undergone a combined liver and kidney transplantation.

Study IV. A total of 111 RTx recipients with ABPM data were analyzed. In case of missing data at 5 years post-Tx we used the nearest subsequent monitoring up to 10 years post-Tx. Four subjects had undergone a combined kidney and liver Tx and their data were analyzed in the RTx subgroup.
Table 5. Features of the study population.

<table>
<thead>
<tr>
<th>Study</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study issue</td>
<td>Puberty</td>
<td>Male fertility</td>
<td>Metabolic</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>Number of patients</td>
<td>109</td>
<td>24</td>
<td>210</td>
<td>111</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Follow-up &gt;1 y Age &gt;8 y Age at RTx &lt;16 y</td>
<td>Follow-up &gt;5 y Age &gt;18 y Age at RTx &lt;18 y</td>
<td>Follow-up &gt;1.5 y No re-RTx</td>
<td>Follow-up &gt;5 y</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>Lack of sufficient data on puberty and growth</td>
<td>Associated co-morbidity No</td>
<td>RTx within 1 y Lack of ABPM data</td>
<td>Acute rejection within 3 months</td>
</tr>
<tr>
<td>Control group</td>
<td>No</td>
<td>Healthy men without history of immunosuppression No</td>
<td>Other solid organ Tx recipients</td>
<td></td>
</tr>
<tr>
<td>Number of controls</td>
<td>-</td>
<td>56</td>
<td>-</td>
<td>29 HTx patients 13 LTx patients</td>
</tr>
</tbody>
</table>

Y, year; RTx, renal transplant, HTx, heart transplant, LTx, liver transplant.
Figure 5. Flow chart of patient selection in Study II. Sixteen men could not be contacted with repeated invitation letters and phone calls.

4.2 Methods

4.2.1 Clinical data collection

A retrospective analysis of patient data on the underlying disease before RTx, initiation and length of dialysis, age at RTx, kidney graft function, growth, and immunosuppressive medication was performed in all the Studies I-IV. In addition, data on other medication (antihypertensive, GH), bone age, and pubertal development were collected, as appropriate, concerning the study protocols. In Study II, a questionnaire concerning marital status, wish for having children, possible infertility treatments, and number of children fathered was filled out by the subjects. Patients and controls were assessed similarly.

Growth was assessed by measuring height and weight, and determining hSDS and height-adjusted weight ratio in percentages of the median using the Finnish growth curves for children (Sorva et al. 1990, Pere 2000). BMI was calculated by dividing weight by height squared (kg/m²). Bone age was determined from a plain radiograph of the left hand according to Greulich and Pyle (1959). Cut-off for short stature was defined as hSDS below -1.5 in Study I and -2.0 in Study III. Overweight was defined as weight-for-height exceeding 20% of the gender- and height-adjusted median (relative weight >120%),
except for Study IV in which overweight was defined, in line with the International Obesity Task Force Criteria recommendations, as BMI exceeding the percentile passing through BMI 25 kg/m² at the age of 18 years, according to the new Finnish growth references (Cole et al. 2000, Saari et al. 2011). Concerning pubertal growth, peak height velocity (PHV) was defined in line with Aksglaede et al. (2008) as the age at maximal height velocity after the age of 8.0 and 10.0 years for girls and boys, respectively. The age at minimum height velocity rate before PHV was defined as the onset of growth spurt (OGS). For FH (adult height) we recorded the height when growth velocity was less than 0.5 cm/year and Tanner stage 5 was reached. Data on RTx recipients transplanted after the onset of puberty were excluded when analyzing pubertal growth and factors affecting the pubertal onset in Study I.

**Pubertal maturation** was clinically evaluated according to Tanner (1962). Achievement of Tanner stage 2 was defined as the onset of puberty and the length of puberty as time between Tanner stage 2 and first evaluation of completed puberty (stage 5). In case of delayed onset of puberty or menarche, the age of the patient at the hallmark was more than 2 SD above the average in the Finnish population (Ojajärvi 1982). Testes were measured with a ruler and testicular volume was calculated using the common formula for prolate spheroid: Volume (mL) = 0.52 x longitudinal diameter (cm) x transversal diameter squared (cm²) (Hansen, With 1952). The mean volume of both testes was used for data analyses except in case of scrotal hydrocele or preceding unilateral orchiectomy, when the volume of the intact testicle was used.

### 4.2.2 Blood pressure measurements

**Office BP** was measured at an outpatient visit or at the beginning of annual follow-up visit. In Study IV, office BP data were from the visit during which the ABPM was performed, or a preceding outpatient visit not more than 3 months previously. Office BP measurement was done three times using an automated oscillometric device and a size-appropriate cuff after the patient had remained seated for five minutes and the average BP was used in the analyses.

The **24-hour ABPM** was performed with one of the three automated devices used at the study center one at a time over consecutive time periods: ABPM 5100 or ABPM 6100 (Welch 122 Allyn Inc., Skaneateles Falls, NY, USA) or Schiller BR-102 (Schiller AG, Baar, Switzerland), which ensures the accuracy of auscultator results by using oscillometric measurements as backup. The devices have been tested to meet the Association for the Advancement of Medical Instrumentation US National Standard or the British Hypertension Society Standard (Denchev, Simova & Matveev 2007, Modesti et al. 1996). The ABPM was disqualified if a longer than 2-hour continuous interruption existed, or if the device- or user-approved measurement count was less than 70% of the total. The ABPM device was programmed to measure BP every 30 minutes between 7 am and 10 pm and hourly thereafter until 7 am the following morning. The patients kept a
diary based on which daytime and nighttime periods were defined. In case the information was not provided by the patient, daytime was defined as the period from 8 am to 8 pm and nighttime as 12 am to 6 am in order to rule out bias by individual bedtime habits as suggested by Jones and Sinha (2011).

Office BP values were indexed by dividing the systolic and diastolic BP values by the gender- and height-adjusted cut-off values according to the criteria of the fourth report on the diagnosis, evaluation, and treatment of high BP in children and adolescents (National High Blood Pressure Education Program (NHBPEP) Working Group on High Blood Pressure in Children and Adolescents 2004). Similarly, daytime and nighttime ambulatory BP indices were calculated by using the cut-off values for healthy European Caucasian children provided by Wühl et al. (2002). BP load was calculated by dividing the count of measurements exceeding those 95th percentile cut-off values by the count of measurements during the study period. Nocturnal dipping was denoted as the difference between average daytime and nighttime BPs.

**Hypertension** was defined in Study III as the use of antihypertensive medication or office BP levels above the 95th percentile cut-off by the abovementioned NHBPEP fourth report. In Study IV, the ABPMs were classified according to the recent update on the 2008 AHA recommendation (Flynn et al. 2014). Office BP was normal in case of being under the 90th percentile and hypertensive if over 95th percentile according to the normative data provided by the NHBPEP. Being equal to or above the 90th percentile or 120/80 mmHg but below the 95th percentile cut-off value, the office BP was considered prehypertensive. Regardless of antihypertensive medication, mean ambulatory BP was defined hypertensive if at least one of the daytime or nighttime, systolic or diastolic BP index was 1.0 or more. Similarly, if any of the BP loads was 25–50% it was regarded hypertensive or severely hypertensive in case of being over 50%. Thus, patients with two related patterns on ABPM remained unclassified: 1. Hypertensive office BP, normal BP indices, but elevated BP loads; and 2. Normotensive office BP, normal BP indices, but elevated BP loads.

### 4.2.3 Biochemistry

**Reproductive hormone** levels (testosterone, estradiol, FSH, LH, prolactin, and sex hormone-binding globulin (SHBG)) were assessed using standardized methods in Studies I and II. Inhibin B (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) and anti-Müllerian hormone (Immunotech, Beckman Coulter Ltd., UK) were measured using a commercially available enzyme-linked immunosorbent assay. Assessment of hormone levels was made in respect to normal values reported by three Danish studies (Hagen et al. 2010, Andersson et al. 1997, Aksølæde et al. 2009). Androgen deficiency was defined by serum testosterone level <10 nmol/L in Study II (Bhasin et al. 2010).
Semen samples were analyzed within an hour after ejaculation according to World Health Organization guidelines (2010). Sperm DNA fragmentation index (DFI) was assessed by sperm dispersion test by counting the proportion of sperm cells with fragmented DNA (Fernandez et al. 2003). A DFI over 30% was considered abnormal, based on a previous report of lower pregnancy rate with results exceeding that cut-off (Evenson, Wixon 2008).

Glucose and lipid metabolism were assessed from blood samples drawn after an overnight fasting. Analyses were performed using standardized methods for blood glucose, insulin, glycosylated hemoglobin (GHbA1c), total cholesterol, HDL and low-density lipoprotein (LDL), triglyceride and uric acid. In Study III, 2-hour oral glucose tolerance test (OGTT; glucose load 1.75 g/kg up to 75 g) with the measurement of glucose and insulin levels at 0, 60 and 120 minutes postload was performed at 5 years post-RTx. According to the OGTT results, patients were classified as having normal glucose tolerance (fasting blood glucose <5.6 mmol/L and 2-hour glucose value <6.7 mmol/L), impaired fasting glucose (IFG; fasting blood glucose 5.6–6.0 mmol/L and 2-hour glucose value <6.7 mmol/L), impaired glucose tolerance (IGT; fasting blood glucose <6.1 mmol/L and 2-hour glucose value 6.7–9.9 mmol/L), or type 2 diabetes (fasting blood glucose ≥6.1 mmol/L or 2-hour glucose value ≥10.0 mmol/L). MS was diagnosed if a minimum of three of the five risk factors (overweight, hypertension, reduced HDL, elevated triglycerides, or elevated fasting glucose) were abnormal, in line with the diagnostic criteria of the American Heart Association (Grundy et al. 2005).

Kidney function in terms of GFR (mL/min/1.73 m^2) was measured at every follow-up visit by ^51^Cr-EDTA clearance, except in Study II, in which GFR was estimated using the patient’s most recent creatinine value and the calculation formula developed by the Modification of Diet in Renal Disease Study Group (Levey et al. 1999). GFR values were corrected with a one-pool approximation model according to modified Brochner-Mortensen equation (Fleming et al. 2004). Mean annual GFR decline (mL/min/1.73 m^2/year) was calculated by dividing the difference between two subsequent GFR results by the time between the measurements until the last follow-up visit.

4.2.4 Statistical analyses

Descriptive numerical data were reported as frequency and percentage, mean and SD, median and range, or median and interquartile range (25th–75th percentile), as appropriate. Differences in continuous variables between two groups were tested for significance using Student’s t-test or Mann-Whitney U-test, as appropriate according to the distribution of continuous variables. One-way analysis of variance (ANOVA) or Kruskall-Wallis test was applied for comparing three or more groups. Categorical variables were tested with chi-squared test or Fischer’s exact test. Associations between variables were analyzed with Pearson correlation analysis or Spearman’s rank correlation.
In Study III, simple linear regression was used for assessing the ability of hypertension, relative weight, cholesterol, HDL, LDL, triglycerides, fasting glucose, fasting insulin, GHbA1c, or uric acid to predict GFR at 1.5 or 5 years post-RTx or the mean annual GFR decline starting at 1.5 or 5 years post-RTx. Hierarchal multiple regression was further conducted to assess the ability of triglycerides at 1.5 years post-RTx to predict the concomitant GFR, after controlling for the influence of uric acid.

We used SPSS statistics software for Windows (version 17.0 and 19.0; SPSS Inc., Chicago, IL, USA) in data analysis. P-values less than 0.05 were considered statistically significant throughout the study.

4.2.5 Ethical considerations

The study protocol was approved by the Ethics Committee for gynecology and obstetrics, pediatrics, and psychiatry of the Helsinki and Uusimaa Hospital District (418/13/03/03/2008 and 99/13/03/03/2012). All participating patients and controls, or a parent in case of minors, signed a written informed consent form, in accordance with the Declaration of Helsinki.
## 5 Results

The patient characteristics are presented in Table 6. The participation rate was the lowest in the cross-sectional male fertility study (II), but according to the hospital records the non-responders’ clinical characteristics were comparable to those of the responders, except for younger age at the time of the study (data not shown).

### Table 6. Clinical characteristics according to the studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of RTx patients</td>
<td>109</td>
<td>24</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>Male/female ratio, n (%)</td>
<td>72/37 (66/34)</td>
<td>24/0 (100/0)</td>
<td>132/78 (63/37)</td>
</tr>
<tr>
<td></td>
<td>Disease leading to RTx, n (%)</td>
<td>CNF 45 (41)</td>
<td>Urethral valve 15 (14)</td>
<td>Nephronophthisis 9 (8)</td>
</tr>
<tr>
<td></td>
<td>Age at start of dialysis, years</td>
<td>3.9 (0–15)</td>
<td>8.9 (1–18)</td>
<td>NR 2.3 (0–15)</td>
</tr>
<tr>
<td></td>
<td>Age at first RTx, years</td>
<td>4.5 (1–16)</td>
<td>9.6 (2–18)</td>
<td>4.5 (1–18)</td>
</tr>
<tr>
<td></td>
<td>Re-transplantation, n (%)</td>
<td>9 (8)</td>
<td>6 (25)</td>
<td>17 (8)</td>
</tr>
<tr>
<td></td>
<td>Follow-up, years</td>
<td>11.1 (2–19)</td>
<td>18.6 (6–33)</td>
<td>7.0 (2–18)</td>
</tr>
<tr>
<td></td>
<td>Age at the study, years</td>
<td>NA</td>
<td>28.1 (19–42)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are median (range) or number of patients (%). RTx, renal transplantation; CNF, congenital nephrotic syndrome of the Finnish type; NR, not recorded; NA, not applicable.
5.1 Pubertal development (I)

5.1.1 Clinical progression

The mean chronological age was significantly (p < 0.0001 and p = 0.001) ahead of the mean bone age at onset of puberty in both boys and girls, respectively (Table 7). During puberty, the mean difference between chronological and bone age diminished from the mean of 1.7 (range: -0.8–4.2) to 1.1 (range: -1.3–3.3) years in boys and from the mean of 1.1 (range: -0.8–4.7) to 0.2 (range: -1.8–2.0) years in girls. The boys with delayed onset of puberty had undergone RTx on average at older age than the others (10.9 ± 4.5 vs. 6.6 ± 5.0 years, p = 0.01). One boy and two girls were treated with leuprolein acetate due to precocious puberty.

Table 7. Characteristics of puberty in RTx recipients.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset of puberty, years</td>
<td>12.7 (9.4–16.2)</td>
<td>10.7 (8.9–12.7)</td>
</tr>
<tr>
<td>Delayed onset of puberty, n (%)</td>
<td>12 (22%)</td>
<td>0</td>
</tr>
<tr>
<td>Bone age at onset of puberty, years</td>
<td>11.2 (8.0–13.0)</td>
<td>9.4 (8.0–12.0)</td>
</tr>
<tr>
<td>Length of puberty, years</td>
<td>3.9 (1.3–6.8)</td>
<td>4.7 (2.2–8.4)</td>
</tr>
<tr>
<td>Age at menarche, years</td>
<td>NA</td>
<td>12.5 (10.5–14.5)</td>
</tr>
</tbody>
</table>

Values are mean (range) unless otherwise stated; NA, not applicable.

In boys, age at RTx correlated significantly with the age at pubertal onset (Spearman’s rank correlation coefficient $r_s = 0.33$, p = 0.01). Similarly, boys transplanted before the age of 5 years reached puberty earlier (12.3 ± 1.2 years) than those transplanted at the age of 5 to 15 years (13.4 ± 1.5 years, p < 0.01). The onset of puberty was similar in short boys and in obese boys in comparison with the others (Table 8). MP daily dose, GH therapy, or GFR did not correlate with the age at pubertal onset.

In girls, age at RTx correlated significantly with the age at pubertal onset ($r_s = 0.49$, p < 0.01). The girls having undergone RTx before the age of five years showed a tendency for earlier pubertal onset compared to the girls transplanted at the age of 5 to 15 years (10.3 ± 0.9 vs. 11.0 ± 1.0 years, p = 0.08). Obese girls reached puberty later than the others (p = 0.04) (Table 8). None of the other factors studied was found to be related to the age at pubertal onset.
Table 8. Age at the onset of puberty in relation to various associating factors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Grouping value</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height SD score</td>
<td>≥-1.5 SD</td>
<td>12.7 ± 1.5</td>
<td>10.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>&lt;-1.5 SD</td>
<td>13.1 ± 1.4</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>Relative weight</td>
<td>&lt;120%</td>
<td>12.8 ± 1.4</td>
<td>10.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>≥120%</td>
<td>13.0 ± 1.7</td>
<td>11.1 ± 0.5*</td>
</tr>
<tr>
<td>MP dose</td>
<td>&lt;0.10 mg/kg/day</td>
<td>12.6 ± 1.5</td>
<td>10.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>≥0.10 mg/kg/day</td>
<td>12.7 ± 2.2</td>
<td>10.5 ± 0.8</td>
</tr>
<tr>
<td>GH therapy after RTx</td>
<td>No</td>
<td>12.7 ± 1.3</td>
<td>10.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>13.0 ± 1.6</td>
<td>10.8 ± 1.5</td>
</tr>
<tr>
<td>GFR</td>
<td>≥40 mL/min/1.73 m²</td>
<td>12.5 ± 1.3</td>
<td>10.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>&lt;40 mL/min/1.73 m²</td>
<td>13.3 ± 1.7</td>
<td>10.5 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Variables are means of 3 years preceding puberty; SD, standard deviation; Relative weight is percentage of the gender- and height-adjusted median; MP, methylprednisolone; GH, growth hormone; GFR, glomerular filtration rate. * p < 0.05.
5.1.2 Growth and adult height

Growth continued relatively long and resulted in acceptable FH in both boys and girls (Table 9). FH, or the age at reaching it, was not related to age at RTx in either boys or girls.

Table 9. Characteristics of pubertal growth.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset of growth spurt, years</td>
<td>12.4 (8.4–15.7)</td>
<td>9.8 (7.2–13.5)</td>
</tr>
<tr>
<td>Height at onset of growth spurt, cm</td>
<td>141.0 (113.9–155.5)</td>
<td>127.4 (115.5–145.5)</td>
</tr>
<tr>
<td>Age at peak height velocity, years</td>
<td>14.7 (10.2–17.6)</td>
<td>11.6 (9.5–13.6)</td>
</tr>
<tr>
<td>Peak height velocity, cm/year</td>
<td>8.6 (5.0–14.5)</td>
<td>8.4 (6.0–14.0)</td>
</tr>
<tr>
<td>Age at reaching final height, years</td>
<td>18.1 (15.8–24.4)</td>
<td>16.0 (13.9–17.6)</td>
</tr>
<tr>
<td>Final height, cm</td>
<td>168.7 (151.6–181.5)</td>
<td>154.3 (148.1–167.4)</td>
</tr>
<tr>
<td>Final height, standard deviation score</td>
<td>-1.2 (-3.8–0.4)</td>
<td>-1.7 (-2.7–0.5)</td>
</tr>
</tbody>
</table>

Values are mean (range).

In boys transplanted during adolescence (age 10–15 years) OGS and PHV occurred later than in those transplanted earlier (13.6 ± 1.4 vs. 12.1 ± 1.7 years, p = 0.009 and 15.7 ± 1.0 vs. 14.4 ± 1.8 years, p = 0.03, respectively). Furthermore, the boys in the first group were taller at OGS than those in the latter group (145.8 vs. 139.3 cm, p = 0.03). Boys having had GH therapy after RTx were significantly older than those without GH therapy at OGS (13.0 ± 1.9 vs. 12.0 ± 1.5 years, p = 0.04).

In girls, those transplanted at the age of 5–15 years were only slightly older at OGS than the recipients who had been operated younger (10.2 ± 1.6 vs. 9.7 ± 1.7 years, p = 0.49). GH therapy after RTx was not related to OGS, PHV, or FH in girls.
5.1.3 Reproductive hormones

Testosterone levels were normal in 51/54 (94%) of the boys (Figure 6). The three males with decreased concentration of testosterone had normal levels of FSH and LH. The gonadotropins LH and/or FSH were moderately elevated (>10 IU/L) in 4 (7%) boys.

Figure 6. Individual serum concentrations in males (A–D): testosterone (A), inhibin B (B), LH (C), and FSH (D), and in females (E–H): estradiol (E), anti-Müllerian hormone (AMH) (F), LH (G), and FSH (H). Solid lines are mean ±2 SD in normal Scandinavian population according to Andersson et al., Aksglaede et al., and Hagen et al. (Hagen et al. 2010, Andersson et al. 1997, Aksglaede et al. 2009). Filled circles indicate patients having had RTx operation after pubertal onset.
5.2 Male fertility (II)

Of the 24 study patients, eight had received cyclophosphamide therapy (median cumulative dose: 4.7 g/m$^2$, range: 1.7–9.6 g/m$^2$). The 24 RTx patients were shorter and lighter than the 56 controls (median: 170 cm vs. 180 cm, p <0.001 and 73 kg vs. 83 kg, p = 0.002, respectively) but the median BMIs were comparable (24.3 kg/m$^2$ vs. 25.3 kg/m$^2$, p = 0.209, respectively).

5.2.1 Reproductive endocrine function

The 24 RTx patients had lower median testosterone (17.5 nmol/L) and calculated free testosterone levels (322 pmol/L) than the controls (18.5 nmol/L and 399 pmol/L, p = 0.048 and p = 0.001, respectively). Correspondingly, the LH levels were higher in RTx men than in controls (7.6 vs. 3.3 IU/L, p <0.001). Clinically abnormal testosterone levels (<10 nmol/L) were detected similarly in RTx patients and controls (13% vs. 4%, p = 0.156). Elevated serum LH levels, however, were not found among the controls as opposed to the RTx patients (17%, p = 0.007). The cyclophosphamide-treated RTx recipients had higher serum LH levels and a tendency for higher serum testosterone levels in comparison with those not treated with cyclophosphamide (Figure 7, Table 10).

![Figure 7](image_url)

**Figure 7.** Testosterone and luteinizing hormone levels in renal transplant patients according to history of cyclophosphamide therapy and in healthy controls. The black horizontal lines represent the cut-off levels of clinical normality and the red lines indicate the median in each subgroup.
The median inhibin B and FSH levels were similar among the RTx patients and the controls (111 vs. 157 ng/L and 4.4 vs. 3.2 IU/L, p = 0.08 in both). Clinically abnormal inhibin B (<100 ng/L) and FSH (>10 IU/L) levels were more frequently found in RTx patients than the controls (38% vs. 10%, p = 0.008 and 38% vs. 2%, p <0.001, respectively). The inhibin B levels of the cyclophosphamide-treated RTx patients were lower than those of the other RTx recipients (Table 10, Figure 8).

![Figure 8. Inhibin B and follicle-stimulating hormone levels in renal transplant patients according to history of cyclophosphamide therapy and in healthy controls. The black horizontal lines represent the cut-off levels of clinical normality and the red lines indicate the median in each subgroup.](image)

The median adult testis volumes of the RTx recipients were significantly smaller than those of the controls (11.4 mL vs. 33.9 mL, p <0.001). Two thirds (n = 16) of the 24 RTx patients had a testicular volume below 15 mL. The testicular volumes did not associate with prior cyclophosphamide therapy (Table 10).

A significant correlation was found between testicular volume and serum FSH (r = -0.507, p = 0.012), LH (r = -0.456, p = 0.025), and inhibin B (r = 0.426, p = 0.038) levels in the RTx patients. Testicular size did not correlate with age at dialysis, cumulative dialysis duration, age at RTx, time of follow-up, age at the study time, GFR, or cumulative cyclophosphamide dose. Furthermore, no significant correlation was found between testicular volume and semen volume, semen concentration, total sperm count, motility, DNA fragmentation index, testosterone, or free testosterone levels.

Testicular volumes were similar among the RTx patients divided into two subgroups by RTx before or after the onset of puberty (n = 17 and n = 7, respectively), or by age at dialysis under or above 8 years (n = 12 for both groups).
Table 10. Reproductive hormone levels and testicular volumes in study subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>No cyclophosphamide</th>
<th>Cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 56</td>
<td>n = 16</td>
<td>n = 8</td>
</tr>
<tr>
<td>Luteinizing hormone (IU/L)</td>
<td>3.3 (2.8–4.2)</td>
<td>6.9 (6.0–8.7)c</td>
<td>9.3 (7.6–12.4)c,d</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>18.5 (14.8–23.9)</td>
<td>14.4 (11.6–20.1)a</td>
<td>18.7 (16.1–20.6)</td>
</tr>
<tr>
<td>Free testosterone (pmol/L)</td>
<td>399 (329–481)</td>
<td>312 (279–366)b</td>
<td>323 (282–406)b</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>3.2 (1.9–4.1)</td>
<td>2.7 (1.8–8.2)</td>
<td>12.6 (6.5–17.1)c</td>
</tr>
<tr>
<td>Inhibin B (ng/L)</td>
<td>157 (137–209)</td>
<td>188 (108–262)</td>
<td>50 (18–101)c,d</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>33 (24–48)</td>
<td>30 (22–40)</td>
<td>42 (31–53)</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>70 (60–80)</td>
<td>65 (60–80)</td>
<td>75 (53–108)</td>
</tr>
<tr>
<td>Prolactin (mU/L)</td>
<td>216 (173–274)</td>
<td>298 (244–368)c</td>
<td>354 (238–594)a</td>
</tr>
<tr>
<td>Testicular volume (mL)</td>
<td>34 (29–40)</td>
<td>13 (9–18)b</td>
<td>9 (7–15)b</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).a p < 0.05, b p < 0.01, c p < 0.001 compared to the control group; d p < 0.05 compared to the patient group without cyclophosphamide treatment. FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin.

5.2.2 Semen quality

Altogether 18 RTx patients and 54 controls provided a semen sample. The RTx recipients had significantly lower median semen volumes, semen concentrations, and total sperm counts (2.0 mL, 2.0 $10^6$/mL, and 1.3 million, respectively) in comparison with the controls (3.0 mL, 49.5 $10^6$/mL, and 135.5 million, respectively) (p = 0.003, p < 0.001, and p < 0.001, respectively). The cyclophosphamide-treated patients also had lower sperm motility than the controls (Table 11).
In the RTx patients, total sperm count correlated with serum FSH ($r = -0.602$, $p = 0.008$), inhibin B ($r = 0.668$, $p = 0.002$), and cumulative cyclophosphamide dose ($r = -0.658$, $p = 0.003$) (Figure 9). Age at dialysis, age at RTx, timing of RTx (before or after puberty), cumulative dialysis duration, follow-up time, age at time of study, or GFR were not found to be related to semen quality. In addition, mean testicular volume did not predict total sperm count in RTx patients.

![Figure 9](image)

**Figure 9.** Total sperm counts in renal transplant patients according to cumulative cyclophosphamide therapy and in healthy controls. The black horizontal lines represent the cut-off levels of clinical normality and the red lines indicate the median in each subgroup.

The majority of the semen samples of the RTx recipients were abnormal, including nine (50%) oligozoospermia (sperm concentration $<20 \times 10^6$/mL) and 5 (28%) azoospermia. Of the men with azoospermia, 4 men had once been on cyclophosphamide therapy and one was currently on sirolimus. Only 4 (22%) of the semen samples had normal sperm count.

Half of the RTx patients were living in a relationship and four men had tried to become a parent. Three men, none of whom had ever received cyclophosphamide, had fathered a biological child of their own.
Table 11. Semen quality in renal transplant patients and healthy controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Renal transplantation patients</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 56</td>
<td>No cyclophosphamide n = 12</td>
<td>Cyclophosphamide n = 6</td>
<td></td>
</tr>
<tr>
<td>Semen volume (mL)</td>
<td>3.0 (2.5–4.5)</td>
<td>2.1 (1.1–3.9)^a</td>
<td>2.0 (1.0–3.3)</td>
<td></td>
</tr>
<tr>
<td>Semen concentration (10^6/mL)</td>
<td>50 (26–65)</td>
<td>9 (1–24)^b</td>
<td>0 (0–1)^b</td>
<td></td>
</tr>
<tr>
<td>Total sperm count (10^6)</td>
<td>136 (77–206)</td>
<td>16 (1–47)^b</td>
<td>0 (0–3)^b</td>
<td></td>
</tr>
<tr>
<td>Sperm motility (PR + NP) (%)</td>
<td>65 (58–71)</td>
<td>60 (34–75)</td>
<td>17 (15–)^b</td>
<td></td>
</tr>
<tr>
<td>DNA fragmentation index (%)</td>
<td>NR</td>
<td>11 (6–33)</td>
<td>34 (34–34)</td>
<td></td>
</tr>
<tr>
<td>Abstinence (days)</td>
<td>3 (2–4)</td>
<td>5 (3–5)</td>
<td>3 (2–4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are median (interquartile range). Only 2 cyclophosphamide-treated patients had motile sperm. ^a p < 0.05, ^b p < 0.001 compared to the control group. ^c p < 0.05 compared to the patient group without cyclophosphamide treatment. NR, not recorded.
5.3 Metabolic risk factors (III)

5.3.1 Metabolic syndrome

We recorded the metabolic variables (lipid and glucose metabolism markers and relative weight) and BP levels at 1.5, 5, 9, and 13 years post-RTx in order to compile an analysis of the patients’ metabolic condition in the long run (Table 12). According to the criteria, 19% (28/147) and 14% (18/127) of the patients were diagnosed with MS at 1.5 and 5 years post-RTx, respectively.

Overweight was diagnosed in a fifth of the patients (20–23%) during the post-Tx follow-up (Table 12). At the same time, the prevalence of obesity (as relative weight >140% of the age and height adjusted median) showed a descendent trend (12–2%).

Elevated lipid levels, concerning total cholesterol, LDL, and triglycerides, were frequent as up to a third of the patients had hyperlipidemia at control visits (Table 12). None of the patients received statins, and during the follow-up the prevalence of abnormal total cholesterol levels (>5.0 mmol/L), LDL levels (≥3.0 mmol/L), and triglyceride levels (≥1.7 mmol/L) varied between 22–39%, 9–31%, and 31–34%, respectively. Low HDL levels (<1.0 mmol/L), however, were somewhat scarcer, occurring in 8–13% of the patients.

Glucose levels were relatively stable as abnormal values (fasting glucose ≥5.6 mmol/L or insulin >15 mU/L) were observed during the follow-up in 10–20% and 13–26%, respectively (Table 12). None of the patients were diagnosed with type 1 diabetes, and abnormal GHbA1c values (>6.0%) were also rare. Furthermore, the IGT prevalence increased from 18% at 5 years to 40% at 13 years post-RTx, but type 2 diabetes was detected in only 3–5% of the patients.

Hypertension, as defined by the indexed office BP levels, was found among the majority (87% and 62%) of the patients at 1.5 and 5 years post-RTx, respectively (Table 12). At the same time, 60% and 35% of the patients used antihypertensive medication. The systolic and diastolic office BP indexes averaged 1.04 and 0.91 (117/66 mmHg) at 1.5 years and 0.99 and 0.86 (116/66 mmHg) at 5 years post-RTx, respectively.
Table 12. Metabolic characteristics of 210 patients with pediatric renal transplantation during long-term follow-up.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal value</th>
<th>1.5 years post-RTx</th>
<th>5 years post-RTx</th>
<th>9 years post-RTx</th>
<th>13 years post-RTx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>Abnormal (%)</td>
<td>Median (range)</td>
<td>Abnormal (%)</td>
</tr>
<tr>
<td>Relative weight*</td>
<td>≤120%</td>
<td>3 (-21–165)</td>
<td>44/194 (23)</td>
<td>7 (-16–170)</td>
<td>35/152 (23)</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>≤5.0 mmol/L</td>
<td>4.8 (2.7–11.4)</td>
<td>76/192 (39)</td>
<td>4.6 (2.6–12.5)</td>
<td>48/153 (31)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>≥1.0 mmol/L</td>
<td>1.4 (0.6–2.6)</td>
<td>15/192 (8)</td>
<td>1.5 (0.7–2.9)</td>
<td>8/153 (5)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>≤3.0 mmol/L</td>
<td>2.6 (0.7–7.2)</td>
<td>60/191 (31)</td>
<td>2.4 (1.1–8.9)</td>
<td>37/153 (24)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;1.7 mmol/L</td>
<td>1.3 (0.4–7.9)</td>
<td>64/191 (34)</td>
<td>1.3 (0.5–5.0)</td>
<td>43/153 (28)</td>
</tr>
<tr>
<td>Glucose metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>&lt;5.6 mmol/L</td>
<td>4.7 (2.6–6.5)</td>
<td>17/171 (10)</td>
<td>4.8 (3.1–7.3)</td>
<td>16/134 (12)</td>
</tr>
<tr>
<td>2-hour blood glucose</td>
<td>&lt;6.7 mmol/L</td>
<td>NR</td>
<td>NR</td>
<td>6.1 (3.5–8.0)</td>
<td>67/130 (52)</td>
</tr>
<tr>
<td>GHbA1c</td>
<td>≤6.0%</td>
<td>5.1 (4.0–6.5)</td>
<td>4/168 (2)</td>
<td>5.2 (4.2–6.5)</td>
<td>1/134 (1)</td>
</tr>
<tr>
<td>Fasting serum insulin</td>
<td>≤15 mU/L</td>
<td>8.5 (0.6–120.0)</td>
<td>38/166 (23)</td>
<td>9.4 (2.0–103.0)</td>
<td>35/134 (26)</td>
</tr>
<tr>
<td>2-hour serum insulin</td>
<td>&lt;75 mU/L</td>
<td>NR</td>
<td>NR</td>
<td>30.2 (0.5–137.8)</td>
<td>9/125 (7)</td>
</tr>
</tbody>
</table>

* Relative weight is the percentage of the gender- and height-adjusted median weight, overweight is >20% deviation of the median; NR, not registered; GHbA1c, glycosylated hemoglobin.
The association between the metabolic factors and GFR was analyzed with respect to both concomitant GFR values at 1.5 and 5 years post-RTx and the GFR decline rate subsequently. The mean concomitant GFR values of the patients with MS at 1.5 and 5 years post-RTx tended to be lower than those of the others (Figure 10B and Table 13). In simple regression analysis a more pronounced annual GFR decline was found in the patients with MS at 1.5 years post-RTx.

Overweight or hypertension did not predict the concomitant or consecutive GFR levels during the follow-up (Table 13). Also, the associations between the total cholesterol and HDL levels with the different GFR parameters were weak. On the other hand, the LDL level at 1.5 years post-RTx associated with the annual GFR decline in the regression analysis, but the finding remained sporadic. Hypertriglyceridemia associated with worse concomitant GFR levels (Table 13 and Figure 10F). The fasting glucose level associated with the concomitant GFR value but overall the GFR levels and the subsequent GFR decline rates were similar in patients with normal or aberrant glucose parameters. In parallel, the GFR levels and the annual GFR decline rates were similar among patients with or without IGT at 5 years.
Figure 10. Mean glomerular filtration rates (GFR) during long-term follow-up in two subgroups of patients divided at 1.5 years post-transplant according to GFR (A), metabolic syndrome (B), glycosylated hemoglobin (GHbA1c) (C), hypertension (D), cholesterol (E), and triglycerides (F).
Table 13. Simple linear regression analysis of association between metabolic risk factors and concurrent GFR level or subsequent annual GFR decline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1.5 years post-RTx</th>
<th></th>
<th>5 years post-RTx</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual GFR, mL/min/1.73 m²</td>
<td>B (SE)</td>
<td>P</td>
<td>Actual GFR decline, mL/min/1.73 m²/year</td>
</tr>
<tr>
<td>Metabolic syndrome, yes/no</td>
<td>-7.75 (4.28)</td>
<td>0.072</td>
<td>4.28 (1.41)</td>
<td>0.003</td>
</tr>
<tr>
<td>Relative weight, %</td>
<td>-0.06 (0.06)</td>
<td>0.362</td>
<td>0.03 (0.02)</td>
<td>0.127</td>
</tr>
<tr>
<td>Hypertension (OBP), yes/no</td>
<td>1.21 (4.60)</td>
<td>0.792</td>
<td>1.79 (1.52)</td>
<td>0.242</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>-1.94 (1.39)</td>
<td>0.165</td>
<td>0.60 (0.39)</td>
<td>0.127</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>4.19 (3.86)</td>
<td>0.279</td>
<td>-0.52 (1.30)</td>
<td>0.691</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>-0.44 (1.70)</td>
<td>0.795</td>
<td>1.63 (0.49)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>-6.45 (1.82)</td>
<td>0.001</td>
<td>0.12 (0.55)</td>
<td>0.824</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>-4.99 (2.48)</td>
<td>0.046</td>
<td>0.34 (0.84)</td>
<td>0.685</td>
</tr>
<tr>
<td>GHbA1c, %</td>
<td>-0.27 (3.75)</td>
<td>0.942</td>
<td>0.24 (1.26)</td>
<td>0.847</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>-0.05 (0.20)</td>
<td>0.487</td>
<td>0.09 (0.07)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

B (SE), unstandardized coefficient beta (standard error); Relative weight is the percentage of the gender- and height-adjusted median weight; OBP, office blood pressure; GHbA1c, glycosylated hemoglobin A1c. The variables are continuous except for metabolic syndrome and hypertension (dichotomous).
5.4 Blood pressure and graft function (IV)

5.4.1 Ambulatory blood pressure profiles

The proportions of recipients in the ABPM categories according to the AHA classification were statistically similar between the RTx, HTx, and LTx groups (p = 0.280). Severe ambulatory hypertension was fairly common occurring in 38%, 28%, and 23% of the RTx, HTx, and LTx recipients, respectively. Indicating poor BP control, normal BP was found only in 13%, 21%, and 0% among these groups, respectively. Masked hypertension was also common (26–46%), whereas white coat hypertension was very rare (0–2%) among the Tx groups.

Data on BP indices and loads according in the Tx patients are presented in Figure 11. The BP profiles of the Tx groups were similar. The RTx and HTx patients had higher BP indices and loads at nighttime as compared to daytime (p <0.010 in all). Furthermore, the HTx patients showed clearly blunted systolic and diastolic dipping averaging only 3.9% and 5.3%, respectively. The RTx and LTx groups had thus significantly higher dippings than the HTx recipients.

The 45 RTx recipients with and the 66 without antihypertensive medication showed no major differences in terms of BP profiles. The systolic BP indices and loads were somewhat surprisingly higher in those receiving antihypertensive medication than in the others (p = 0.001–0.032). The diastolic nighttime measures, however, were constantly higher than the daytime countermeasures regardless of antihypertensive medication (p < 0.001 in all). The diastolic BP indices, loads, and both the dipping values were similar among the patients with or without antihypertensive medication.
5.4.2 Hypertensive BP parameters

In all the Tx groups, hypertensive BP indices (≥1.0) and BP loads (≥25% or ≥50%) occurred markedly more frequently during nighttime than daytime. Eighty-six percent of the recipients (76–100% among the subgroups) had at least one BP load measure exceeding 25%, whereas 64% of the patients (62–69% among the subgroups) had at least one BP load equal to or above 50%.

The BP indices and loads correlated highly significantly (r = 0.836–0.919; p < 0.001 in all groups), as shown in Figure 12. Importantly, up to 23–49% of the patients with a BP index <1.0 had already the correspondent BP load above 25%, but only 1–4% had the correspondent BP load above 50%. Thus, the vast majority (91–97%) of patients with a normal BP index had the concomitant BP load between 25–50%.

The lack of normal systolic or diastolic dipping (≥10%) was common in the Tx patients (49–83%). RTx patients without a 10% dipping had significantly higher nighttime BP indices and loads than those with at least a 10% dipping, but the daytime values were comparable between these groups, thus explaining the difference (data not shown).
Figure 12. Linear correlations between systolic (A) and diastolic (B) daytime, and systolic (C) and diastolic (D) nighttime blood pressure (BP) indices and corresponding BP loads in RTx, HTx, and LTx patients. The correlation coefficients are for the study patients as a whole. The dashed lines indicate the thresholds of abnormal values (vertical: ≥1.0 in index, lower horizontal: ≥25% in load, and upper horizontal: ≥50% in load).

5.4.3 Relation between blood pressure parameters and clinical variables in RTx recipients

No significant correlations between any of the 10 ABPM parameters (day- or nighttime, systolic or diastolic, indices, loads, or BP dippings) and the GFR at the time of the ABPM
were found among the RTx patients. This was confirmed by analyses showing that GFR below 60 mL/min/1.73 m² did not predict significantly any of the BP parameters (index, load, or dipping) being abnormal. In diastolic dipping, however, 85% of the patients with abnormal (<10%) dipping value had a decreased GFR under 60 mL/min/1.73 m² at the time of the ABPM. The proportion was higher compared to that of those with normal diastolic BP dipping and decreased GFR (68%, p = 0.04).

Triglycerides correlated with nighttime diastolic BP index (r = 0.215; p = 0.026). No significant correlations with any of the ABPM parameters were found among the other metabolic factors (total cholesterol, HDL, LDL, and obesity) and age at the time of the study (data not shown).

The results showed significant correlations between systolic office BP index and all the daytime and nighttime BP indices and loads (r = 0.252–0.578; p <0.001–0.008). We verified the usability of the office BP results in predicting BP parameter levels by logistic regression analyses and the results were consistent showing that hypertensive office BP do predict hypertensive systolic and diastolic BP values both day- and nighttime (data not shown). No correlation, however, was found between the office BP and the systolic or diastolic dippings (p = 0.117–0.805).
6 Discussion

This thesis reports, in a retrospective and partly cross-sectional manner, the endocrinologic and metabolic long-term outcomes in pediatric RTx patients. The results showed that female puberty appeared normally after RTx, but in males a delayed pubertal timing occurred in a fifth of the patients. This was reflected further as decreased testicular function and fertility prospects in young men. Impairment of metabolic factors and hypertension occurred frequently, but importantly, the deleterious effect of kidney graft function remained moderate in the long run.

6.1 Puberty

The pubertal development in pediatric RTx recipients started on average at 12.7 years in boys and at 10.7 years in girls. The ages are very similar to those of healthy Finnish population (12.2 and 10.8 years) as well as to other Scandinavian populations (Ojajärvi 1982, Lindgren 1996, Juul et al. 2006). None of the girls had a delayed onset of puberty or menarche, whereas 22% of the boys were regarded as late developers. Of the 55 boys only two received testosterone therapy due to moderately delayed development, however. The mean length of puberty in RTx patients resembled that of healthy Finnish population reported by Ojajärvi in 1982 (3.9 vs. 3.4 and 4.7 vs. 4.5 years in boys and girls, respectively).

Previous studies have reported of markedly delayed puberty in adolescents with RTx (Rees et al. 1988, Van Diemen-Steenvoorde, Donckerwolcke 1988, Nissel et al. 2004, Ghanem et al. 2010). Our results are in contradiction to this, but may in part be explained by the difference in the patient material. In our center, the CNF children form the largest cohort and are transplanted at the age of 1–2 years after a relatively short period of uremic phase (peritoneal dialysis lasting 3–12 months from nephrectomy to RTx). In other centers, school-aged children with a quite long-lasting uremic state form the largest group of RTx patients. The earlier maturation of our adolescents, however, was obvious also in those transplanted at school age. Another possible explanation for the difference is that the previous studies were conducted on smaller patient cohorts and many of the recipients had undergone RTx after pubertal onset. The graft function was not strongly associated with the timing of puberty in our patients transplanted prepubertally, opposing interestingly the results of studies reporting on renal failure impairing physical maturation (Turenne et al. 1997, Hokken-Koelega et al. 1994b, Ferraris et al. 1980, Maxwell, Haffner & Rees 1998, Harambat, Cochat 2009). We did, however, see a tendency towards later pubertal onset in boys with a deteriorating GFR.

GH therapy-induced bone maturation and earlier onset of puberty in children with idiopathic short stature was reported in 2002 by Kamp et al. In contradiction, we found no difference in pubertal onset between groups with or without GH therapy after RTx. In
accordance, our results showed similar timing of pubertal development in subjects with short or normal stature and in those who were overweight or normal weight.

The mean age at OGS in our male patients was slightly delayed compared to healthy Scandinavian boys (12.4 and 11.8 years, respectively) but in females the timing was similar (9.8 years) to that (10.2 years) reported by Aksglaede et al. (2008). In agreement with the studies by Nissel et al. (2004) and Schaefer et al. (1990), delayed OGS was associated with a late RTx also in our study. In girls, the PHV and age at reaching it resembled those reported in the Finnish population, but in boys the average PHV and the age at reaching it were blunted and delayed in comparison with their healthy peers (Ojajärvi 1982). Importantly, the growth period was somewhat extended, so that the FH was reached on average at the age of 18.1 and 16.0 years in males and females, respectively. Ojajärvi reported in 1982 that the FH was reached at a mean age of 16.8 and 15.2 years in boys and girls, respectively. In agreement with a previous report, bone age was delayed compared to the chronological age (Englund et al. 2003).

The serum levels of reproductive hormones were normal in the majority of males and only four had hypergonadotrophic FSH and/or LH levels (>10 IU/L). The hormone levels of females were all normal, suggesting normal ovarian reservoir and prospect of fertility. The ovarian function was assessed by anti-Müllerian hormone levels, which do not fluctuate significantly during the menstrual cycle (Hagen et al. 2010, Hehenkamp et al. 2006).

6.2 Male fertility

Our results suggest that life-long immunosuppressive therapy started after pediatric RTx may decrease the fertility of RTx recipients. On the other hand, medical history in terms of age at dialysis, duration of dialysis, age at RTx, and follow-up time seem to have a lesser impact on reproductive functions. Eighteen RTx patients provided a semen sample, including five azoospermic and nine oligozoospermic (low sperm concentration) samples. Semen quality in patients who had undergone RTx as a child was significantly worse in comparison to the healthy controls. Sperm counts were significantly decreased in those with or without a history of cyclophosphamide therapy compared to the values of the controls. This is in contrast to earlier studies reporting restoration of gonadal function in adult patients after RTx (Ferraris et al. 1980, De Celis, Pedron-Nuevo 1999, Saha et al. 2002).

Our RTx patient cohort had lower testosterone and calculated free testosterone levels than their healthy peers, but the levels were still within the normal reference ranges. The Leydig cells secrete testosterone under regulation by the pituitary hormone LH. The observed higher serum LH levels, stimulating the maintenance of normal serum testosterone level, may indicate subclinical Leydig cell damage as previously described among cancer survivors (Kenney et al. 2001). In parallel with other reports, all our patients had had a normal pubertal development and had normal sexual characteristics,
suggesting that RTx does not disturb testosterone production (Wang et al. 2010, Yadav et al. 2008).

Decreased testicular volumes were evident among the RTx recipients regardless of the history of cyclophosphamide exposure. The results in semen quality, serum inhibin B levels, and adult testicular volumes were even worse in those who had previously received cyclophosphamide. The vast majority (80%) of the azoospermic men had a history of cyclophosphamide therapy and none of the cyclophosphamide-treated men had fathered a child. According to the results, the adult testicular volumes of the RTx men were smaller (11 mL) and the total sperm counts were lower (0 million) than those previously reported on leukemia survivors treated with similar doses of cyclophosphamide (22 mL and 120 million, respectively) (Jahnukainen et al. 2011). In contrast to our results, a high spermatogenetic recovery rate has also been reported in cyclophosphamide-treated patients with nephrotic syndrome without continuous long-term immunosuppressive therapy (Bogdanovic, Banicevic & Cvoric 1990).

Testicular volume did not correlate with the semen parameters, age at RTx, or the kidney function at the time of the study. The lack of correlation is contradictory to previous reports on other infertile cohorts (Bahk et al. 2010, Abramsson, Duchek 1989). A testicular volume under 15 mL has been reported to lead to incomplete recovery of normal gonadal function and sperm production and as a sign for fertility counseling (Arai et al. 1998). Our results indicate, however, that adult testicular volume may not predict spermatogenetic potential as accurately as described among childhood cancer survivors (Jahnukainen et al. 2011).

6.3 Metabolic consequences

In our study, the prevalence of MS was 19% and 14% at 1.5 and 5 years after RTx, respectively. The numbers are lower compared to those reported previously in pediatric patients. Recently, Wilson et al. (2010) reported the results of a multi-center study according to which the prevalence of MS was 19% at the time of RTx and 38% at one year post-Tx. In a smaller Mexican study, a quarter (8/32) of the patients fulfilled the criteria for MS at two years after RTx (Ramirez-Cortes et al. 2009). Glucocorticoids are known to predispose Tx recipients to metabolic problems, and in a study by Maduram et al. (2010), MS was diagnosed in only 15% of those without a glucocorticoid and in 68% of patients receiving prednisone at one year post-RTx. The essential difference between our study and the three previous ones is that we focused on the maintenance phase, when the immunosuppressive drug dosing was already reduced to a minimum.

Especially during the early maintenance phase post-Tx, overweight is common due to the excessive exposure to glucocorticoids. According to previous reports, however, the association between obesity and kidney graft function is contradictory in children (Mitsnefes, Khoury & McEnery 2002, Kasap et al. 2006, Dart et al. 2010, Boschetti et al. 2009).
A fifth of our patients were moderately overweight at 1.5 and 5 years after RTx, but no clear association with impaired concomitant or future graft function was found. This is in contrast to the previous reports on obesity at the time of RTx. Mitsnefes et al. reported in 2002 that obese patients at the time of RTx and 1 year thereafter had lower GFR levels compared to those who became obese during the first year post-RTx. More recently, the opposite finding was reported by Boschetti et al. (2013): those who gained weight after the operation had worse graft survival at 36 months. Thus, it seems that moderate overweight appearing years after RTx affects kidney graft function less than severe obesity during the early postoperative phase.

Hypertension, mainly induced by CNIs and glucocorticoids, has been associated with subsequent graft failure (Sorof et al. 1999, Mange et al. 2000, Mitsnefes, Khoury & McEnery 2003). Sorof et al. reported in 1999 that hypertension associates with higher graft failure rates and the use of antihypertensive medication predicts subsequent graft failure in children with RTx. In another study on pediatric patients, systolic hypertension at one year post-RTx predicted poor long-term graft survival (Mitsnefes, Khoury & McEnery 2003). In line with the results of Krmar et al. (2008) reporting similar GFR decline rates in hypertensive and normotensive patients, we did not find a clear association between hypertension and later graft function (Krmar, Berg 2008).

Post-Tx diabetes prevalence in children with RTx is reportedly 2–35% (Greenspan et al. 2002, Kasiske et al. 2003). According to our results, none of the patients developed type 1 diabetes and type 2 diabetes was diagnosed only in 12 patients (3–5%) during the long-term follow-up. The low prevalences may be explained by the fact that the majority of the patients received CsA instead of tacrolimus. Furthermore, the patients were also on a low-dose alternate-day MP minimizing the glucocorticoid exposure. In OGTT performed at 5 years post-RTx, only one of the 6 patients diagnosed with impaired fasting glucose later developed type 2 diabetes. The insulin level of ≥75 mU/L in OGTT predicted a more rapid subsequent GFR decline, but fasting glucose or insulin levels or GHbA1c did not correlate with GFR, in parallel with previous results of both pediatric and adult studies (Gerhardt, Grosse Huttmann & Hohage 1999, Prokai et al. 2008, Wiesbauer et al. 2010). IGT was commonly (18–40%) found in OGTT but, again, it did not correlate with the GFR levels.

According to our results, decline in GFR could be predicted by hypertriglyceridemia at 1.5 post-RTx. This is in accordance with the multivariate analysis observations in adult patients showing hypertriglyceridemia to be independently associated with impaired renal allograft function beyond the first post-Tx year (de Vries et al. 2004). Recently, a register study by Bonthuis et al. (2014) confirmed the inverse association between triglyceride levels and estimated GFR in pediatric RTx recipients. In our patients, total cholesterol, HDL, or LDL did not associate with GFR. While the prevalence of hypercholesterolemia (39–22%) and elevated LDL levels (17–4%) decreased during the follow-up, a third of the patients remained hypertriglyceridemic. In light of the present study, the importance of dietetic guidance of Tx children and adolescents is emphasized.
6.4 Blood pressure and graft function

ABPM is a feasible method to confirm the office BP results suspecting hypertension in school-aged children and older subjects. Lack of uniformly accepted criteria for the definition of hypertension in respect to ABPM has been a major problem, however. BP indices and loads as well as several combinations of these parameters have previously been used, complicating the comparison of the results of different studies with each other (Seeman et al. 2006, Lingens et al. 1997, Sorof, Poffenbarger & Portman 2000). Especially in patients with secondary hypertension, blunted nocturnal BP dipping has been regarded as an important indicator for hypertension (Seeman et al. 2005). The AHA recommendations helped in the classification but raised some concerns. Some of these issues, such as prehypertension, were taken into account in the recent update of this classification which also introduced an unclassified patient group with normal or hypertensive office BP, normal mean BP but elevated loads (Flynn et al. 2014).

The ABPM profiles of RTx, HTx, and LTx patients resembled each other. To the best of our knowledge, no study to date has compared the BP profiles of different Tx recipient groups with each other. The predominance of nocturnal hypertension and reduced BP dipping was evident in all the Tx groups. This is in line with the results reported previously in RTx patients (Morgan et al. 2001). The cause of reduced nocturnal dipping, however, has been under debate. The ABPM may interfere with the sleep of a study subject, thus, obviously leading to increase in the BP levels during the night. According to our results (not shown), patients with essential hypertension undergoing similar BP monitoring showed more prominent circadian variation in BP profile than the Tx recipients, suggesting that the nocturnal predominance in the Tx patients was not a technical bias. There is also some evidence on disturbances in the autonomic BP regulation in diabetic population leading to nighttime hypertension, which may also be the case in Tx population (Kario et al. 1997).

We found a strong correlation between the BP index and the corresponding BP load. Concurrently, our results suggested that the limit of 25% in BP load may emphasize hypertension. This is in line with the results by Koshy et al. (2005), which showed that BP load of 50% as a cut-off is in better agreement with BP index than the limit of 25%. The use of higher cut-off would also be justified by the fact that BP load takes into account only the proportion of hypertensive measures and not the magnitude of excess of the actual BP measure. Furthermore, in our study only sporadic patients had a BP load below 25% with the corresponding index being equal to or above one.

In line with the previous results in RTx patients, the majority of our RTx patients were considered hypertensive (Giordano et al. 2000, McGlothan et al. 2006, Seeman 2009). Hypertension was also often detected in RTx recipients who were on antihypertensive medication, in concert with the previous reports. The relatively high prevalence of uncontrolled hypertension reflects the fact that ABPM is commonly used as an adjuntive method. Previously, when the actual BP levels in most cases have been looking “quite
normal” and no strict criteria for interpreting ABPM results have existed (until recently), the antihypertensive therapy has rarely been intensified. In addition, nocturnal hypertension among the patients treated with antihypertensive drugs is most likely due to the fact that the effect of the medication (most often calcium channel blocking agent) taken in the morning is insufficient to last the entire day and night.

The ABPM data on other Tx recipients than RTx are relatively scarce (Roche et al. 2008, Del Compare et al. 2004). Two previous studies have reported 30% and 50% prevalence of hypertension in LTx and HTx patients, respectively (Bayrakci et al. 2012, Walker et al. 2005). In our study, the frequency of abnormal ABPM in the LTx and HTx patients was 100% and 76%, respectively, resembling the high prevalence of hypertension observed in RTx patients.

The major limitation of this particular study are its retrospective nature and the low number of LTx patients. Currently, annual ABPM is routinely performed only in RTx and HTx patients in the study center. In LTx patients, ABPM is done only when hypertension is suspected. This explains the high prevalence of abnormal ABPM results among the LTx patients. In addition, the relatively long time period of 5 to 10 years post-Tx for performing the ABPM may introduce a time bias as the graft function decrease over time. Eighty-four percent of the measurements were performed 5 to 7 years post-Tx decreasing the potential risk for bias, however. The main goal of the present study, however, was to compare the ABPM profiles among the different Tx patient groups and to illuminate the possible differences in the information provided by the numerous ABPM parameters.

Decreased kidney graft function (defined by measured GFR <60 mL/min/1.73 m²) could be predicted by a decreased diastolic BP dipping among RTx patients. We found no correlations, however, between the other BP parameters and the GFR or several other clinical variables. An association between GFR and hypertension has been previously reported in adult Tx patients (Mange et al. 2000, Mitsnefes, Khouy & McEnery 2003, Jacobi et al. 2000, Fernandez-Fresnedo et al. 2001). On the other hand, two Swedish studies have reported that the velocity of decline in graft function was not affected by hypertension compared to normotension, or hypertension during day- or nighttime(Krmar, Berg 2008, Cameron et al. 2014). In line with the conclusion of Roche et al. (2008), the high incidence of hypertension in all the three Tx groups, however, suggests that the immunosuppressive medication (CNIs and MP), as such, is largely responsible for the elevated BP levels.

6.5 Methodology

The principal strength of this study is that it takes into account all the pediatric patients receiving a kidney graft in Finland. All transplantations are performed at the Children’s Hospital in Helsinki and the subsequent follow-up of the patients is centralized to the study center. In addition, another strength of the study is the exceptionally long follow-up
due to the high proportion of CNF children undergoing RTx before the age of two years. Our different Tx groups receive very similar immunosuppressive medication (CNI, azathioprine or a mycophenolate, and low-dose MP), which makes the comparison of these cohorts interesting.

The intensive and robust follow-up protocol allowed virtually complete data collection on the national cohort of Tx patients. Thanks to the centralization of follow-up to our institution, we were also able, in the case of missing data, to compare the clinical information of participants and non-participants. We therefore believe that our studies represented well the entire national pediatric RTx recipient cohort. Furthermore, kidney function was assessed by direct GFR measurement by means of $^{51}$Cr-EDTA clearance. Also, the data were collected and assessed by the same investigator, reducing the possibility of errors and differences in measuring techniques.

Naturally, this study also has several limitations. The majority of the data were collected retrospectively, which limits reaching firm conclusions on some of the associations found. In cases of expected results remaining nonsignificant, the small subgroup population size may have led to type II statistical errors. For instance in Study II, the higher cumulative doses of cyclophosphamide among the tacrolimus-treated patients did not allow us to compare the effect of CsA and tacrolimus on semen quality. As for Study II, one additional weakness was the relatively small study population and low participation rate, which may have caused the lack of correlation, for example, between semen quality and GFR. Furthermore, the cohort included three possible outliers, but the re-analysis of the data without these patients did not distort the essential findings of the study.

6.6 Future perspectives

Because allograft tolerance is not yet in the horizon, immunosuppressive medication will continue to be needed in the future. Avoidance of the deleterious effects of immunosuppression in parallel with avoiding chronic rejection should thus be pursued. Along with the improved long-term patient and graft survival, more precise knowledge is also needed on the long-term impact of the underlying disease as well as on general health. Despite the improved results in both graft and patient survival, the kidney function declines inevitably in pediatric and adult RTx patients by both immunological and non-immunological mechanisms. The current half-life of kidney graft after pediatric RTx is reportedly at its best less than 40 years (Vats et al. 2002). In practice the half-life is far less and children transplanted at a very young age, such as those with CNF, need a new graft as young adults. Avoidance and treatment of post-Tx problems in childhood is therefore important for the success of later therapies.

Further studies on fertility and sexuality of young adults with RTx, especially males, are needed. One explanation for the poor semen quality and small testicular volumes may be the continuous immunosuppressive therapy. Experimental studies on CsA- and
tacrolimus-treated rats have shown various degrees of atrophy of seminiferous tubes and problems with spermiation (Masuda et al. 2003, Hisatomi et al. 1996), and therapeutic levels of CsA and sirolimus have also been reported to have even stronger toxicity on rat spermatogenesis than tacrolimus (Chen et al. 2013). These findings would require confirmation with a study on human testes. The cumulative effect of the combination of cyclophosphamide therapy and continuous use of other immunosuppressants on spermatogenetic recovery would also be of interest.

Studies on metabolic risk factors in RTx children and adolescents are important in at least two respects. Firstly, the metabolic factors may have a deleterious effect on the graft function and subsequently lead to graft loss, and secondly, they form a long-term risk for CVD among RTx patients (Li et al. 2003). The challenge is to find factors that could predict the graft and patient outcomes, which justifies larger prospective studies on the long-term effect of metabolic factors on graft and patient survival.
7 Conclusions

The main conclusions of this thesis are:

1. Pubertal maturation was quite normal in all female and most male adolescents after RTx during childhood or adolescence. Patients having undergone RTx in early childhood reached puberty earlier than others. Growth continued relatively long in comparison with healthy population resulting in acceptable adult height. Reproductive hormone levels were normal in girls and in a great majority of boys.

2. Testicles were small and semen quality was poor in young adult men after pediatric RTx. A third of the patients showed signs of hypogonadism. Continuous life-long immunosuppressive medication, especially cyclophosphamide therapy, may be more deleterious for the testicular endocrine system and sperm production than the time with ESRD or deteriorating graft function. The prospect of fertility is often decreased, thus sperm cryopreservation, optimally before RTx, should be considered for all men who are physically mature enough to produce sperm.

3. Metabolic syndrome and its components (overweight, hypertension, abnormalities in lipid and glucose metabolism) associated modestly with the long-term kidney graft function beyond the first postoperative year. Triglyceride levels correlated with the consequent measured GFR levels, and hypertriglyceridemia at the early maintenance phase later on predicted the decrease of graft function.

4. Hypertension was common and the ABPM profiles were similar in RTx, LTx, and HTx recipients. The BP load threshold of 50% is a superior cut-off value because the cut-off of 25% overstated the high prevalence of hypertension. The BP variables provided by the ABPM associated weakly with clinical metabolic factors and kidney graft function but, on the other hand, supported each other when assessing hypertension. The predominance of nocturnal hypertension emphasizes the superiority of ABPM over the office BP measurement as an essential tool for long-term follow-up.
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