Lifestyle, the next target for intervention in renal transplant recipients

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Lifestyle, the next target for intervention in renal transplant recipients.
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Lifestyle, the next target for intervention in renal transplant recipients

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Contents

1

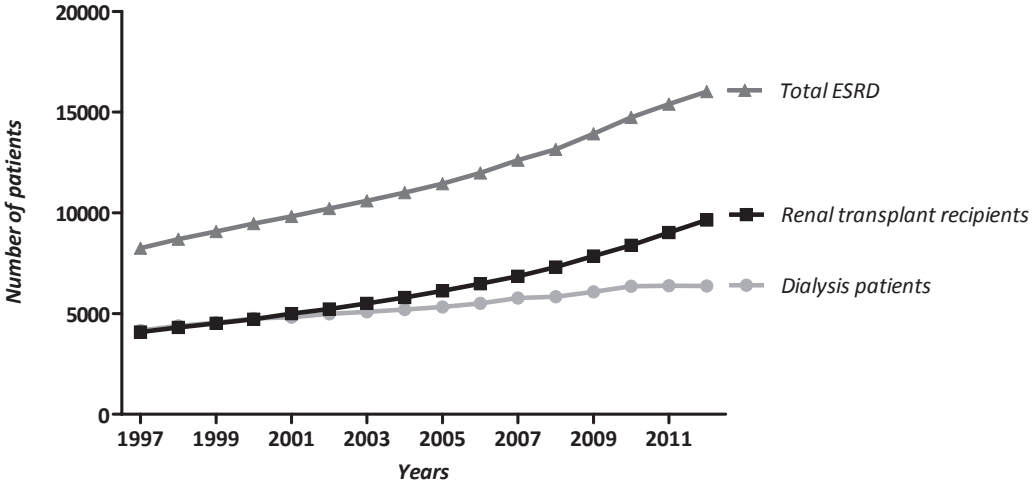
Introduction and Aims of the Thesis

Introduction

The number of patients with chronic kidney disease (CKD) is increasing worldwide, causing CKD to become a serious public health problem (1-3). Patients with CKD have an increased risk of cardiovascular disease (CVD), kidney failure and premature death (4,5). Risk factors for CKD include age, diabetes mellitus, hypertension, smoking, obesity and history of CVD (6-9).

In the past decades, the number of patients with End Stage Renal Disease (ESRD) in the United States (US) increased rapidly from 196,000 in 1991 to 571,414 in 2009 (10). The number of patients with ESRD in Europe is also increasing steadily, with trends resembling those in the US (2,11). According to the Renal Replacement Registration of the Netherlands (RENINE), the number of patients with ESRD in the Netherlands nearly doubled from 8,243 in 1997 to 16,018 in 2012 (Figure 1)(12). This increase in patients with ESRD cannot be fully explained by ageing of the population and is supposed to reflect the alarming increase in obesity and diabetes that occurs worldwide (13,14).

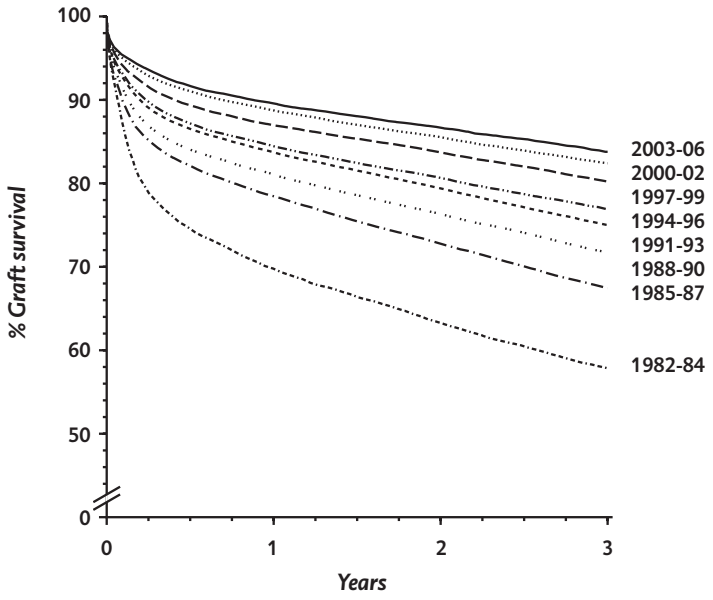
Figure 1. Number of patients on the first of January, 1997-2012. Dialysis patients and patients with a functioning donor kidney. Adapted from RENINE



Renal transplantation is the best treatment for patients with ESRD. Renal transplantation improves quality of life and increases survival, when compared to dialysis treatment (15,16). The annual death rate for renal recipients (RTR) is estimated to be 4.2 times lower than for patients on dialysis treatment but still considerably exceeds the risk in the general population (16). The first concern after renal transplantation is acceptance and functioning of the new donor organ. Graft survival improved tremendously during the last decades, from approximately 40% in the 1970's up to 90% in 2012 (12,17,18). The improvements in overall graft survival are mainly a consequence of better short-term graft survival. These short-term improvements were particularly possible due to

the introduction of new immunosuppressive drugs such as cyclosporine and later mycohenolate mofetil and tacrolimus (19-21). No 'real' improvements occurred in long-term graft and patient survival during the last decades. Beyond one year after transplantation, survival curves of patients transplanted in 1982 run parallel to those transplanted in 2003 (Figure 2). Moreover, approximately half of all cadaveric renal allografts are still lost within 10-12 years after transplantation (18).

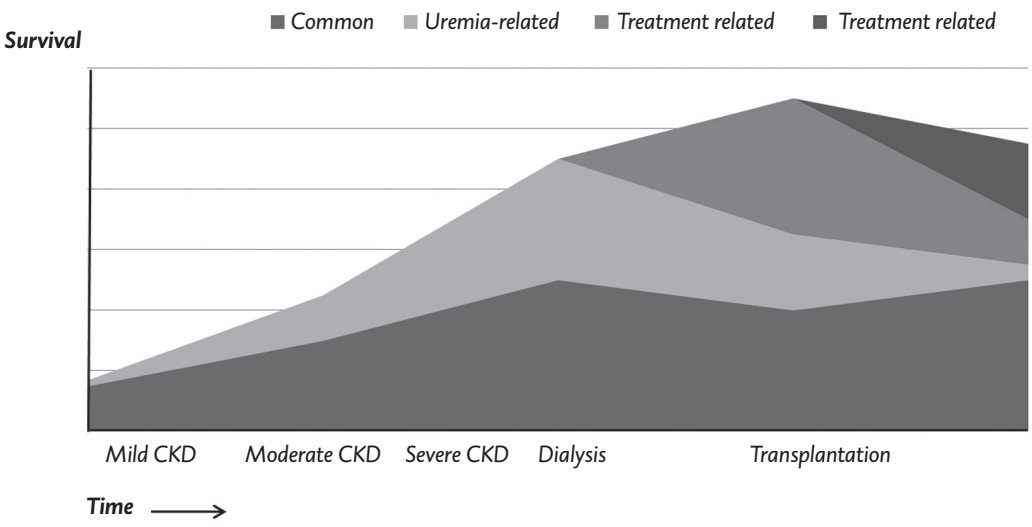
Figure 2. Graft survival of first cadaver kidney transplants according to different years of transplantation. Figure is available online at: http://www.ctransplant.org (Figure: K-14101-0208)



Increased cardiovascular mortality and chronic transplant dysfunction are the two main reasons why long-term graft survival did not improve during the past years (17,18,22). Various categories of cardiovascular risk factors like obesity and high blood pressure contribute to this increased risk but cannot fully explain the incidence of CVD in RTR. Pre-existent risk and specific post-transplant risk factors together comprise the overall risk for cardiovascular mortality after transplantation. Figure 3 displays the contribution of various categories of risks factors over time for CKD, dialysis and transplantation, illustrating that the factors contributing to cardiovascular risk in the renal patients change over time. The common cardiovascular risk factors are present in all stages. With the progression of CKD uremia related risk increases as well as the common risk factors. Start of dialysis is an important point in transition of risks. The uremia related risk decreases drastically, but the risk associated with the dialysis treatment such as bioincompatibility and adverse cardiac effects, rises (23). Transplantation is the second transition to a new phase. Uremia related risk usually decreases

further but common risk factors like high blood pressure, dislipidemia and obesity increase again. Use of immunosuppressive medication and viral infections like Cytomegalovirus, also adds to the total risk after transplantation. Illustrating that for CKD patients, and transplant recipients in particular, it is not warranted to simply extrapolate cardiovascular risk in non-renal populations to renal patients. This warrants focus on the determinants of long-term outcome as a target for further improvement with particular emphasis on modifiable risk factors of cardio metabolic risk.

Figure 3. Contribution of diverse risks for different stages of CKD, dialysis and transplantation



Risk for cardiovascular disease, and mortality

The incidence and prevalence of CVD are estimated to be 4-6 times higher in RTR than in the general population (24,25). The main cause of death after renal transplantation is CVD (22). Data from our own cohort of RTR, (606, mean age, 51 ±12 years; 55% male) show that survival is significantly shorter than in the general population. During a median follow-up of 7.0 [6.2 – 7.5] years, 137 (23%) RTR died, 52.6% of the deaths were due to CVD, 17.5% due to infectious causes, 23.4% due to malignancies and 6.6% was unknown. Classical cardiovascular risk factors such as dyslipidemia, hypertension, hyperglycemia and obesity, which commonly coexist as the metabolic syndrome (MS), contribute to this excess in CVD (26,27). In clinical practice, these risk factors are being treated in line with the current guideline of KDIGO (Kidney Disease: Improving Global Outcome). These guidelines are mainly based on studies in the non-renal or non-transplant population. Other factors more specific for transplantation, such as dialysis duration, inflammation, and use of immunosuppressive drugs

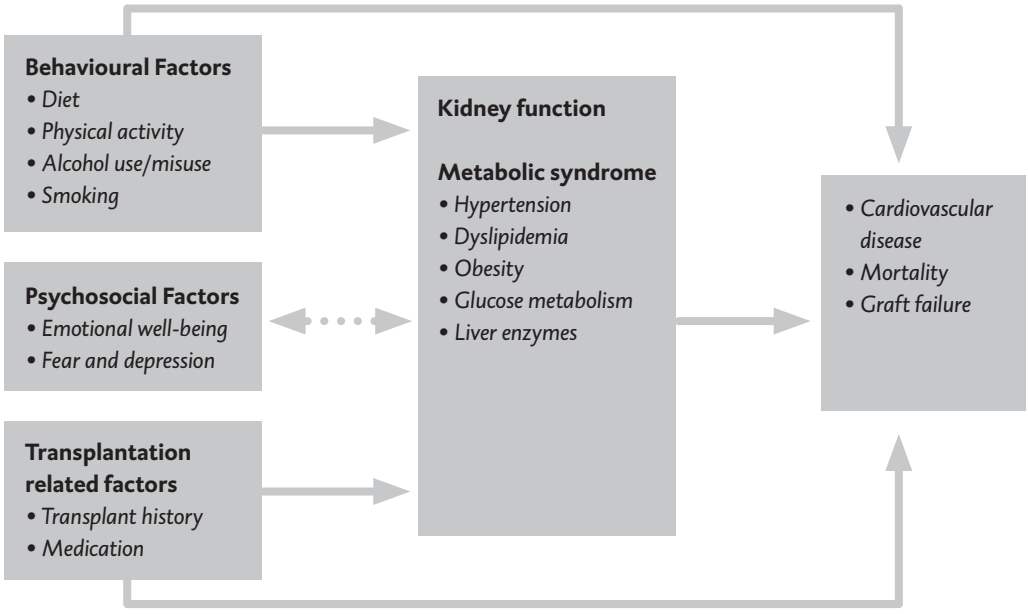
might also be relevant to this high risk profile (28,29). Immunosuppressive medication used by RTR is 'toxic' for the cardiovascular system, promoting atherosclerosis (30). It also makes patients more susceptible for infections and cancer (31,32).

Despite the fact that cardiovascular risk factors are carefully treated in clinical care, RTR are still facing an alarming high risk for CVD and mortality. Prevention and treatment of CVD in RTR is based on studies in the general population, since no specific data in RTR are available: hence the treatment might not be optimally suited to the needs of the RTR population. This could contribute to the suboptimal CVD outcome. Moreover, we could have overlooked some important factors such as lifestyle that can be of great influence in determining cardiovascular risk after transplantation.

One way to combat the occurrence of CVD is to promote a as good as possible, physical condition. In this respect it is of relevance that various chronic conditions that occur after renal transplantation share the same risk factors and pathogenesis. Figure 4 shows the most common risk factors associated with CVD, graft failure and mortality. Physical activity and diet act on many metabolic risk factors at the same time offering a great opportunity for intervention. Thus lifestyle modification for RTR could be a powerful tool to optimize the physical condition of the RTR, as it targets various risk factors simultaneously.

We therefore investigated the effects of behavioural-, psychosocial- and biological factors on metabolic profile and survival after renal transplantation.

Figure 4. Cluster of risk factors and determinants of outcome



Behavioural Factors

Management of post transplant obesity, MS and new onset of diabetes after transplantation (NODAT) is of great importance after transplantation. Diet and physical activity are important to maintain the energy balance in the human body and thereby prevent overweight. The role of diet and physical activity in post transplant weight gain is unknown and needs to be determined. If these modifiable determinants are involved in post transplant weight gain, they can be important targets for lifestyle intervention. The beneficial effects of regular physical activity in the general population are well studied. Physical activity is associated with various health benefits. It can lower the risk of CVD and can positively influence blood pressure, lipid profile and insulin sensitivity (33,34). However, little known about the influence of physical activity on metabolic health and outcome in RTR and only few studies are available on physical activity level after renal transplantation. From these studies we know that RTR enhance their level of physical activity after transplantation, but it still remains below the level of the general population (35). Both transplantation surgery and immunosuppressive medication have negative effects on muscle mass, fat mass and bone density and thereby negatively influence spontaneous recovery of the physical activity level. In order to better understand the low physical activity levels in RTR, barriers and determinants of physical activity need to be addressed. Other modifiable behavioural factors after transplantation are smoking and alcohol use. In a previous study we showed that smoking after renal transplantation is a risk factor for graft failure and mortality (36). Along with cigarette smoking, alcohol consumption after transplantation is controversial. Although the KDIGO Practice Guidelines for the Care of Kidney Transplant Recipients does not mention specific alcohol restrictions, advices on the internet are different and recommending RTR to not use alcohol at all. It is not known whether moderate alcohol consumption after transplantation should be avoided or could be protective for NODAT and mortality similar to the general population.

Psychosocial Factors

Monitoring patients' psychological health is important to assure optimal patient treatment and patient satisfaction. A successful transplantation can offer patients a great improvement in quality of life (15). Renal transplantation itself is often experienced as a 'second birth' offering the patient a new opportunity for life. On the other hand, years and sometimes decades of CKD, insecurity about availability of a donor, and transplant surgery contributes to a high psychological burden of the patient (37). After transplantation patients and their families experience psychological distress, such as fear of losing the graft and complications after transplantation (38-41). Common problems after renal transplantation are unemployment, tiredness, changes in body composition, facial changes, a demanding treatment and depression (37,42-45). It is therefore important to understand the impact of renal transplantation and its complications on patients' quality of life and psychological well-being.

Transplantation related

The most important transplantation related factor that contributes to the cardiovascular risk is use of immunosuppressive medication. The standard medication used for prevention of graft rejection in our hospital are: cyclosporine microemulsion (Neoral, Novartis Pharma B.V., Arnhem, The Netherlands); mycophenolate mofetil (Cellcept, Roche B.V., Woerden, The Netherlands) and prednisolone. Besides the great therapeutic effects, immunosuppressants also have several negative side effects. A very general side effect of immunosuppressive medication is immunodeficiency. This makes RTR more vulnerable for infections. Infectious complication can be serious and also decrease the chance of a successful outcome (46). Cytomegalovirus is the most common virus occurring after transplantation and active Cytomegalovirus infection can contribute to cardiovascular risk (47,48). Other common side effects of immonosuppresants are osteoporosis and factors that can contribute to cardiovascular risk including hypertension, dyslipidemia, hyperglycemia (49-52).

Biological Risk Factors: Obesity, Diabetes and Metabolic syndrome

Weight gain after transplantation is very common and occurs in the majority of RTR. Obesity is associated with cardiovascular risk, inflammation, hypertension, dyslipidemia, insulin resistance and thereby plays an important role in the development of MS and NODAT (53-55). Post transplant obesity has also adverse effects on graft and patient survival. A Dutch study in 1871 RTR showed that patients with BMI over 34 kg/m2 at the time of transplantation, have more than twofold risk for mortality and graft failure compared to RTR with a BMI < 25 (56). Also in the absence of overt obesity, a higher BMI is a risk factor for a worse outcome (57). The use of corticosteroids in RTR only explains a small part of the weight gain (58,59); other causes of weight gain still need to be investigated. MS, characterized by a clustering of risk factors, is recognized as a risk factor for CVD in the general population (60). Various studies investigated the prevalence of MS and its risks after renal transplantation and suggest that MS is related to worse outcome (61,62). MS is very common before and after renal transplantation. Data from our own cohort showed that at a median of 6 years post transplant MS was present in 63% of the RTR (63). MS is an independent risk factor for NODAT and is associated with reduced long-term kidney function (61,64). Obesity as well as the chronic exposure to calcineurin inhibitors and corticosteroids increases the risk for development of NODAT (65,66). NODAT occurs in approximately 20% of the RTR and places RTR at an increased risk of infections, CVD, graft failure and mortality (52,67,68). The presence of pre-transplantation insulin resistance in the final stage of kidney failure is also a mechanism in the development of NODAT (69). Additionally, NODAT can be a consequence of an acquired defect in insulin secretion by beta cell dysfunction (69,70). In addition to this several other risk factors play a role including age, family history of diabetes and smoking (67,71,72).

Aims of the thesis

This thesis and outline addresses the relationship between various modifiable risk factors for mortality and graft failure. Furthermore, we aimed to draw attention to less recognized but very important psychological an lifestyle related factors after renal transplantation such as: physical activity, diet, depression, fear of movement and alcohol intake.

Metabolic syndrome is highly prevalent after renal transplantation (62). Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic component of MS in the general population and is associated with a higher risk for mortality (64,65). In **chapter 2** we therefore assessed the association between liver enzymes and MS, in addition the association between the hepatic component of the MS and mortality was investigated. In **chapter 3** we investigated the association between beta-cell dysfunction, insulin resistance and development of NODAT in RTR. NODAT places RTR at an increased risk of infections, cardiovascular disease, graft failure and mortality. Early identification of patients at risk for NODAT allows for start of treatment which may prevent other complications. Therefore we additionally investigated whether proinsulin, as a marker for beta-cell function, had additive value in prediction of NODAT. In **chapter 4** we determined alcohol consumption after renal transplantation. Furthermore, we investigated the association of alcohol consumption with NODAT, mortality and graft failure. In **chapter 5** we studied post transplant weight gain and it relation with cardiovascular risk factors. Additionally, the most important modifiable causes of weight gain were determined to provide targets for treatment and prevention. In **chapter 6** we draw attention to the importance of physical activity after transplantation. We assessed the level of physical activity after transplantation and investigated whether physical activity was associated with mortality. Moreover important determinants of physical activity will be addressed. **Chapter 7** examines the association between kinesiophobia and physical activity. Kinesiophobia refers to the anxiety that individuals can experience, with regard to engaging in activities or physical movements. Fear of movement can be an important barrier to engage in physical activity after renal transplantation and therefore target of intervention. In **chapter 8** we assessed the prevalence of depression after renal transplantation and its relationship with mortality and graft survival. Renal transplantation offers great advantages but on the other hand also introduces new uncertainties which can lead to emotional distress. Emotional distress may influence all aspects of treatment and the impact of depression after transplantation is greatly underestimated. Finally, **chapter 9** summarizes the most important findings of the previous chapters and provides suggestions for further research.

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2

Markers of the Hepatic Component of the Metabolic Syndrome as Predictors of Mortality in Renal Transplant Recipients

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Abstract

Background: *Cardiovascular disease (CVD) is a leading cause of mortality in renal transplant recipients (RTR). Metabolic syndrome (MS) is highly prevalent in RTR. Non-alcoholic fatty liver disease (NAFLD) is considered the hepatic component of MS. We investigated associations of NAFLD markers with MS and mortality.*

Methods: *RTR were investigated between 2001-2003. NAFLD markers, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (AP) were measured. Bone and non-bone fractions of AP were also determined. Death was recorded until August 2007.*

Results: *602 RTR were studied (age 52±12 years, 55% men). At baseline 388 RTR had MS. Prevalence of MS was positively associated with liver enzymes. During follow-up for 5.3 [4.5-5.7] years, 95 recipients died (49 cardiovascular). In univariate Cox-regression analyses, GGT (HR=1.43 [1.21-1.69], P<0.001) and AP (HR=1.34 [1.11-1.63], P=0.003) were associated with mortality, whereas ALT was not. Similar associations were found for cardiovascular mortality. Adjustment for potential confounders, including MS, diabetes and traditional risk factors did not materially change these associations. Results for non-bone AP mirrored that for total AP.*

Conclusion: *ALT, GGT and AP are associated with MS. Of these 3 enzymes, GGT and AP are associated with mortality, independent of MS. These findings suggest that GGT and AP are independently related to mortality in RTR.*

INTRODUCTION

Despite major improvements in short term survival after renal transplantation, long-term prognoses are still poor. Many renal transplant recipients (RTR) die prematurely from cardiovascular disease (CVD) (1). This excess in mortality can only partly be explained by the classical risk factors such as dyslipidemia, hypertension and smoking (1-3). The metabolic syndrome (MS), characterized by a clustering of risk factors, is a herald of CVD in the general population. Prevalence of MS and its accompanying cardiovascular risks is increased in the renal transplant population (3).

All components of MS, including waist circumference, glucose, triglycerides, HDL cholesterol levels and blood pressure have been shown to correlate with liver fat in the general population (4). In this population, non alcoholic fatty liver disease (NAFLD) is a common hepatic disorder characterized by liver fat accumulation and an increased risk for cardiovascular death (5). NAFLD is considered to be the hepatic component of MS (6). Prevalence of NAFLD is varying between studies, but it is estimated that approximately one fourth of healthy adults are affected (4). Prevalence of NAFLD is much higher in patients with type 2 diabetes mellitus and in morbidly obese patients (7). In most cases of NAFLD enzyme activity of alanine aminotransferase (ALT) in the circulation is elevated and consequently it is used as a marker of NAFLD (8-12). Serum gamma glutamyl transferase (GGT) is well known as a marker of hepatobiliary disease and excessive alcohol consumption (13), but recent studies in the general population indicate that GGT is also associated with NAFLD and increased risk for CVD (9,13-18). In the same population, both ALT and GGT are strongly correlated with MS (19-22). Alkaline phosphatase (AP) is most commonly used to monitor metabolic bone disease, but is also associated with MS in the general population and with increased risk for death in hemodialysis patients (23,24).

We aimed to cross-sectionally investigate whether the liver enzymes ALT, GGT and AP are associated with MS in RTR. We furthermore aimed to prospectively investigate in these patients whether serum activities of ALT, GGT and AP are associated with mortality, and if so, whether this association depends on components related to MS.

MATERIALS AND METHODS

Research Design and Subjects

In this prospective cohort study we invited all RTR, with a functioning graft for more than one year, who visited our outpatient clinic between 2001 and 2003. The group that did not sign informed consent was comparable with the group that did sign informed consent with respect to age, sex, body mass index (BMI), serum creatinine, creatinine clearance, and proteinuria. In patients with

fever or other signs of infection (e.g. complaints of upper respiratory tract infection or urinary tract infection), baseline visits were postponed until symptoms had resolved. Patients with overt congestive heart failure and patients diagnosed with cancer other than cured skin cancer were not considered eligible for the study. A total of 606 out of 847 eligible RTR signed written informed consent. Liver enzymes were available in 602 recipients. Full details on the study design have been previously reported (25). The Institutional Review Board approved the study protocol (METc 2001/039).

Endpoints of the study

The primary endpoint of this study was RTR mortality. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of death. We contacted general practitioners or referring nephrologists in case the status of a patient was unknown. Mortality was recorded until August 2007. Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9). Cardiovascular death was defined as deaths in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 410-447. There was no loss due to follow up.

Renal Transplant Characteristics

Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. Current medication was taken from the medical record. Smoking status and CVD history were obtained using a self-report questionnaire. CVD history was considered positive if participants had a myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA).

Measurements and definitions

BMI was calculated as weight in kilograms, divided by height in meters squared. Hip circumference was measured at the widest point at the level of the trochanter major and waist circumference was measured at the point halfway between the spina iliaca and the lower rib using a plastic tape measure. Blood pressure was measured as the average of three automated (Omron M4; Omron Europe B.V., The Netherlands) measurements with 1-min intervals after a 6-min rest in supine position. Diabetes was defined according to the guidelines of the American Diabetes Association as a fasting plasma glucose ≥7.0mmol/l or the use of antidiabetic medication (26).

Blood was drawn after an overnight fasting period. Serum creatinine concentrations were

determined using the Jaffé method, and serum triglycerides were determined with the GPO-PAP method. Total cholesterol was determined using the CHOD PAP method (MEGA AU 510; Merck Diagnostica, Darmstadt, Germany). High density lipoproteins (HDL) cholesterol was determined with the CHOD PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, the Netherlands) and low density lipoproteins (LDL) cholesterol was calculated using the Friedewald formula (27). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH, USA). HbA1c was determined by highperformance liquid chromatography (VARIANT™ HbA1c Program with Bio-Rad CARIANT Hb Testing System, Bio-Rad, Hercules, CA). Serum high sensitive C-reactive protein (hsCRP) was assessed with a high sensitivity CRP ELISA assay as described before (28).

Uniform measurement of ALT, GGT and total AP activity in serum was performed using IFCC-method (37°C). Serum bone specific AP kit (Metra™ Bone AP assay (Quidel Corporation, San Diego, CA) was used for assessment of the bone specific part of AP activity. Non-bone AP was calculated by subtracting bone AP from total AP. Non-bone AP is usually considered equivalent to liver AP, because intestinal and kidney iso-enzymes contribute little to total non-bone AP activity (29,30).

Creatinine clearance was calculated from 24-hour urinary creatinine excretion and serum creatinine. Total urinary protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany) and proteinuria was defined as urinary protein excretion ≥0.5 g per 24 hours. In this study MS was defined by the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII) (31). MS is defined by the NCEP-ATPIII when three or more of the following components are present: (1) a waist circumference >102 cm in men and > 88 cm in women; (2) serum triglycerides ≥ 1.70 mmol/L; (3) serum HDLc < 1.03mmol/L in men and < 1.29 mmol/L in women; (4) blood pressure ≥ 130/85 mmHg or use of antihypertensive medication; and (5) fasting plasma glucose ≥6.1 mmol/L or use of antidiabetic medication. Recently the American Diabetes Association (ADA) lowered the cut-off point for impaired fasting glucose to ≥ 5.6 mmol/L (26). For our analysis of the prevalence of the MS we used this ADA cut-off point. In our laboratory, the normal ranges for ALT, GGT and AP are < 45 U/L for ALT, < 40 U/L in females and < 55 U/L in males for GGT and < 120 U/L for AP.

Statistical Analysis

Data were analyzed with SPSS version 16.0 (SPSS Inc.,Chigago, IL) and GraphPad Prism version 4.03 (GraphPad Software Inc., San Diego, CA). Continuous variables were summarized using means (standard deviations) and medians (interquartile range); percentages were used to summarize categorical variables. Log transformation was used for variables with a skewed distribution. Hazard ratio's (HR) are reported with 95% confidence interval [95% CI].

Recipient-related and transplantation-related characteristics were analyzed separately for quartiles of liver enzymes. Student's t-test or Kruskal Wallis test was used to compare means for continuous variables and with Chi- square for categorical variables. The association between quartiles of ALT, GGT and AP and prevalence of MS was tested with Chi-square test. Subsequently, the relationship between single components of MS and liver enzymes were analysed and tested with Chi-square test.

To analyse whether ALT, GGT and AP are associated with mortality, we first performed Kaplan-Meier analyses with Log-rank test. For these analyses, levels of ALT, GGT and AP were divided into quartiles. Multivariate Cox regression analyses were performed to investigate whether ALT, GGT and AP were independently associated with all-cause and cardiovascular mortality. For these analyses ALT, GGT and AP were first log transformed to achieve a normal distribution and then transformed into z-scores, which results in expression of hazard ratios per standard deviation change in the log transformed variables. In this way strengths of associations can be compared between variables. In the Cox-regression analyses, we adjusted for recipient age, sex, creatinine clearance, urinary protein excretion (Model 2), additionally adjusted for presence of diabetes, HbA1c, fasting glucose, fasting insulin, use of anti-diabetic drugs and duration of diabetes (Model 3), all individual components of the MS (Model 4) and other cardiovascular risk factors (Model 5). As secondary analyses, we also performed similar Cox regression analyses with all-cause mortality as endpoint, after exclusion of all subjects with diabetes. To allow for comparison of strength of associations of GGT and AP with mortality with associations of more classical risk factors for mortality, we performed age and sex adjusted analyses for several risk factors, including HbA1c and HDL-cholesterol (Table 4).

RESULTS

Cross-Sectional association of ALT, GGT and AP with features of the MS

A total of 602 RTR were studied (mean age 52±12 years, 55% men). Baseline characteristics according to quartiles of serum activity levels of ALT, GGT and AP are shown in table 1. Median [interquartile range] activity of ALT, GGT and AP were 18.0 [14-25] U/L, 24.0 [17.8-39.0] U/L and 72.0 [57.0-93.3] U/L resp., with bone and non-bone AP resp. accounting for 42.9 ± 11% and 57.1 ± 12% of total AP activity. Enzyme activities exceeding the normal ranges of ALT, GGT and AP resp. were present in 29 (4.8%), 118 (19.7%) and 59 (9.8%) of RTR. Levels of ALT are positively associated with male gender, MS, BMI, waist, fasting triglycerides, use of statins, fasting insulin concentrations, prevalence of post-transplant diabetes, use of anti-diabetic drugs and creatinine clearance, whereas there are inverse associations with smoking status and prevalence of polycystic renal disease as primary renal disease. Similar associations were found for GGT, except for absence

of associations with smoking, use of statins, and creatinine clearance and a positive rather than an inverse association with polycystic renal disease. GGT appeared also positively associated with age, fasting glucose and HbA1c. For AP, also similar associations were present as for ALT, except for absence of associations with male gender, smoking status and polycystic renal disease, presence of inverse associations with alcohol intake and HDL-cholesterol and positive associations with systolic blood pressure, fasting glucose, HbA1c, pre-transplant diabetes, use of insulin and hsCRP.

A total of 388 out of 602 recipients suffered from MS. The association between liver enzymes and MS is shown in figure 1, with significantly increasing prevalence of MS over respective quartiles of liver enzymes. Prevalence of MS in the 1st vs. 4th quartile of liver enzymes was 57.9 vs. 72.7% for ALT, 53.3 vs. 73.7% for GGT and 50.3 vs. 82.2% for AP. To investigate which component of the MS

Table1. Baseline characteristics according to quartiles of liver enzymes

	ALT			GGT			AP		
	1st N= 140	2nd+3rd N= 308	4th N=154	1st N=150	2nd+3rd N=300	4th N=152	1st N=153	2nd+3rd N=297	4th N=152
Recipient demographics									
Age (years)	50.5 ± 13	51.8 ± 12	51.8 ± 11	47.6 ± 13	52.4 ± 11*	53.6 ± 11*	50.6 ± 12	51.2 ± 12	53.0 ± 11
Male (%)	42.9	55.5*	63.6 *	47.3	58.0*	54.6*	51.3	54.5	58.6
Smoking (%)	31.4	21.4*	14.9*	24.0	21.0	22.4	22.2	20.2	25.7
Alcohol (%)	15.1	13.4	15.1	14.8	15.4	11.4	21.6	11.4*	12.3*
MS (%)	57.9	63.3	72.7*#	53.3	65.3*	73.7*#	50.3	62.6*	82.2*#
History of CVD									
MI (%)	7.1	7.2	10.5	6.8	8.0	9.2	11.8	5.8	8.6
TIA/CVA (%)	6.4	5.2	5.2	4.7	5.4	6.6	6.6	4.1	7.2
Body composition									
BMI (kg/m2)	24.8 ± 4	26.3 ± 4*	26.9 ± 5*	24.9 ± 4	26.2 ± 4*	27 ± 5*#	24.9 ± 4	25.2 ± 4*	27.1 ± 5*#
Waist (cm) women	90 ± 14	96 ± 15*	96 ± 15*	88 ± 12	96 ± 14*	99 ± 16*	89 ± 14	95 ± 14*	99 ± 15*#
Waist (cm) men	94 ± 12	100 ± 12*	103 ± 13*	95 ± 12	99 ± 12*	105 ± 12*#	97 ± 13	99 ± 12	104 ± 13*#
Blood pressure									
SBP (mmHg)	153 ± 25	153 ± 22	153 ± 21	151 ± 22	154 ± 23	153 ± 22	149 ± 20	153 ± 23	157 ± 23*#
DBP (mmHg)	90 ± 10	90 ± 10	90 ± 9	90 ± 10	90 ± 10	89 ± 10	89 ± 10	90 ± 10	91 ± 10
Use of ACE-i (%)	27.1	40.9	26.6	37.3	34.0	30.9	39.2	33.0	30.9
Use of b-blocker (%)	57.9	62.7	63.6	59.3	61.0	65.8	62.1	60.9	63.2
Number of AHD	1.9 ± 1	2.0 ± 1	1.8 ± 1	1.8 ± 1	1.9 ± 1	2.0 ± 1	1.9 ± 1	1.9 ± 1	2.0 ± 1
Lipids									
TC (mmol/L)	5.6 ± 1	5.6 ± 1	5.6 ± 1	5.5 ± 1	5.6 ± 1	5.7 ± 1	5.5 ± 1	5.7 ± 1	5.6 ± 1
LDL-C (mmol/L)	3.6 ± 1	3.5 ± 1	3.5 ± 1	3.6 ± 1	3.6 ± 1	3.5 ± 1	3.5 ± 1	3.7 ± 1	3.4 ± 1
HDL-C (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.3*#
Triglycerides (mmol/L)	1.8 [1-2]	1.9 [1-3]	2.0 [2-3]*#	1.7 [1-2]	1.9 [1-3]	2.1 [2-3]*#	1.7 [1-2]	1.9 [1-3]	2.2 [2-3]*#
Use of statin %	37.9	54.2*	51.3*	37.3	54.3*	52.6*	51.0	47.8	52.0

	ALT			GGT			AP		
	1st	2nd+3rd	4th	1st	2nd+3rd	4th	1st	2nd+3rd	4th
	N= 140	N= 308	N=154	N=150	N=300	N=152	N=153	N=297	N=152
Glucose homeostasis									
Glucose (mmol/L)	4.7 ± 1	4.8 ± 1	5.0 ± 2	4.6 ± 1	4.8 ± 1	5.1 ± 2*	4.5 ± 1	4.8 ± 1	5.3 ± 2*#
Insulin (μmol/L)	10 [7-14]	11 [8-16]*	13 [9-18]*#	10 [8-14]	11 [8-16]*	13 [9-18]*#	11 [7-15]	11 [8-15]	12 [9-19]*#
HbA1c (%)	6.4 ± 1	6.5 ± 1	6.6 ± 1	6.2 ± 1	6.5 ± 1*	6.8 ± 1*#	6.3 ± 1	6.5 ± 1*	6.9 ± 1*#
Post-Tx DM (%)	5.7	15.6*	22.1*#	8	12.7	26.3*#	10.5	12.1	25.0*#
Pre-Tx DM (%)	3.6	2.6	2.6	2.0	3.3	2.6	0.0	3.0*	5.3*
Duration of diabetes†	2.9 [0-26]	4.1 [0-8]	1.4 [0-4]	4.1 [0-12]	2.3 [0-10]	1.5 [0-6]	0.0 [0-4.3]	2.7 [1-12]	1.8 [0-7]
Use of ADD (%)	6.4	14.0*	18.2*	7.3	12.0	21.7*#	5.2	12.8*	22.4*#
Use of insulin (%)	4.3	7.1	7.1	6.7	6.0	7.2	2.6	6.4	10.5*
hsCRP (mg/L)	2.5 [1-6]	1.9 [1-4]	2.1 [1-4]	1.4 [1-4]	1.8 [1-5]	3.2 [1-8]	1.4 [1-3]	2.3 [1-5.]*	2.9 [1-7]*
Renal transplant									
Creatinine (μmol/L)	156 ± 74	146 ± 58	143 ± 44	154 ± 70	144 ± 53	148 ± 59	151 ± 67	147 ± 58	145 ± 54
Creat Clear (mL/min)	56 ± 22	64 ± 22*	65 ± 23*	60 ± 22	64 ± 23	61 ± 22	61 ± 22	62 ± 22	63 ± 23
UPE (g/24hr)	0.3 [0-1]	0.2 [0-1]	0.3 [0-1]	0.2 [0-1]	0.2 [0-1]	0.2 [0-1]	0.3 [0-1]	0.2 [0-1]	0.3 [0-1]
Proteinuria (%)	27.1	29.4	26.0	28.0	29.1	25.8	29.4	27.8	27.0
Dialysis time (mo)	29 [15-48]	25 [11-50]	29 [16-48]	25 [11-47]	28 [14-50]	29 [15-48]	25 [11-44]	27 [14-50]	35 [18-52]
Prior transplants (n)	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.3	1.2 ± 0.5	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.5
Primary renal disease									
Polycystic vs other (%)	19.3	19.8	12.3 #	9.3	15.7	30.3*#	22.2	16.2	16.4

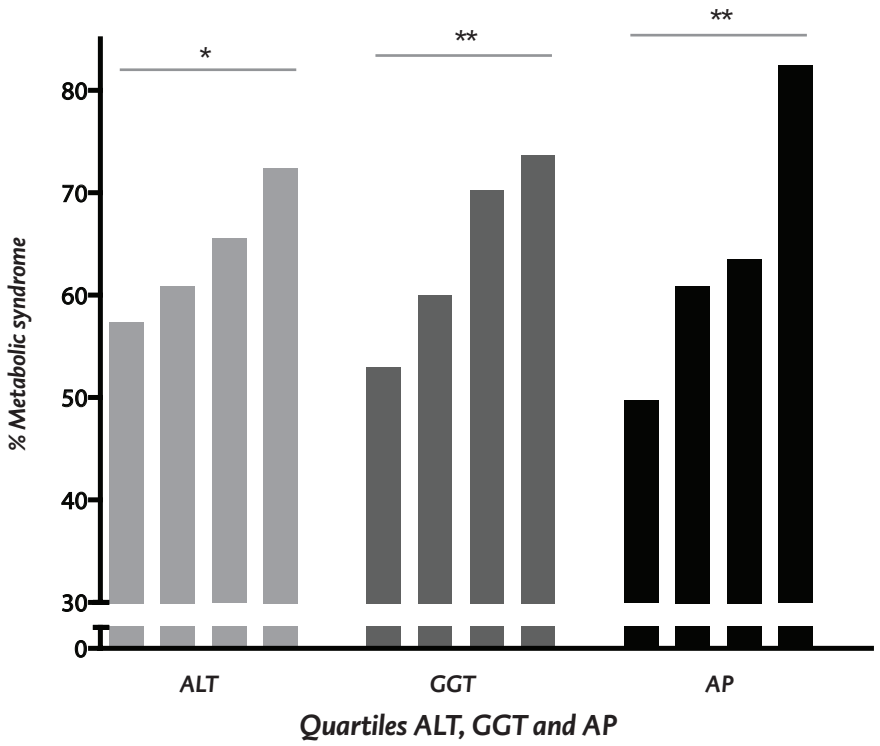
Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t test or Kruskal Wallis test for continuous variables and with Chi- square for categorical variables. * Quartile significantly different from 1ste quartile, P <0.05 # Quartile 4 is different from quartile 2 plus 3, P <0.05

Ranges for ALT 1st quartile: 4-13 U/l, 2nd and 3rd quartile: 14-24 U/l, 4th quartile 25-189 U/l. Ranges for GGT 1st quartile: 8-17 U/l, 2nd and 3rd quartile: 18-38 U/l, 4th quartile 39-1626 U/l. Ranges for AP 1st quartile: 21-57 U/l, 2nd and 3rd quartile: 58-93 U/l, 4th quartile 94-513 U/l. ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; AP, alkaline phosphatase; Alcohol, alcohol consumption more than 7 units per week; MS, metabolic syndrome; MI, myocardial infarction; TIA/CVA, Transient Ischemic Attack/ Cerebo Vasculair Accident; BMI, body mass index; Waist (cm), waistcircumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACE-i, Angiotensin Converting Enzyme inhibitor; AHD, anti-hypertensive drugs; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C., high density lipoprotein cholesterol; Post TDM, post transplant diabetes mellitus; ADD, anti-diabetic drugs; hsCRP, high sensitive C-reactive protein; Creat Clear, Creatinine Clearance; UPE, urinary protein excretion.

†Duration of diabetes is expressed in years and only applicable to patients with diabetes.

contributed most to the relationship with liver enzymes, components were analyzed separately. Percentages of patients meeting the component criteria of MS according to quartiles of liver enzymes are shown in table 2. Levels of ALT, GGT and AP were significantly associated with abdominal obesity, hypertriglyceridemia and impaired fasting glucose (Table 2). Higher levels of AP were also

*Figure 1: Prevalence of the metabolic syndrome according to quartiles of ALT, GGT and AP. Differences tested with Chi-square * P= <0.001, ** P= <0.005*



associated with low HDL-cholesterol concentrations. If the in total 107 RTR with either pre- or post transplant diabetes were excluded, existing associations of liver enzymes with abdominal obesity, hypertriglyceridaemia and HDL-cholesterol remained essentially unchanged, whereas associations with the impaired fasting glucose criterion of the MS became insignificant. If after exclusion, analyses were not restricted to individual components but to prevalence of MS overall, the association with ALT lost significance (prevalence of MS 53.5, 53.7, 57.6 and 63.3% according to increasing quartiles, P = 0.36), whereas associations with GGT (45.1, 59.9, 57.7 and 64.8% resp., P = 0.02) and AP (43.5, 53.7, 57.1 and 74.4% resp., P < 0.001) remained.

Prospective association of ALT, GGT and AP with mortality

During (median) follow-up for 5.3 [4.5-5.7] years, 95 recipients died, with 49 deaths cardiovascular in origin. We first performed Kaplan-Meier analyses for quartiles of liver enzymes. The incidence of death was almost equally distributed over the different quartiles of ALT, with respective numbers of 21 (14.9%), 25 (14.8%), 29 (20.3%) and 20 (13.2%) according to respective quartiles (Log-rank test: P = 0.46). It, however, significantly increased according to increasing quartiles of GGT, with respective

Table 2. Percentage of patients meeting MS criteria[1] according to quartiles ALT, GGT and AP

	% meeting criteria of MS				
Quartiles	**Waist**	**Triglycerides**	**HDLc**	**BP**	**Glucose**
ALT					
Q1	38.3	66.7	64.5	97.2	15.6
Q2	53.2	76.3	55.6	97.0	20.1
Q3	57.7	77.5	66.2	98.6	21.1
Q4	53.3**	80.9*	63.2	99.3	30.9*
GGT					
Q1	37.3	62.7	61.3	96.7	15.3
Q2	49.7	77.2	57.9	97.2	10.3
Q3	54.8	81.3	60	97.4	29.7
Q4	62.5***	82.2***	69.1	99.3	32.9***
AP					
Q1	39.9	69.9	52.9	97.4	13.1
Q2	45.7	77.5	56.9	98.7	19.9
Q3	52	69.6	67.6	96.6	18.9
Q4	66***	85**	71.2**	99.3	36.6***

[1] MS criteria: waist circumference >102 cm in men and > 88 cm in women; serum triglycerides ≥ 1.70 mmol/L; serum HDL cholesterol < 1.03mmol/L in men and < 1.29 mmol/L in women; blood pressure ≥ 130/85 mmHg or use of antihypertensive medication;and fasting plasma glucose ≥5.6 mmol/L ore use of antidiabetic medication. Q1; quartile 1, Waist; waist circumference, BP; blood pressure

Differences were tested with Chi-square * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

numbers of 14 (9.3%), 17 (11.7%), 29 (18.7%) and 35 (23.0%) (Log-rank test: P = 0.001, Figure 2a). A similar association with death was present for total AP, with incidences of death of 18 (11.8%), 24 (16.0%), 21 (14.3%) and 32 (21.1%) according to increasing quartiles resp. (Log-rank test: P=0.02, Figure 2b). This association was not present for bone specific AP, with incidences of death of 20 (12.9%), 27 (18.4%), 25 (16.6%) and 23 (15%) according to increasing quartiles resp. (Log-rank test: P = 0.30). For non-bone AP, however, a similar – albeit somewhat weaker – association was present as for total AP, with incidences of death of 15 (10.0%), 21(14.0%), 28(18.4%) and 31(20.8%) according to increasing quartiles resp. (Log-rank test: P = 0.04). Of note, increases in GGT and total AP within the normal range are already associated with higher mortality, because the 2nd and the 3rd quartile in which levels are still within the normal range (the 2nd and 3rd quartile were analyzed together because lines were comparable) are at increased risk compared to the 1st quartile.

Results of univariate and multivariate Cox-regression analyses are shown in table 3. In univariate analyses, GGT strongly predicted all-cause and cardiovascular mortality in RTR (Model 1). These associations weakened after adjustment for age, sex creatinine clearance and urinary protein excretion, but remained significant (Model 2). The same was true for further adjustment for diabetes and factors related to diabetes (Model 3), further adjustment for all separate components

of the MS (Model 4) and further adjustment for cardiovascular risk factors, number of previous transplantations and total time on renal replacement therapy (Model 5). If analyses were restricted to 64 deaths in 495 RTR with neither pre- nor post transplant diabetes, results for univariate and multivariate analyses remained essentially unchanged (Table 3, last column).

Table 3. Hazard ratios for mortality according to Z-scores of ALT, AP and GGT

	All-cause Mortality N=602		Cardiovascular Mortality N=602		All-cause Mortality* N= 495	
ALT	**HR [95% CI]**	**P**	**HR [95% CI]**	**P**	**HR [95% CI]**	**P**
1	1.03 [0.8-1.3]	0.8	1.06 [0.8-1.4]	0.7	0.96 [0.7-1.2]	0.7
2	1.13 [0.9-1.4]	0.2	1.15 [0.9-1.5]	0.3	1.04 [0.8-1.3]	0.8
3	1.10 [0.9-1.3]	0.4	1.10 [0.8-1.4]	0.5	1.04 [0.8-1.3]**	0.8
4	1.07 [0.9-1.3]	0.5	1.05 [0.8-1.4]	0.7	1.04 [0.8-1.3]***	0.8
5	1.07 [0.9-1.3]	0.5	1.04 [0.8-1.3]	0.8	1.04 [0.8-1.4]	0.8
GGT						
1	1.43 [1.2-1.7]	<0.001	1.55 [1.3-1.9]	<0.001	1.51 [1.2-1.9]	<0.001
2	1.32 [1.1-1.6]	0.001	1.40 [1.1-1.7]	0.001	1.36 [1.1-1.7]	0.006
3	1.30 [1.1-1.5]	0.003	1.35 [1.1-1.7]	0.008	1.34 [1.1-1.7]**	0.01
4	1.21 [1.0-1.4]	0.03	1.27 [1.0-1.6]	0.04	1.35 [1.1-1.7]***	0.01
5	1.21 [1.0-1.4]	0.03	1.25 [1.0-1.4]	0.05	1.38 [1.1-1.8]	0.01
AP						
1	1.34 [1.1-1.6]	0.003	1.50 [1.2-1.9]	0.002	1.30 [1.0-1.7]	0.05
2	1.39 [1.2-1.7]	<0.001	1.51 [1.2-1.9]	<0.001	1.43 [1.1-1.9]	0.01
3	1.32 [1.1-1.6]	0.005	1.37 [1.1-1.8]	0.01	1.40 [1.1-1.8]**	0.01
4	1.24 [1.0-1.5]	0.03	1.33 [1.0-1.7]	0.03	1.39 [1.1-1.8]***	0.02
5	1.29 [1.1-1.6]	0.01	1.36 [1.1-1.8]	0.02	1.42 [1.1-1.9]	0.02

Model 1: Univariate analyses
Model 2: Model 1 + adjustment for recipient age, sex, creatinine clearance and urinary protein excretion
Model 3: Model 2 + adjustment for presence of diabetes, HbA1c, glucose, use of insulin, use of anti diabetic drugs and years of diabetes.
Model 4: Model 2 + adjustment for all components of the MS: waist circumference, triglycerides, HDL cholesterol, blood pressure and impaired fasting plasma glucose or diabetes.
Model 5: Model 4 + adjustment for HbA1c, LDL cholesterol, hsCRP, smoking, previous transplantations, total time on renal replacement therapy.

* Patients with pre- and post transplant diabetes were excluded from these analyses.
** Only additional adjustment for HBA1c and glucose, because all subjects with diabetes were excluded in this column.
*** No additional adjustment for diabetes, because all subjects with diabetes were excluded in this column.

Figure 2a: Kaplan-Meier curve of mortality in quartiles of GGT tested with Log-rank test (P= 0.001). Cut-off points for quartiles of GGT were: 1st quartile: 8-17 U/L, 2nd quartile: 18-23 U/L, 3rd quartile: 24-38 U/L and 4th more than 39 U/L.

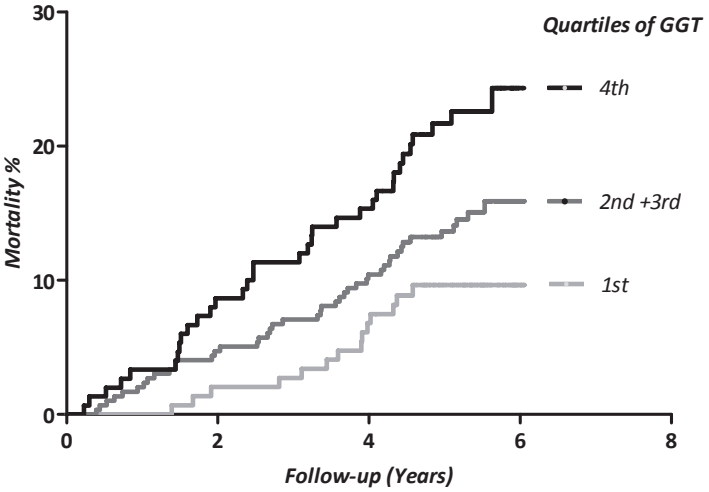
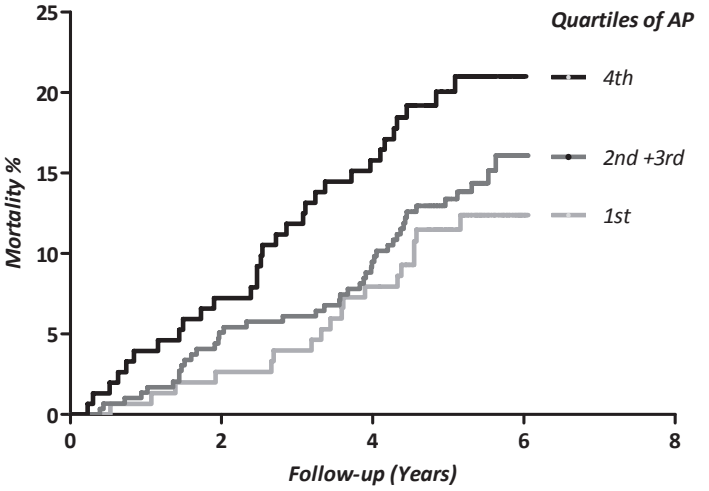


Figure 2b: Kaplan-Meier curve of mortality in quartiles of AP tested with Log-rank test (P= 0.02) Cut-off points for quartiles of AP were: 1st quartile: 21-57 U/L, 2nd quartile: 58-72 U/L, 3rd quartile: 73-93 U/L and 4th more than 94 U/L.



In univariate analyses, AP was also strongly associated with all cause and cardiovascular mortality (Model 1). Adjustment for age, sex, creatinine clearance and urinary protein excretion strengthened the association (Model 2), whereas it was weakened, but remaining significant after adjustment for

diabetes and factors related to diabetes (Model 3), components of MS (Model 4) and cardiovascular risk factors, number of previous transplantations and total time on renal replacement therapy (Model 5). In univariate analysis, bone specific AP was associated with neither all-cause mortality (HR = 1.10 [0.90-1.35], P 0.36) nor cardiovascular mortality (HR = 1.09 [0.82-1.44], P = 0.56). Contrastingly, non-bone AP was strongly associated with all-cause and cardiovascular mortality, with hazard ratios of 1.45 [1.20-1.76], P <0.001 and 1.63 [1.27-2.08], P < 0.001 resp. for univariate analyses. Results for multivariate Cox regression analyses with non-bone AP were essentially similar to those for total AP.

In table 4 results of age and sex adjusted Cox regression analyses for z-scores of classic cardiovascular risk factors, including HbA1c, HDL-cholesterol, systolic blood pressure, C-reactive protein and glucose are compared with those for GGT and AP. By using z-scores, strengths of associations are comparable because derived hazard ratios are expressed per standard deviation change in variables. Hazard ratios of GGT and AP appeared of similar strength as those of HbA1c and hsCRP.

Table 4. Age- and sex-adjusted hazard ratios for prediction of all-cause mortality expressed per standard deviation change in risk factor.

Risk factors	All cause Mortality	
	HR [95% CI]	P
HBA1c	1.30 [1.10-1.54]	0.002
SBP	1.28 [1.05-1.55]	0.01
hsCRP	1.26 [1.12-1.42]	<0.001
Glucose	1.16 [1.00-1.34]	0.05
HDL-C	0.80 [0.64-0.99]	0.04
GGT	1.32 [1.12-1.56]	0.001
AP	1.31 [1.08-1.59]	0.007

SBP, systolic blood pressure; hsCRP, high sensitive C-reactive protein; HDL-C, high density lipoprotein cholesterol; GGT, gamma glutamyl transferase; AP, alkaline phosphatase.

DISCUSSION

This study shows that liver enzymes ALT, GGT and AP are positively associated with prevalence of MS in RTR. Not all components of the MS contributed equally to this relationship. The strongest contributors for associations of MS with liver enzymes were abdominal obesity, hypertriglyceridemia and impaired fasting glucose. Only for AP low HDL-cholesterol concentrations had significant impact. If RTR with diabetes were excluded, the association of ALT with prevalence of MS lost significance, but associations of GGT and AP remained. This study furthermore shows that liver enzymes GGT

and AP strongly predicted mortality in RTR. Increasing levels of GGT and AP – even within the normal range – are associated with increasing all-cause and cardiovascular mortality. Separate analyses for bone and non-bone AP – mainly representing liver isoenzyme activity – showed that associations present for total AP also exist for non-bone AP, whereas bone AP is unrelated. Associations of liver enzymes with all-cause and cardiovascular mortality were not materially affected by adjustments for age, sex, creatinine clearance, urinary protein excretion, diabetes and factors related to diabetes, MS and other cardiovascular risk factors. Analyses performed after exclusion of all patients with diabetes showed that the associations of GGT and AP with mortality are independent of diabetes. When GGT and AP are compared with classical cardiovascular risk factors, including HbA1c, HDL cholesterol, systolic blood pressure and C-reactive protein, GGT and AP appeared to have associations of similar strength as the strongest of established cardiovascular risk factors.

Cardiovascular disease is the main cause of death in renal transplant patients (1). Traditional risk factors like diabetes mellitus, dyslipidemia and hypertension are often seen in transplantation patients but could not fully explain the high prevalence of cardiovascular death among this patient group (3). The clustering of risk factors in the MS is associated with an increased risk of CVD and type 2 diabetes mellitus (32). It is also associated with a broad spectrum of other cardiovascular risk factors such as coagulation abnormalities, chronic inflammation, increased oxidative stress and endothelial dysfunction (33). There is a body of evidence that supports that NAFLD is the new hepatic component of the MS (6,20,22). Our findings are consistent with earlier findings where higher GGT levels predicted CVD, mortality and development of the MS in the general population (15,18-20). Other prior community based studies found that increased levels of GGT were associated with insulin resistance and an increased risk of coronary heart disease and stroke (17,21). Our observations relating AP to mortality in RTR complement a prior study by Regidor et al., where a rise in AP was associated with an increased risk for death in hemodialysis patients (23). In this study, no data on GGT were available, and it could not be investigated whether there was an association between AP and GGT, neither was it investigated whether AP was associated with MS and its components.

We hypothesized that the hepatic manifestation of the MS could be a new risk factor for cardiovascular related death. As our findings point towards an association between GGT, AP and mortality independent of the components of MS, it can be suggested that other underlying mechanisms play a role. Our observations relating GGT and AP to mortality could be elucidated by the mechanism of oxidative stress. Serum GGT activity levels within its normal range might be related to oxidative stress (34). Recent studies indicate that GGT may have a direct involvement in atherosclerotic plaque formation and thereby could be a key factor in the pathogenesis of CVD (35,36). This is in line with evidence for an association of cytosolic triglycerides with oxidative stress (37). Increased storage of cytosolic triglycerides in non- adipose tissues such as the liver leads to

elevated concentrations of cytosolic long-chain acetyl-CoA esters. An increase in these esters would lead to inhibition of mitochondrial adenine nucleotide translocators, causing an ADP deficiency. It was speculated that this deficiency stimulates production of free oxygen radicals, which leads to atherosclerosis (37).

RTR are prone to develop high bone turnover, which can lead to elevations in AP activity. In separate analyses for the bone and non-bone part of AP activity, we found that the association with all-cause and cardiovascular mortality was only present for non-bone AP. Non-bone AP is usually considered equivalent to liver AP, because intestinal and kidney iso-enzymes contribute little to total non-bone AP activity (29,30). Alcohol abuse can also alter the level of GGT but in the present study alcohol consumption was not higher in the group with elevated levels of GGT. We also explored the possibility that polycystic renal disease as underlying disorder could have influenced the relationship between GGT and mortality; nevertheless it did not remain significant in our regression analyses.

The strength of our study is its prospective design. RTR in this study were closely monitored by regular check-up in our clinic, this gives complete information on patient status. A limitation of our study is that we only measured liver enzymes in baseline samples. Most epidemiological studies use a single baseline measurement to predict outcome, which adversely affects predictive properties of variables associated with outcome. If intra-individual variability of predictive biomarkers is taken into account, this results in strengthening of predictive properties that – despite sometimes considerable intra-individual variation day-to-day variation – also existed for single measurements of these biomarkers. The higher the intra-individual day-to-day variation would be, the greater one would expect the benefit of repeated measurement for prediction of outcomes. Further, the present study is a single center study and the predictive value of GGT and AP need to be confirmed in other centra and/or multicenter studies. Our study includes RTR that were transplanted in multiple immunosuppressive eras. However, immunosuppressive therapy was not significantly associated with liver enzymes. Furthermore, our study only includes stable RTR, relatively long-term after transplantation. Future studies could investigate whether liver enzymes, measured in the early post transplant period also predict mortality.

In conclusion, liver enzymes ALT, GGT and AP are strongly related to prevalence of the MS however waist circumference, triglycerides and glucose levels were the strongest contributors to this relationship. Increasing levels of GGT and AP within the normal ranges are associated with a higher risk for mortality independently of age, sex, creatinine clearance, protein excretion, diabetes and factors related to diabetes, MS and classic cardiovascular risk factors. These findings suggest that a raise in serum GGT and AP levels are independent risk factors for early all-cause and cardiovascular mortality in RTR.

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3

Beta-cell function and risk for New Onset Diabetes After Kidney Transplantation

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Abstract

Background: *Chronic exposure to calcineurin inhibitors and corticosteroids poses renal transplant recipients (RTR) at high risk for development of new onset diabetes after transplantation (NODAT). Pancreatic β-cell dysfunction may be crucial to the pathophysiology of NODAT and specific markers for β-cell dysfunction may have additive value for predicting NODAT in this population. Therefore, we prospectively investigated whether proinsulin, as a marker of pancreatic β-cell dysfunction, is associated with future development of NODAT and improves prediction of it.*

Methods: *All RTR between 2001-2003 with a functioning graft for ≥1 year were considered eligible for inclusion, except for subjects with diabetes at baseline who were excluded. We recorded incidence of NODAT until April 2012.*

Results: *A total of 487 RTR (age 50 ±12 years, 55% men) participated at a median [IQR] time of 6.0 [2.6-11.5] years post-transplant. Median fasting proinsulin levels were 16.6 [11.0-24.2] pmol/L. During median follow-up for 10.1(9.7-10.4) years, 42 (35%) RTR developed NODAT in the highest quartile of the distribution of proinsulin versus 34 (9%) in the lowest three quartiles (P<0.001). In Cox-regression analyses, proinsulin (Hazard ratio [95% confidence interval] = (2.29 [1.85-2.83], p<0.001) was strongly associated with NODAT development. This was independent of age, sex, calcineurine inhibitors, prednisolon use, components of the metabolic syndrome or HOMA.*

Conclusions: *In conclusion, fasting proinsulin is strongly associated with NODAT development in RTR. Our results highlight the role of β-cell dysfunction in the pathophysiology of NODAT and indicate the potential value of proinsulin for identification of RTR at increased risk for NODAT.*

INTRODUCTION

New onset diabetes mellitus after transplantation (NODAT) is one of the main metabolic complications of renal transplantation (1). It is estimated to affect approximately 20% of the renal transplant recipients (RTR) (2). NODAT places RTR at an increased risk of infections, cardiovascular disease, graft failure and mortality (2-4). Comparable with diabetes mellitus type 2, NODAT may be a result of increased insulin resistance and decreased insulin production by the pancreatic β-cell (5). Early identification of increased risk for NODAT, allowing for early intervention, could be of great importance to renal transplant healthcare considering the detrimental effects associated with NODAT.

The presence of pre-transplantation insulin resistance in the final stage of kidney failure is seen as a mechanism in the development of NODAT (6). The chronic exposure to calcineurin inhibitors and corticosteroids aggravates the insulin resistance and poses RTR at high risk for NODAT development (7,8). Another potential mechanism in NODAT is a defect in insulin secretion as a consequence of pancreatic β-cell dysfunction, leading to an inability to compensate for insulin resistance (5,6).

As a precursor of insulin, intact proinsulin has been proposed as a specific marker of β-cell dysfunction (5). In the past, nonspecific assays showed high cross-reactivity which could lead to incorrect conclusions on β-cell dysfunction and prediction of diabetes. A new specific intact proinsulin enzyme-linked immunisorbent assay (Elisa) (no cross-reactivity) has been developed which can easily be used in routine laboratories (9).

It is unknown whether proinsulin is a good marker of β-cell dysfunction in RTR and whether it is independently associated with future development of NODAT and/or predicts NODAT beyond established clinical risk predictors. Therefore, we prospectively investigated the association between β-cell dysfunction, insulin resistance and NODAT development in RTR. Furthermore, we investigated whether proinsulin had additive value in the prediction of NODAT.

RESEARCH DESIGN AND METHODS

Design and Subjects

Study design and inclusion/exclusion criteria have been described previously (10). In brief, for this prospective cohort study all adult allograft recipients between August 2001 and July 2003 who survived with a functioning allograft beyond the first year after transplantation were eligible to participate at their next visit to our outpatient clinic. A total of 606 from an eligible 847 RTR (72% consent rate) signed written informed consent. We excluded 105 recipients with existing diabetes (defined as fasting plasma glucose ≥7.0 or antidiabetic medication) at baseline from analysis.

Proinsulin levels were available in 487 RTR leaving 487 non-diabetic RTR for analysis. Baseline data were collected between August 2001 and July 2003 and RTR were followed for several years. The Institutional Review Board approved the study protocol (METc 2001/039).

Renal Transplant Characteristics

The Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. We found current medication information in the medical record and obtained information on employment status, living situation, smoking and alcohol consumption, and cardiovascular history by self-report questionnaire.

Standard immunosuppressive treatment consisted of the following: prednisolon and azathioprine (100 mg/day) from 1968 to 1989; cyclosporine standard formulation (Sandimmune, Novartis Pharma B.V., Arnhem, The Netherlands, 10 mg/kg; trough levels of 175 to 200 mg/L in the first 3 months, 150 mg/L between 3 and 12 months post transplant, and 100 mg/L thereafter) and prednisolone (starting with 20 mg/day, rapidly tapered to 10 mg/day) from January 1989 to February 1993; cyclosporine microemulsion (Neoral, Novartis Pharma B.V., Arnhem, The Netherlands; 10 mg/kg, trough levels idem) and prednisolone from March 1993 to May 1997; mycophenolate mofetil (Cellcept, Roche B.V., Woerden, The Netherlands; 2 g/day) was added from May 1997 to present date. In some specific situations, immunosuppressive medication deviated from the standard protocol. Cyclosporine was converted to tacrolimus in the event of acute rejection, hypertrichosis, gingival hypertrophy or intolerance of cyclosporine. Target trough levels of tacrolimus were 6 to 10 μg/L. Sirolimus was used when frequent skin malignancy occurred. Target trough levels of sirolimus were 4 to 6.5 μg/L.

Measurements and definitions

Body Mass Index (BMI) was determined as a measure of overall obesity. BMI was calculated as weight in kilograms, divided by height in meters^2. Waist circumference was measured on bare skin midway between the iliac crest and the 10th rib. Muscle mass was estimated by 24-hr creatinine excretion as described earlier (11). Blood pressure was measured after a 6-minute rest in supine position as the average of three automated measurements at 1-minute intervals (Omron M4; Omron Europe B.V., the Netherlands). Blood was drawn after an overnight fasting period. Plasma glucose, insulin, high-density lipoproteins (HDL) cholesterol, low-density lipoproteins (LDL) cholesterol, hs-CRP and serum creatinine were measured as described previously (10). Homeostasis model assessment (HOMA) was calculated as: [glucose (in millimoles per liter) × insulin (in microunits per milliliter)]/22.5 (12). In this study, metabolic syndrome (MS) was defined according to the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII) (13). Renal allograft function was determined as discribed before (10).

Proinsulin

Proinsulin levels were measured with the Mercodia-Proinsulin ELISA. Mercodia-Proinsulin ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the proinsulin molecule. Proinsulin in the sample reacts with anti-proinsulin antibodies bound to microtitration wells and peroxidase-conjugated anti-insulin antibodies in the solution. Cross-reactivity for insulin is < 0.03% and cross-reactivity for C-peptide is < 0.006%.

NODAT

The International Expert Panel Meeting (14) proposed recommendations to define NODAT based on the American Diabetes Association criteria 2003 (15). The diagnosis of NODAT was based on one of the following criteria are: 1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l). The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss; 2. Fasting plasma glucose ≥126 mg/dl (7.0 mmol/l) or use of antidiabetic medication. Fasting is defined as no caloric intake for at least 8 h. NODAT was recorded until April 2012.

Statistical analyses

Data were analyzed with SPSS version 16.0 (SPSS Inc., Chicago, IL), STATA Version 11 (StataCorp LP, Texas) and GraphPad Prism version 4.03 (GraphPad Software Inc., San Diego, CA). Recipient-related characteristics were analyzed separately for quartiles of proinsulin. For analyses, we combined quartile 1–3 to one group and compared this group with quartile 4. Differences between groups were tested for statistical significance with Student's t-test for normally distributed variables, Mann–Whitney test for skewed distributed variables, and chi-squared test for categorical variables. We performed multivariate Cox-regression analyses to investigate whether proinsulin is independently associated with NODAT. In subsequent multivariate analyses, we investigated whether the association of proinsulin is independent of age, sex, use of cyclosporine, tacrolimus, dose of prednisolone, trough levels of cyclosporine and tacrolimus, HOMA or components of the metabolic syndrome. Death was regarded as a competing risk for NODAT. Patients were censored at date of last follow-up or death.

As there are no validated clinical models for the prediction of NODAT in RTR, we used a commonly used prediction model for the general population. This clinical model includes: sex, smoking, waist circumference, hypertension and family history of diabetes (16). To assess the added value of proinsulin, we examined improvement of diabetes prediction in terms of discrimination and integrated discrimination improvement (IDI). Discrimination was evaluated using the Harrell's c-index for censored data, a statistic similar to the area under a receiver operating characteristic

curve (17). In general, discrimination refers to the ability of a model to distinguish well between individuals with and without incident diabetes; a value of 1 implies a perfect discrimination and a value of 0.5 implies performance no better than chance. We used IDI as a continuous measure of reclassification, calculated by subtracting the mean difference of predicted risk between the clinical model (16) and the model including different biomarkers, for those who developed NODAT from the corresponding risks for those who did not develop NODAT.

RESULTS

The study cohort was composed of 487 RTR (55% men) aged 50 ± 12 years at a median [IQR] time of 6.0 [2.6-11.5] years post-transplant. Median [IQR] concentration of fasting proinsulin was 16.6 [11.0-24.2] pmol/L. Baseline characteristics of the RTR according to the two groups of proinsulin are shown in table 1. RTR with high proinsulin (4^{th} quartile versus quartiles 1-3) were more obese with a higher BMI and waist circumference. High proinsulin was positively associated with use of beta-blocker, use of statin, history of MI, hsCRP, glucose, insulin, HOMA and pro-insulin-insulin ratio. RTR with high proinsulin had significantly lower creatinine clearance and higher serum creatinine. We found other differences in lipid-profile with lower HDL and LDL-cholesterol and higher triglyceride in subjects with high proinsulin. There was a trend towards lower use of tacrolimus in subjects with high proinsulin. No differences were found in other components of immunosuppressive treatment. Out of the 487 RTR, 309 (75%) fulfilled the criteria for MS. Prevalence of MS was 101 (86%) in the highest quartile of proinsulin versus 208 (57%) in the lowest three quartiles ($p<0.001$).

NODAT developed during median follow-up for 10.1 [9.7 - 10.4] years in 76 (16%) RTR. Incidence of NODAT was 42 (35%) in the highest quartile versus 34 (9%) in the lowest three quartiles of pro-insulin ($p<0.001$) (Figure 1). Cumulative percentage of NODAT at one, three, five and ten years after baseline were: 1.2, 4.6, 7.4 and 14.8% respectively.

Subsequently, we proceeded with prospective analyses for proinsulin and development of NODAT during follow-up. Proinsulin (HR: 2.29 [1.85-2.83], $p<0.001$) strongly predicted NODAT development in RTR in univariate analyses. Multivariate Cox regression analyses for proinsulin and NODAT development are shown in table 2. Adjustment for age and sex did not materially influence the associations (Model 2). We adjusted for use of cyclosporine, tacrolimus and prednisolone dose in model 3. This adjustment did also not materially influence the association of proinsulin with NODAT development. Of note, use of tacrolimus was significantly associated with NODAT development (HR: 2.84 [1.37-5.89], $p=0.005$), in this Cox-regression model. This was independent of proinsulin, age, sex, use of cyclosporine and prednisolone dose. We found no significant association of use of cyclosporine with development of NODAT. In further analyses, in which we

Table 1. Recipient characteristics according to groups of proinsulin.

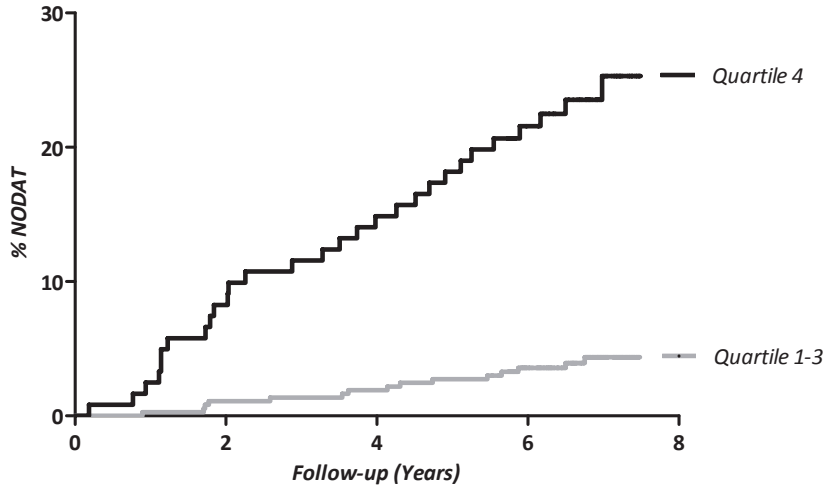
	Quartiles of Proinsulin		
	1-3rd (N= 366)	**4rd** (N= 121)	**P**
General characteristics			
Age (years)	50.4 ± 12.5	51.1 ± 11.0	0.6
Male gender, n (%)	203 (55.5)	68 (56.2)	0.9
Lifestyle			
Physical activity (METS)	124.5 [31.2 – 312.1]	118.7 [26.7 – 294.1]	0.4
Metabolic Syndrome, n (%)	208 (57)	101 (86)	<0.001
Smoking, n (%)	150 (41.3)	48 (39.7)	0.8
Alcohol consumption			
- Abstainers, n (%)	160 (44)	63 (53)	0.2
- <10 g/d, n (%)	145 (40)	44 (37)	
- 10-30 g/d, n (%)	52 (14)	13 (11)	
- >30 g/d, n (%)	6 (2)	0 (0)	
Body composition			
BMI (kg/m^2)	25.1 ± 3.9	27.8 ± 4.2	<0.001
Waist circumference (cm) women	90.4 ± 13.3	98.8 ± 13.8	<0.001
Waist circumference (cm) men	96.6 ± 11.1	105.7 ± 10.9	<0.001
Urinary creatinine excretion (mmol/24hr)	11.9 [9.5 – 14.4]	11.9 [9.8 – 13.9]	0.9
Blood pressure			
Systolic pressure (mmHg)	152.0 ± 23.1	149.6 ± 20.9	0.3
Diastolic pressure (mmHg)	90.0 ± 10.1	89.3 ± 9.3	0.5
Use of ACE-inhibitor or Angiotensin II-antagonist, n (%)	117 (32)	49 (40.5)	0.09
Use of β-blocker, n (%)	216 (59.0)	87 (71.9)	0.01
History of cardiovascular disease			
MI[a], n (%)	24 (6.6)	16 (13.2)	0.02
TIA/CVA[b], n (%)	12 (3.4)	8 (6.6)	0.1
Inflammation			
Hs CRP (mg/L)	1.8 [0.7-4.3]	2.8 [1.2-5.9]	0.007
Lipids			
Total cholesterol (mmol/L)	5.7 ± 1.1	5.6 ± 1.0	0.2
LDL (mmol/L)	3.7 ± 1.0	3.4 ± 0.9	0.007
HDL (mmol/L)	1.1 ± 0.3	1.0 ± 0.7	<0.001
Triglycerides (mmol/L)	1.8 [1.3-2.4]	2.4 [1.7-3.2]	<0.001
Use of statin at index, n (%)	165 (45.1)	71 (58.7)	0.009

	Quartiles of Proinsulin		
	1-3rd (N= 366)	4rd (N= 121)	P
Glucose homeostasis			
Glucose (mmol/L)	4.5 ± 0.6	4.8 ± 0.8	<0.001
Insulin (μmol/L)	9.4 [7.0-12.4]	15.0 [11.1-21.1]	<0.001
HOMA	1.81 [1.29-2.52]	3.28 [2.31-4.39]	<0.001
Proinsulin (pmol/L)	13.8 [9.6-17.9]	36.4 [28.4-51.5]	<0.001
Proinsulin/insulin ratio	1.4 [1.0-1.9]	2.6 [1.7-3.4]	<0.001
Family history of diabetes			
Parent or sibling with diabetes, n (%)	86 (23)	32 (26)	0.5
Renal allograft function			
Serum creatinine concentration (μmol/L)	134.0 [111.8-163.3]	146.0 [123.0-174.0]	0.007
Urinary creatinine excretion	11.9 [9.5-14.4]	11.9 [9.8-13.9]	1.0
Creatinine clearance (mL/min)	60.0 [47.0-78.0]	57.0 [43.0-71.5]	0.04
Urinary protein excretion (g/24hr)	0.2 [0.0-0.5]	0.3 [0.1-0.5]	0.1
Proteinuria, n (%)	98 (26.8)	36 (29.8)	0.5
Transplantation and history			
Number of transplantations >1, n (%)	39 (10.7)	12 (9.9)	0.2
Time after transplantation (years)	6.4 [2.9 – 12.2]	5.7 [2.2 -12.2]	0.8
Dialysis duration (months)	26 [12 – 49]	33 [19 – 49]	0.06
Immunosuppression			
Calcineurine inhibitor, n (%)	286 (78.1)	93 (76.9)	0.8
Cyclosporine, n (%)	234 (61.6)	82 (67.8)	0.3
Cyclosporine (trough level, ug/l)	108 [80-138]	104 [77-149]	0.9
Tacrolimus, n (%)	57 (15.6)	11 (9.1)	0.07
Tacrolimus (trough level, ug/l)	8.7 [6.4-10.2]	8.6 [6.0-9.7]	0.9
Proliferation inhibitor, n (%)	278 (87)	72 (72)	0.4
Azathioprine, n (%)	124 (33.9)	38 (31.4)	0.6
Mycophenolate mofetil, n (%)	154 (42.1)	49 (40.5)	0.8
Sirolimus, n (%)	8 (2.2)	2 (1.7)	0.7
Sirolimus (trough level, ug/l)	9.0 ± 4.9	7.3 ± 6.3	0.7
Prednisolon dose, (mg/day)	9.1 ± 1.4	9.3 ± 1.3	0.3

Data are represented as mean ± SD or median [95% confidence interval]. Differences were tested by ANOVA or Kruskal-Wallis test for continuous variables and with Chi- square for categorical variables.

additionally adjusted for trough levels of tacrolimus and cyclosporine (Model 4), we found no additional association of trough levels of tacrolimus with NODAT. However, trough levels of cyclosporine were associated, with increased risk for higher concentrations (HR=1.07 [1.01-1.13], p=0.02). In further analyses, it appeared that the association between proinsulin and NODAT was independent of HOMA (Model 5). Interestingly, in this model HR for HOMA was also associated with NODAT independent of proinsulin (HR: 1.23 [1.09-1.40], p=0.001). Adjustments for factors of the MS (Model 6) slightly weakened the association, but proinsulin remained independently associated with NODAT. Waist circumference (HR: 1.02 [1.01-1.04], p=0.01), triglycerides (HR: 1.21 [1.05-1.40], p=0.01), and glucose (HR: 2.26 [1.63-3.11], p<0.001), were significantly associated with NODAT, independent of proinsulin.

Figure 1. Kaplan-Meier curve de novo diabetes in quartiles of Proinsulin tested with Log-rank test (P=< 0.001). Cut-off points for quartiles of Proinsulin were: 1st-3rd quartile: 2.4-24.4 (pmol/L), and 4th more than 24.5(pmol/L).



Evaluation of prognostic value of proinsulin is summarized in table 3. Harrell's c-index of discrimination improved from 0.71 [0.65-0.77] to 0.80 [0.75-0.85], (p<0.01) after adding proinsulin to a clinical prediction model including sex, smoking, waist circumference, hypertension and family history of diabetes (16). IDI analysis shows that the clinical risk score with proinsulin predicted NODAT more accurately than the clinical risk score alone. We found similar results when HOMA or glucose was added to the clinical model. When proinsulin was added on top of glucose, IDI was positive and remained significant. These results show that proinsulin is a promising biomarker for predicting NODAT beyond established clinical risk predictors in RTR.

Table 2. Proinsulin independently predicts NODAT in RTR

	Proinsulin	
Cox-regression	**HR [95% CI]**	**P-value**
Model		
1	2.29 [1.85-2.83]	<0.001
2	2.26 [1.83-2.81]	<0.001
3	2.43 [1.94-3.04]	<0.001
4	2.44 [1.95-3.04]	<0.001
5	1.76 [1.34-2.32]	<0.001
6	1.58 [1.25-1.99]	<0.001

Model 1: Univariate analyses
Model 2: Model 1 + adjustment for recipient age, sex
Model 3: Model 2 + adjustment for cyclosporine, tacrolimus and prednisolon dose
Model 4: Model 3 + adjustment for trough levels of cyclosporine and tacrolimus
Model 5: Model 2 + adjustment HOMA
Model 6: Model 2 + adjustment all components of the MS: waist circumference, triglycerides, HDL cholesterol, blood pressure and glucose concentration

Table 3. Additive value of proinsulin for the prediction risk of developing NODAT

	C-statistic (95% CI)	p-value for change in C-statistic	IDI	p value
Model 1	0.71 [0.65-0.77]	-	-	-
Model 1 + proinsulin	0.80 [0.75-0.85]	<0.01	0.077	<0.01
Model 1 + HOMA	0.77 [0.71-0.82]	<0.01	0.077	<0.01
Model 1 + glucose	0.78 [0.73-0.84]	<0.01	0.075	<0.01
Model 1 + glucose and proinsulin	0.80 [0.75-0.85]	0.06	0.046	<0.01
Model 1 + proinsulin and glucose	0.80 [0.75-0.85]	0.06	0.038	<0.01

Model 1: Clinical model including: sex, smoking, waist circumference, hypertension and family history of diabetes.

CONCLUSIONS

Our study shows that proinsulin is an independent predictor of NODAT in RTR. Proinsulin levels predicted development of NODAT in RTR, after adjustment for risk factors for NODAT. Our findings emphasize the importance of β-cell dysfunction in the pathophysiology of NODAT in RTR.

In our study, adjustment for MS (waist circumference, triglycerides, HDL cholesterol, blood

pressure and glucose concentration) attenuated the association of proinsulin with NODAT. This supports the notion that MS and particularly waist circumference, triglycerides and glucose, partly contributes to this association. Factors of the MS could be modified by regular physical activity and a healthy diet, showing the importance of lifestyle interventions after renal transplantation. The association between proinsulin and NODAT was independent of HOMA which suggests that the relationship is driven by β-cell dysfunction.

It has become clear that both insulin resistance and β-cell dysfunction are present early in the natural history of diabetes. There is a hyperbolic relationship between insulin sensitivity and insulin secretion that depends on a negative feedback loop. Pancreatic β-cells compensate for changes in insulin sensitivity (18,19). In healthy subjects plasma glucose levels are maintained near to normal, even with low insulin sensitivity, as a consequence of a compensatory increase in insulin secretion. RTR are more insulin resistant than the general population. Oterdoom et al showed that obesity, waist to hip ratio and prednisolon treatment are the predominant determinants of insulin resistance after transplantation (20). Furthermore, proinsulin can be used as a marker of pancreatic β-cell dysfunction. In this light, increased circulating levels of proinsulin are seen as a marker of β-cell stress when insulin demands required for maintenance of glycaemic control are relatively high for the prevailing β-cell capacity. This is accompanied by increased "spill-over" of proinsulin (5).

Calcineurin inhibitors impair insulin secretion, produce β-cell toxicity, cause insulin resistance and thereby contribute to an increased risk for NODAT (21-23). Various studies comparing cyclosporine and tacrolimus showed that use of cyclosporine is associated with a significantly lower incidence of NODAT than tacrolimus after renal transplantation (3,8,24). Interestingly, proinsulin levels tended to be lower in RTR receiving tacrolimus, despite the fact that use of tacrolimus was significantly associated with increased risk for NODAT development. Use of tacrolimus increased the risk for NODAT almost by three-fold (HR: 2.84 [1.37-5.89], p=0.005). This observation could point to the known interference of tacrolimus with insulin production by pancreatic β-cells at the level of synthesis rather than at the level of conversion of proinsulin to insulin (21-23). The low variation in steroid doses in the population we investigated did not allow for us to find a relationship between steroid dose and NODAT.

Based on the results from the UKPDS, it was suggested that β-cell dysfunction was reduced up to 50% at time of diagnosis (25). Loss of β-cell dysfunction starts many years before diagnosis. Therefore, proinsulin can be used to identify patients at risk for NODAT development several years later. Elevated levels of proinsulin can be present despite normal glucose values. Proinsulin can still bind to the insulin receptor and has a glucose-lowering effect of 10-20% compared to insulin (26). Because of this minor but evident effect, patients with β-cell dysfunction are not always diagnosed with diabetes mellitus. Although no cut-off points for proinsulin are described in the literature,

there is one study in which cut-off values are given for defining insulin resistance. Data from the IRIS-II (Study on Insulin Resistance and Insulin Sensitivity) show that elevated proinsulin levels (>10 pmpl/l) are a good indirect marker for insulin resistance (27). In our study 80% of the RTR had proinsulin levels above this threshold of >10 pmpl/l. This may reflect the fact that β-cell function is impaired in almost all RTR because of increased metabolic demands on the β-cells and the chronic exposure to immunosuppressive drugs which poses RTR at high risk for development of NODAT (2).

Sharif et al validated important insulin resistance indexes in RTR maintained on tacrolimus (28). Rodrigo et al analyzed the performance of two general population risk scores for prediction of diabetes in RTR (29). None of these three evaluations included a marker of β-cell dysfunction. Chakkera et al recently developed a pre-transplant risk score for the prediction of post transplant diabetes (30). Also in this study no marker of β-cell dysfunction was included. The performance of the risk score developed by Chakkera et al was modest with areas under receiver operating curves varying between 0.70 and 0.72. Our study is the first to include a marker of β-cell dysfunction in addition to insulin resistance indexes in the prediction of NODAT in RTR. In our analyses we found that both proinsulin as marker of β-cell dysfunction and HOMA as a marker for insulin resistance are independently associated with increased risk for NODAT. The prediction model with proinsulin had good discrimination, showing that 80% of the RTR were adequately classified as at risk for NODAT. Integrated discrimination improved with 8% by adding proinsulin to the model. Proinsulin is a promising marker for early detection of patients at risk for NODAT and possibly future studies may also identify it useful in the clinic to monitor β-cell function early after transplantation. Monitoring β-cell function could allow for early intervention and treatment strategies to preserve β-cell function. Interestingly, Hecking at al recently showed in a randomized controlled study that basal insulin therapy may be a good strategy to reduce HbA1c and decrease the incidence of NODAT, presumably by protection of the β-cells (31). Patients were randomized to immediate-postoperative isophane insulin (treatment group) or short-acting insulin and/or oral anti-diabetic agents (standard care). The treatment group had 73% lower odd for NODAT and HbA1c was 0.38 % lower than in the control group. Besides pharmacological strategies lifestyle, interventions could play an important role in prevention of NODAT beyond the first year after transplantation. Exercise training decreases insulin resistance and the risk of diabetes in the general population and it can be assumed that it has similar effects in RTR. Sharif et al. showed that lifestyle modification is beneficial for high-risk RTR with glucose intolerance. Intensive lifestyle modification (dietician, exercise program and weight loss advice) resulted in 15% improvement in 2-hr postprandial glucose (32). Lifestyle interventions targeting physical activity and diet after transplantation as well as individualized choice of immunosuppressive agents could help in the prevention of NODAT.

The strength of our study is its prospective design. RTR in this study were closely monitored through regular check-ups at our clinic, which gives complete information on patient status. Our

study population is a crosscut of all the RTR that visited our outpatient clinic, giving a variation of RTR with different times after transplantation, also including stable RTR late after transplantation. On the other hand, our study is limited by the heterogeneousness of our study population, with variable time post transplantation and immunosuppressant medication. The healthy survivor effect is another drawback of our study. We used multivariate Cox regression modelling to adjust for confounders of NODAT. This modelling cannot fully correct for the fact that RTR without NODAT late after transplantation may be a healthier group. Future studies could investigate whether proinsulin levels, measured earlier post-transplant also predict NODAT.

Proinsulin is strongly related to development of NODAT in RTR. Our results highlight the role of β-cell dysfunction in the pathophysiology of NODAT in RTR. Considering that the development of NODAT is associated with a higher risk of complications and worse survival, identifying RTR at increased risk for development of NODAT is needed. Proinsulin as a marker of β-cell dysfunction has potential value for identification of RTR at increased risk for NODAT. Prevention of NODAT by lifestyle interventions, early identification of patients at risk and choice of immunosuppressive medication are all important to control and manage NODAT in RTR.

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Alcohol consumption, new onset of diabetes after transplantation, and all-cause mortality in renal transplant recipients

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Abstract

Background: *Renal transplant recipients (RTR) are often advised to refrain from alcohol because of possible interaction with their immunosuppressive medication. While moderate alcohol consumption is associated with reduced risk of diabetes and mortality in the general population, this is unknown for RTR. Therefore, we investigated the association of alcohol consumption with new onset of diabetes after transplantation (NODAT), mortality and graft failure in RTR.*

Methods: *RTR were investigated between 2001-2003. Alcohol consumption was assessed by self-report. Mortality and graft failure was recorded until May 2009.*

Results: *600 RTR were studied (age 51±12 years, 55% men). Of these RTR, 48% were abstainers, 38% had light alcohol intake, 13% had moderate intake and 1% were heavy consumers. Moderate alcohol consumption was associated with a lower risk of developing NODAT over the follow-up period than was abstention (OR=0.36 [0.2-0.6], P=<0.001). During follow-up for 7.0[6.2–7.5] years, 133 recipients died. In Cox-regression analyses moderate alcohol consumption was associated with lower mortality than was abstention (HR=0.40 [0.2-0.8], P=0.009). Adjustment for confounders, including age and smoking did not materially change this association. No association was found between alcohol consumption and graft failure.*

Conclusions: *Moderate alcohol consumption is associated with low prevalence of NODAT and reduced risk for mortality in RTR, in line with findings in the general population. These findings refute the common advice to refrain from alcohol in RTR.*

INTRODUCTION

Renal transplantation is the treatment of choice for patients with end-stage renal disease and allows freedom from lifestyle restrictions such as a strict diet (1). Short-term outcome after renal transplantation has improved substantially in the past decades, but long-term graft and patient survival have not improved in a similar manner (2). One main reason for persisting poor long-term outcome is premature death due to cardiovascular disease (CVD), with incidence of CVD estimated to be 4-6 times higher in renal transplant recipients (RTR) than in the general population (3).

Interventions targeting modifiable risk factors such as hypertension, dyslipidemia, and physical activity, can improve outcome in RTR (4-6). Moderate alcohol consumption could be a behavioral lifestyle factor for lowering of risk of premature death, because numerous studies in the general population have demonstrated that moderate alcohol intake (1-3 units per day) is associated with reduced risk of diabetes, mortality and CVD compared to abstainers and sporadic users, while there is again an increased risk with intake above 3 units per day (7).

The KDIGO Practice Guideline for the Care of Kidney Transplant Recipients does not mention specific alcohol restrictions for RTR (8). Accordingly, transplantation centers usually do not specifically advise RTR to refrain from post-transplantation alcohol use. Advice on the internet is, however, different. RTR are advised not to use alcohol for one year after transplantation, to avoid alcohol unless the doctor gives permission, or not to use any alcohol at all (9,10) .

One study showed that alcohol abuse is rare after kidney transplantation and that intake among RTR is generally low (11). No data are available on the relation between post-transplant alcohol use and long-term outcome.

Therefore, we aimed to investigate the prevalence and correlates of alcohol consumption in RTR in a large single-center cohort. Furthermore, we aimed to investigate whether alcohol consumption is associated with new onset of diabetes after transplantation (NODAT), all cause mortality and graft survival in these patients.

MATERIALS AND METHODS

Research Design and Subjects

The Institutional Review Board approved the study protocol (METc 2001/039), which was incorporated in the outpatient follow-up of the Groningen Renal Transplant Program. The outpatient follow-up constitutes a continuous surveillance system in which patients visit the outpatient clinic with declining frequency, in accordance with American Transplantation Society guidelines, i.e. ranging from twice a week immediately after hospital discharge to twice a year long-term after

transplantation (12). All adult allograft recipients between August 2001 and July 2003 who survived with a functioning allograft beyond the first year after transplantation (1 year post transplant was considered baseline) were eligible to participate at their next visit to the outpatient clinic (index date). The group that did not sign informed consent was comparable with the group that did sign informed consent with respect to age, sex, body mass index, serum creatinine, creatinine clearance, and proteinuria. In patients with fever or other signs of infection (e.g. complaints of upper respiratory tract infection or urinary tract infection), baseline visits were postponed until symptoms had resolved. Patients with overt congestive heart failure and patients diagnosed with cancer other than cured skin cancer were not considered eligible for the study. A total of 606 out of 847 eligible renal transplant recipients signed written informed consent. Information on alcohol consumption was available in 600 patients. Alcoholism is considered a contraindication for transplantation in our center.

Endpoints of the study

The primary endpoints of this study were recipient mortality and graft failure. Graft failure was defined as a return to dialysis or re-transplantation. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of death. We contacted general practitioners or referring nephrologists if the status of a patient was unknown. Mortality and graft loss were recorded until May 2009. Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9) (13). Cardiovascular death was defined as death in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 410-447. There was no loss due to follow-up. NODAT was defined by fasting plasma glucose concentration was ≥7.0 mmol/l and/or use of anti-diabetic medication (14).

Renal Transplant Characteristics

Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. Current medication was taken from the medical record. Smoking status and cardiovascular history were obtained using a self-report questionnaire. Cardiovascular disease history was considered positive if participants had a myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA).

Measurements and definitions

Quantitative information on alcohol consumption was obtained by self-report questionnaire.

Accordingly RTR were categorized into 4 groups of alcohol consumption (15): abstainers, light consumption (up to 10 gram per day), moderate consumption (10-30 gram per day) and high alcohol consumption (more than 30 gram per day). BMI, waist circumference and blood pressure were determined as described previously (13). Metabolic Syndrom (MS) was defined by the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII) as described earlier (13).

Blood was drawn after an overnight fasting period. We measured serum creatinine, serum triglycerides, total cholesterol, high-density lipoproteins (HDL) cholesterol, plasma glucose and HbA1c as described previously. Low-density lipoproteins (LDL) cholesterol was calculated using the Friedewald formula (16). Serum high sensitive C-reactive protein (hsCRP) was assessed with a high sensitivity CRP ELISA assay as described before (17). We performed uniform measurement of Gammaglutamyltransferase (GGT), asparaat-amino-transferase (ASAT), alanine-amino-transferase (ALAT), Alkaline phosphatase (AP) and lactaatdehydrogenase (LDH) activity in serum as described before.

Creatinine clearance was calculated from 24-hour urinary creatinine excretion and serum creatinine. Total urinary protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany) and proteinuria was defined as urinary protein excretion ≥0.5 g per 24 hours.

Statistical analysis

Data were analyzed with SPSS version 16.0 (SPSS Inc., Chicago, IL) and GraphPad Prism version 4.03 (GraphPad Software Inc., San Diego, CA). Normally distributed variables are expressed as mean ± standard deviation, whereas skewed distributed variables are given as median (25^{th}-75^{th} percentile), percentages were used to summarize categorical variables. Log-transformation was used for variables with a skewed distribution. Hazard ratio's (HR) are reported with 95% confidence interval [95% CI].

We analyzed recipient-related characteristics separately for the four categories of alcohol consumption. Student's *t*-test or Kruskal Wallis test was used to compare means for continuous variables and with Chi-square for categorical variables. Logistic regression analyses were used to analyze the association between alcohol consumption and risk for NODAT, for this analyses 17 RTR with pre-transplantation diabetes at baseline were excluded. To analyze whether alcohol consumption is associated with mortality and graft failure, we first performed Kaplan-Meier analyses with a Log-rank test. Univariate and multivariate Cox-regression analyses were performed to investigate whether alcohol consumption is independently associated with mortality and graft failure. Each baseline group of alcohol consumption was compared with the group of abstainers as reference group.

RESULTS

A total of 600 RTR were studied (mean age 51±12 years, 55% men). Baseline characteristics according to alcohol consumption are shown in table 1. Of the 600 patients providing information on alcohol use, 288 (48%) were abstainers, 226 (38%) had light intake (< 10 gram per day), 78 (13%) had moderate intake (10-30 g per day) and 8 (1%) – all men – had high intake (>30 g per day).

Moderate alcohol consumers were more likely to have a shorter dialysis time, a living donor and higher LDL-cholesterol. Alcohol consumption was positively associated with male gender, smoking and past smoking, diastolic blood pressure and use of statins and serum creatinine concentrations in men. There was an inverse association between alcohol consumption and prevalence of MS, fasting insulin concentrations and prevalence of NODAT. Total prevalence of NODAT was 125 (21%). Results of univariate and multivariate logistic regression analyses for alcohol consumption and risk for NODAT are shown in table 2. Moderate alcohol consumption was strongly associated with low prevalence of NODAT (Odds ratio= 0.37 [0.17-0.78], P=0.008). A total of 385 RTR (64%) fulfilled the criteria of MS. Prevalence of MS decreased according to increasing alcohol consumption (P= 0.04).

Table 1. Characteristics in groups of alcohol consumption

	Abstainers	<10 g per day	10-30 g per day	>30 g per day	P-value
	N=288	N=226	N=78	N=8	
Recipient demographics					
Age, yr	53 ± 13	50 ± 12	52 ± 9	50 ± 11	0.1
Male, n (%)	105 (36)	226 (67)*	65 (83) *	8 (100) *	<0.001
Being unfit to work, n (%)	78 (27)	62 (27)	21 (27)	2 (25)	0.9
(Pre)transplant history					
Dialysis time, months	29 [14-50]	28 [13-52]	21 [9-33] *	26 [20-38]	0.01
Time between ntx and inclusion , yr	6.0 [3-12]	6.1 [3-11]	5.8 [3-11]	2.6 [1-9]	0.5
Living donor, n (%)	29 (10)	37 (16)	16 (21)	1 (13)	0.06
Acute rejection a, n (%)	121 (42)	106 (47)	40 (51)	5 (63)	0.3
Cardiovascular Disease History					
Myocardial Infarction, n (%)	27 (9)	14 (6)	6 (8)	1 (13)	0.5
TIA/CVA, n (%)	16 (6)	15 (7)	2 (3)	0 (0)	0.5
Substance use					
Current smoking, n (%)	54 (14)	55 (24)	20 (26)	4 (50)	0.08
Past smoking, n (%)	105 (37)	102 (45)*	43 (55)*	3 (38)	0.02
History and current smoking, n (%)	159 (55)	157 (69)*	63 (81)*	7 (88) *	<0.001

	Abstainers	<10 g per day	10-30 g per day	>30 g per day	P-value
	N=288	N=226	N=78	N=8	
Body composition					
BMI, kg/m2	26 ± 5	26 ± 4	25 ± 4	26 ± 2	0.3
Waist circumference, men (cm)	100 ± 13	100 ± 13	99 ± 12	100 ± 10	0.9
Waist circumference, women (cm)	95 ± 15	94 ± 15	86 ± 10	-	0.1
Blood pressure					
Systolic BP, mmHg	153 ± 24	153 ± 21	152 ± 22	166 ± 17	0.5
Diastolic BP, mmHg	89 ± 10	90 ± 9	90 ± 9	104 ± 5*	0.001
Use of antihypertensive drugs, n (%)	246 (85)	201 (89)	69 (88)	8 (100)	0.4
Lipids					
Total cholesterol, mmol/l	5.5 ± 1.0	5.6 ± 1.2	5.7 ± 1.0	5.8 ± 0.7	0.5
HDL cholesterol, mmol/l	1.1 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.3 ± 0.6	0.2
LDL cholesterol, mmol/l	3.5 ± 0.9	3.6 ± 1.1	3.7 ± 1.0	3.3 ± 0.7	0.07
Triglycerides, mmol/l	2.0 [1.4-2.8]	1.9 [1.3-2.5]	1.8 [1.4-2.3]	2.4 [1.8-3.6]	0.1
Statins, n (%)	145 (50)	102 (45)	41 (53)	8 (100)*	0.02
Glucose homeostasis					
Glucose, mmol/l	4.9 ± 1.4	4.8 ± 1.5	4.8 ± 1.1	4.7 ± 0.5	0.9
Insulin, (μmol/L)	12.0 [9-17]	10.6 [8-15]*	9.1 [7-13]*	9.0 [6-15]	0.001
HbA1c, %	6.6 ± 1.1	6.5 ± 1.0	6.5 ± 1.1	6.3 ± 0.8	0.5
NODAT, n (%)	73 (25)	42 (19)	9 (12)*	1 (13)	0.03
Pre transplant diabetes, n (%)	13 (5)	12 (5)	3 (4)	0 (0)	0.8
Metabolic Syndrome n, (%)	196 (68)	145 (64)	40 (51)*	4 (50)	0.04
Inflammation					
CRP, mg/l	2.2 [1-5]	1.9 [1-5]	1.8 [1-4]	1.0 [0-3]	0.2
Immunosuppressive medications					
Daily prednisolone dose, mg/day	10.0 [8-10]	10.0 [8-10]	10.0 [8-10]	10.0 [8-10]	0.3
Calcineurin inhibitors, n (%)	224 (78)	183 (81)	56 (72)	7 (88)	0.3
Proliferation inhibitors, n (%)	205 (71)	170 (75)	62 (79)	7 (88)	0.3
Renal allograft function					
Serum creatinine, Men (μmol/l)	137 [119-171]	146 [125-177]	144 [124-182]	166 [145-214]*	0.03
Serum creatinine, Women (μmol/l)	119 [99-149]	127 [97-156]	127 [106-155]	-	0.7
Creatinine clearance, ml/min	60 [45-75]	60 [47-76]	66 [51-81]	58 [45-76]	0.2
Proteinuria, g/24h	0.2 [0.0-0.5]	0.3 [0.1-0.6]	0.3 [0.1-0.5]	0.4 [0.2-0.8]	0.2

	Abstainers	<10 g per day	10-30 g per day	>30 g per day	P-value
	N=288	N=226	N=78	N=8	
Liver function					
GGT, U/L Men	25 [17-38]	24 [19-38]	26 [18-42]	33 [22-49]	0.4
GGT, U/L Women	24 [17-40]	23 [16-35]	22 [20-34]	-	0.6
ASAT, U/L	23 [19-28]	22 [19-26]	23 [18-27]	21 [18-29]	0.8
ALAT, U/L	17 [13-23]	29 [14-25]	19 [14-26]	22 [14-26]	0.8
AP, U/L	74 [60-96]	71 [55-94]	72 [57-84]	65 [50-102]	0.4
LDH, U/L	260 [233-310]	261 [224-297]	250 [228-286]	266 [228-290]	0.4

Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t-test or Kruskal Wallis test for continuous variables and with Chi-square for categorical variables. *Group significantly different from reference group (abstainers), P <0.05. Time between ntx and inclusion, yr, time between transplantation and inclusion, years; a Acute rejection treatment with high dose corticosteroids. TIA, transient ischemic attack; CVA, cerebrovascular accident; BMI, body mass index; BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; NODAT, new onset of diabetes after transplantation; CRP, C-reactive protein; GGT; gammaglutamyltransferase; ASAT, asparaat-amino-transferase; ALAT, alanine-amino-transferase; AP; alkaline phosphatase LDH, lactaatdehydrogenase;

Table 2. Logistic regression analyses for NODAT according to groups of alcohol consumption

	Alcohol consumption						
	Abstainers	<10 g per day		10-30 g per day		>30 g per day	
	N=278	N=219		N=78		N=8	
	Reference	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value
Model 1	1.0	0.67 [0.43-1.02]	0.06	0.37 [0.17-0.78]	0.008	0.40 [0.05-3.31]	0.4
Model 2	1.0	0.76 [0.48-1.21]	0.25	0.41 [0.19-0.90]	0.026	0.51 [0.06-4.39]	0.5
Model 3	1.0	0.78 [0.49-1.23]	0.30	0.42 [0.19-0.93]	0.03	0.55 [0.06-4.70]	0.6
Model 4	1.0	0.72 [0.44-1.17]	0.20	0.43 [0.19-0.97]	0.04	0.57 [0.07-4.91]	0.6

Model 1: Crude model

Model 2: Model 1 + adjustments for age and sex

Model 3: Model 2 + current smoking and past smoking

Model 4: Model 3 + BMI

During follow-up for 7.0 [6.2 – 7.5] years, 133 RTR died and 52 RTR suffered graft failure necessitating their return to dialysis. In the group with moderate alcohol consumption 9 (12%) RTR died during follow-up, whereas this number was 75 (26%) for the group with no consumption, 47 (21%) in the group with light consumption (Log-rank test P=0.02, Figure 1a), and 2 (25%) in the group with high alcohol consumption. Results of univariate and multivariate Cox-regression analyses for associations of alcohol consumption with mortality are presented in table 3. Moderate alcohol

Table 3. Cox regression analyses for mortality according to groups of alcohol consumption

	Alcohol consumption						
	Abstainers	<10 g per day		10-30 g per day		>30 g per day	
	N=288	N=226		N=78		N=8	
	Reference	HR [95%CI]	P-value	HR [95%CI]	P-value	HR [95%CI]	P-value
Model 1	1.0	0.76 [0.52-1.10]	0.15	0.40 [0.20-0.79]	0.009	0.93 [0.25-3.77]	0.9
Model 2	1.0	0.85 [0.58-1.25]	0.42	0.44 [0.22-0.91]	0.026	1.42 [0.34-5.93]	0.6
Model 3	1.0	0.74 [0.50-1.08]	0.12	0.38 [0.19-0.78]	0.008	1.17 [0.28-4.97]	0.8
Model 4	1.0	0.74 [0.50-1.09]	0.15	0.39 [0.19-0.80]	0.010	1.20 [0.28-5.08]	0.8
Model 5	1.0	0.75 [0.51-1.10]	0.14	0.42 [0.21-0.88]	0.021	1.21 [0.29-5.12]	0.8
Model 6	1.0	0.74 [0.50-1.09]	0.23	0.44 [0.21-0.90]	0.025	1.19 [0.28-5.05]	0.8

Model 1: Crude model

Model 2: Model 1 + adjustments for age and sex

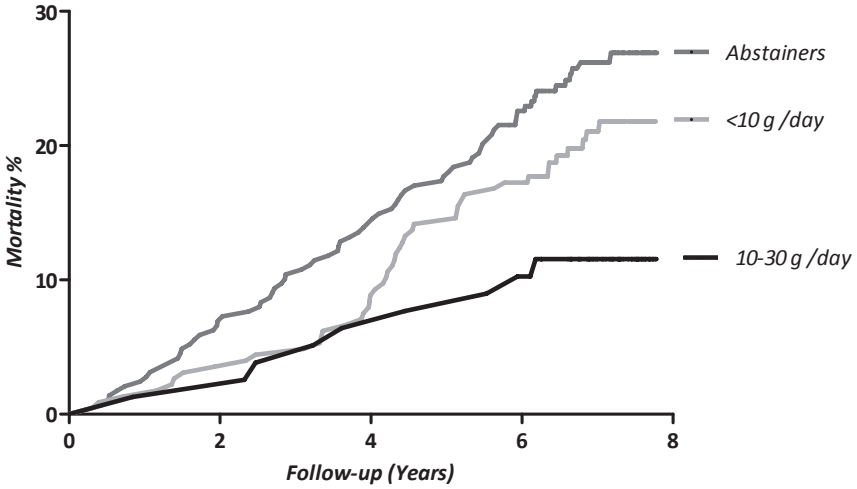
Model 3: Model 2 + adjustments for current smoking and past smoking

Model 4: Model 3 + adjustments for BMI, statin use, and LDL-cholesterol

Model 5: Model 4 + adjustments for living donor and dialysis time

Model 6: Model 5 + adjustments for serum creatinine

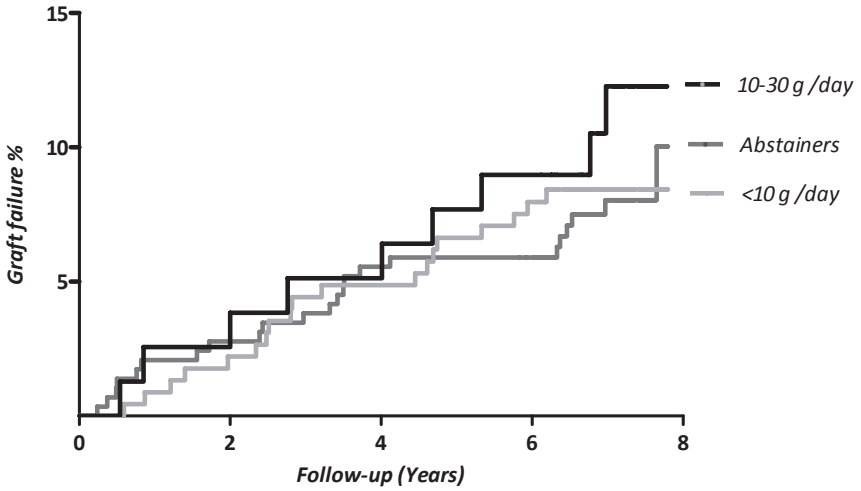
Figure 1a. Kaplan Meier curves of mortality to alcohol consumption, tested with Log-rank test (P= 0.02).



consumption was strongly associated with reduced risk for mortality (Model 1) in univariate analyses (HR=0.40 [0.2-0.8], P=0.009). Upon multivariate analyses, these associations weakened after adjustment for age and sex (Model 2). Adjustment for current smoking and past smoking

strengthened the association (Model 3). Further adjustments for BMI, use of statins and LDL-cholesterol (Model 4) living donor and dialysis time (Model 5) and serum creatinine (model 6) did not materially changed the association.

Figure 1b. Kaplan Meier curves of graft loss according to alcohol consumption, tested with Log-rank test (P= 0.6).



In the group with moderate alcohol consumption 9 (12%) RTR developed graft failure during follow-up, whereas this number was 23 (8%) for the group of no alcohol consumption and 19 (8%) for the group with light alcohol consumption (Log-rank test P=0.6, Figure1b). In the group with high alcohol consumption 1 (12.5%) RTR developed graft failure. In a univariate Cox-regression analysis moderate alcohol intake was not associated with graft failure (HR =1.44 [0.7-3.1], P=0.4).

DISCUSSION

This is the first study to report on habitual alcohol consumption, NODAT and all cause mortality in RTR. It suggests that moderate alcohol consumption has protective effects in RTR that correspond to those in the general population, despite differences in disease burden and medication use. In particular, moderate alcohol consumption is associated with lower risk for NODAT and all-cause mortality. No association was found between alcohol consumption and graft loss.

The prevalence of alcohol consumption in our study population (52%) is similar to the prevalence and severity of alcohol consumption in RTR reported by Fiertz (11), and lower than in the general population, where alcohol consumption is approximately 89% in men and 74% in women (18). This

suggests that RTR limit alcohol consumption, either spontaneously or as a consequence of advice. In agreement with guidelines, we do not actively advise RTR to refrain from post-transplantation alcohol use in our center. On the other hand, advice on the internet does (9,10) and there may be a general tendency for patients and physicians to adhere to this. Reasons for advising against alcohol consumption could be several. Due to the immunosuppressive regime, transplant doctors are often cautious with advice on alcohol consumption. It may for instance be thought that great amounts of alcohol interfere with liver metabolism, in addition to calcineurin inhibitors, such as cyclosporine, stressing the liver. Another reason may be the data provided by the only other prospective study of the effects of alcohol consumption in RTR. In this study, Gueye et al. analyzed renal graft and patient survival in 425 RTR with alcohol dependence before transplantation compared to 60 523 RTR without alcohol dependence (19), rather than average daily intake after transplantation, as we did. They concluded that alcohol dependence before transplantation is a risk factor for renal graft failure and death and advised against use of alcohol in RTR. Our study suggests that average daily intake of alcohol after transplantation cannot be compared to alcohol dependence before transplantation.

New onset diabetes is a common complication of transplantation and a major risk factor for graft failure and mortality in RTR (20,21). Traditional risk factors like obesity, diabetes mellitus, dyslipidemia and hypertension are often seen in transplantation patients and cluster in the MS (22). Our data confirm this with a prevalence MS of 64%.

Although there is no other literature addressing alcohol consumption and long-term outcome in RTR, the inverse association between alcohol consumption and CVD in the general population is well documented. Moderate alcohol consumption is associated with a decreased risk for myocardial infarction (23,24), heart failure (25,26) and ischemic stroke (27-29). It also reduces the risk of myocardial and all-cause mortality (30-34). Moderate alcohol consumption is also associated with a decreased risk of developing type 2 diabetes (35,36). Our study shows that moderate alcohol consumption is associated with 60% decreased risk for all cause mortality, compared to the group of abstainers. The risk reduction of moderate alcohol consumption in the general population is estimated to be between 20-30%. The magnitude of risk reduction seems to be higher in RTR. This is in line with a previous study which showed larger risk reductions for those with an increased cardiovascular risk (37).

There is little known about the influence of alcohol consumption on renal function and graft survival and results are inconsistent. Studies in general population cohorts suggest no adverse effects of alcohol consumption and even a protective effect of alcohol consumption on renal function (38,39) has been described. Other studies suggest a negative link between alcohol intake and kidney function. In an Australian population-based study all alcohol intake of ≥30 g/day was independently associated with an increased risk of albuminuria (40). Another population based study (41) showed a 4-fold increased risk of end-stage renal disease in subjects who consumed

≥2 units alcohol per day. It has been argued that the pressor effect of alcohol might increase the risk for renal disease (42). Indeed, we found evidence for a decreased renal function in the group with high alcohol consumption in our study. Serum creatinine was significantly higher in the group with high alcohol consumption and there was a trend for a lower creatinine clearance and higher proteinuria with high alcohol consumption. Systolic blood pressure was also significantly higher in the RTR with high alcohol consumption. Although we found no significant association between alcohol consumption and graft failure, this is possibly the consequence of the group being very small, increased systolic blood pressure and increased urinary protein excretion are consistent with an adverse effect of heavy alcohol consumption on graft function.

We anticipated that a potential association of moderate alcohol consumption with mortality could be confounded by other risk factors for mortality or a healthier lifestyle. We found, however that the association between alcohol consumption and all-cause mortality was not materially affected by adjustments for current smoking and past smoking, BMI and LDL-cholesterol. Tobacco use is an important confounder in the relationship between alcohol and mortality. Tobacco use is often greater in people who drink alcohol (43). History of smoking and current smoking in our population increased from 159 (55%) to 157 (69%), 63 (81%) and 7 (88%) according to increasing alcohol consumption (P< 0.001). Studies showed that smoking has a major negative impact on mortality and graft survival in RTR (44,45). The strengthening of the association after adjustment for smoking (Table 2, Model 3) suggests that alcohol consumption has a stronger protective effect if it is not combined with smoking. It has been argued that moderate drinkers may represent a relatively healthy subpopulation, with a general healthier lifestyle, related to the fact that people in a poor overall condition are less likely to consume alcohol, which may explain a small proportion of the effect of alcohol consumption on mortality (46). Various studies have removed the effect of so called 'sick quitters' by taking lifelong abstainers as reference group and looked at the effects independent of other healthy lifestyle factors, showing that moderate alcohol consumption is causally related to a lower risk for cardiovascular diseases (47).

There is substantial evidence to support a causal relationship between alcohol consumption and CVD in the general population (48). The protective effect of moderate alcohol consumption can be explained by several mechanisms. An important way by which alcohol consumption can modulate the CVD risk is by changes in lipid profile. Moderate alcohol consumption is associated with higher HDL-cholesterol and apolipoprotein A1 levels (49,50). Alcohol also has been shown to decrease platelet aggregation and lower concentrations of plasma fibrinogen (51,52). Another important mechanism that could modulate CVD risk is by lowering insulin resistance. Moderate alcohol consumption increases the insulin sensitivity and thereby lowers the risk for developing diabetes (35,53,54). The same mechanisms could be relevant to reduce mortality risk in RTR. Our results indicate that RTR with moderate alcohol consumption had lower insulin levels and lower

prevalence of diabetes, implicating a possible effect of alcohol on insulin sensitivity. Moderate consumers also had higher levels of LDL-cholesterol and lower triglycerides (borderline significant). The small differences in lipid levels could partly be explained by the frequent use of statins among our RTR.

The strength of our study is its prospective design. RTR in this study were closely monitored by regular check-ups in our clinic, which give complete information on patient status. Some limitations of our study warrant consideration. First, this study relied on self-reported data. Recall bias and social desirability bias are unfortunately unavoidable and could influence internal validation. Self-reported alcohol intake is generally under reported (55). However, studies have found that questionnaires provide enough validity for ranking participants on alcohol consumption (56). Second, we acquired information on average daily intake with no details on drinking patterns. The cardio protective effect occurs in regular drinkers and not in binge and irregular heavy drinkers (57). Moreover, our study used a single measurement of alcohol consumption and did not take into account any changes in alcohol consumption over time. Finally, it was a single center study in a predominantly Caucasian population. In our study prevalence of diabetes and BMI were relatively low. The contribution of diabetes mellitus to end-stage renal disease in the Netherlands as compared its surrounding countries is relatively low (58-60). This may at least in part explain the low prevalence of diabetes in our study. Similarly, the relatively low BMI may be a reflection of the relatively low contribution of type 2 diabetes to end-stage renal disease in our population.

This study shows that moderate alcohol consumption is inversely associated with NODAT and all cause mortality in RTR. So, in contrast to common belief, drinking moderate amounts of alcohol does not appear to be detrimental and may be protective against diabetes and mortality in RTR, similar to the general population, despite differences in disease burden and medication use. Further research is needed to confirm these findings and to investigate the relationship between alcohol consumption and graft survival.

Acknowledgments

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5

The role of diet and physical activity in post transplant weight gain after renal transplantation

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Abstract

Background: *Long-term survival of renal transplant recipients (RTR) has not improved over the past 20 years. The question rises to what extent lifestyle factors play a role in post-transplant weight gain and its associated risks after transplantation.*

Methods: *26 RTR were measured for body weight, body composition, blood lipids, renal function, dietary intake and physical activity at 6 weeks, and 3, 6 and 12 months after transplantation.*

Results: *Weight gain ranged between -2.4 kg to 19.5 kg, and was largely due to increase in body fat. RTR who remained body fat stable, showed more daily physical activity (P=0.014), tended to consume less energy from drinks and dairy (P=0.054), consumed less mono-and disaccharides (sugars) (P=0.021) and ate more vegetables (P=0.043) compared to those who gained body fat. Gain in body fat was strongly related to total cholesterol (r=0.46, P=0.017) and triglyceride (r=0.511, P=0.011) at one year after transplantation.*

Conclusions: *Gain in adiposity after renal transplantation is related to lifestyle factors such as high consumption of energy-rich drinks, high intake of mono- and disaccharides and low daily physical activity. RCT's are needed to investigate potential benefits of lifestyle intervention on long-term morbidity and mortality.*

INTRODUCTION

Care and treatment regarding transplantation and avoiding rejection have considerably improved over the last decades, leading to more than 95% survival in the first year after transplantation. In contrast, long-term (graft) survival rates after the first year of transplantation have hardly improved (1,2). The increased cardiovascular risk as a consequence of post-transplant weight gain plays an important role. Weight gain after transplantation is a common and worrisome problem (3,4). About 15 years ago it was already known that in the US about 50% of renal transplant recipients (RTR) showed a gain in body weight of over 10% after transplantation (4).

Post transplant weight gain is associated with important health risks (1). Patients with obesity (BMI >30 kg/m^2) at the time of transplantation have increased mortality and lower graft survival compared to patients with a normal BMI (1). One year posttransplant BMI and BMI increment were more strongly related to death and graft failure than pretransplant BMI (5).

It is well recognized that weight gain relates to a clustering of metabolic risk factors including abdominal obesity, elevated triglycerides, reduced high-density lipoproteins (HDL) cholesterol, hypertension and hyperglycaemia, also called the metabolic syndrome, and that the presence of the metabolic syndrome is related to cardiovascular mortality. The metabolic syndrome is often present after transplantation (6), and is likely to become worse due to post-transplant weight gain. Importantly, metabolic syndrome also increases the risk for a deterioration of kidney function. In addition to the weight gain, other factors such as age, the use of certain immune suppressants like tacrolimus, and smoking at the time of transplantation are related to cardio-metabolic complications and graft failure after transplantation (7,8).

With the current increase in obesity and type 2 diabetes, the causes for weight gain and subsequent prevention measures have gained interest (3). Within the transplant population, some determinants for weight gain are known, such as the use of corticosteroids (9). However, medication only partly explains post-transplant weight gain (10,11). We hypothesized that lifestyle factors such as diet and physical activity play an important role in post transplant weight gain. Therefore we aimed to describe the course of post transplant weight gain and to determine the risk factors associated with weight gain. Next, we aimed to identify the most important lifestyle determinants of post-transplant weight gain to provide targets for intervention and prevention.

SUBJECTS AND METHODS

Design and subjects

A total of 119 RTR were included in this observational cohort study. All patients who visited our outpatient clinic between December 2007 and December 2008 were eligible to participate in the

study. Exclusion criteria included auto-immune disease, malignancies with the exception of skin malignancies, and combined transplantations. From this larger cohort, 29 RTR participated in more detailed assessments of dietary intake and daily physical activity.
Measurements at the time of transplantation were derived from patient records. Measurements took place 6 weeks, 3, 6 and 12 months post-transplant. Standard immunosuppressive treatment consisted of the following: cyclosporine microemulsion (Neoral, Novartis Pharma B.V., Arnhem, The Netherlands); mycophenolate mofetil (Cellcept, Roche B.V., Woerden, The Netherlands) and prednisolone. The study was performed according to the declaration of Helsinki, and all participants signed informed consent.

Measurements

Body weight, body composition

Height and weight at the time of transplantation were retrieved from medical records. During follow-up after transplantation, RTR were weighed wearing light clothing with a standardized scale and waist circumference was measured at the level midway between the lowest rib and the iliac crest. Single frequency bio-electrical impedance assessment (BIA, Bodystat 1500, Onchan, UK) was used to estimate body composition (fat free mass, body fat percentage and water content).

Cardio-metabolic risk factors

Serum triglycerides, total cholesterol, HDL-cholesterol, low-density lipoproteins (LDL) cholesterol and glucose were assessed by routine laboratory measurements as described previously (12). Blood pressure was measured after a 6-minute rest in supine position as the average of three automated measurements at 1-minute intervals (Omron M4; Omron Europe).

Graft function and urinary analyses

Serum and urine creatinine concentration were analyzed with a photometric modification with the Jaffe´ method. Creatinine clearance was calculated from 24-hour urinary creatinine excretion and serum creatinine and proteinuria was defined as urinary protein excretion ≥0.5 g/24 h. Renal hemodynamics including glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and filtration fraction (FF) were assessed using clearances of 125I-iothalamate and 131I-hippurate as previously described (13,14).

Lifestyle factors

Dietary intake and daily physical activity were assessed in detail at 6 weeks, and 3, 6 and 12 months after transplantation. To assess habitual food intake, a 24-h recall and dietary history were used. Macro- and micronutrient intake were calculated based on the Dutch Food Composition Database

(NEVO 2006). Collections of 24h urine were used to evaluate protein and sodium intake. Protein intake was calculated by Maroni's formula. Sodium intake was estimated from 24h sodium excretion. Daily physical activity was objectively measured using the SenseWear® (Body Media Inc., Pittsburg, USA). The SenseWear® contains a biaxial accelerometer with additional sensors to detect skin contact to register when and how long the SenseWear® was worn. Only measurements with more than 48 hours of valid recording were included. Habitual physical activity and sports activities were assessed using the validated SQUASH questionnaire (15) and the score was calculated by multiplying duration (minutes) and MET-score. The reported intakes were compared to the Dutch Guidelines for a Healthy Diet 2006. These guidelines indicate sufficient vegetable intake as > 200g/day, 200 g or two pieces of fruit per day, saturated fat intake <10 Energy %, and sodium intake below 2.4 g per day. For physical activity, it is recommended to take on average 10,000 steps per day (16).

Statistical analyses

Participants were divided into two groups: those who remained relatively body fat stable in the first year after transplantation (change in body fat below median, ≤3% increase, N=13) and those who gained body fat mass (more than 3% body fat gain, N=13). Group means were compared using a nonparametric test (Mann-Whitney) because normality is uncertain in groups with a low number of observations. Differences in frequency were analysed by Chi-square test. Changes in time were explored using repeated measures ANOVA and paired sample T-test. With regard to the data for dietary intake, no clear time trends were found. Therefore, the mean of these four measurements was taken to characterize habitual dietary intake of the participant. Regarding the limited number of participants, correlations are presented as the Spearman correlation coefficient. Results are given as mean ± SD. Statistical analysis was performed using SPSS 17.0.

RESULTS

The RTR who participated in these lifestyle examinations were of the same age, and had comparable gender distribution, BMI at hospital discharge, and comparable cholesterol at one year after transplantation as other patients (N = 90, data not published) who received a transplant in the same period. Dialysis duration was shorter (21 ± 23 vs 34 ± 28 months, p = 0.04) and renal function was better (GFR, 60 ± 15 vs 52 ± 18; ERPF, 224 ± 48 vs 196 ± 46, p = 0.03; no difference FF). Although these patients may have been slightly more healthy at the start of the investigations compared to the total transplant population, it is a representative sample with regard to metabolic profile and body weight.

In table 1, the general characteristics and transplantation history of the RTR are presented for

all participants as well as for those who gained fat mass and those who remained body fat stable. No differences were observed with regard to age between the body fat stable and body fat gain group. Two individuals stopped for motivational reasons, of which one completed more than one measurement and is included in the analysis when applicable. One individual died, none had allograft loss.

Table 1. Baseline characteristics.

	All N=29	BF stable N=13	BF gain N=13	P-value
General				
Age (years)	51.4 ± 12.0	51.9 ± 6.3	49.0 ± 16.1	0.80
Male gender, n (%)	48%	46%	46%	1.00
BMI at time of transplantation (kg/m2)	25.8 ± 3.8	26.6 ± 4.2	25.2 ± 3.5	0.45
Use of ACE-inhibitor, n (%)	31%	23%	39%	0.40
Use of β-blocker, n (%)	61%	46%	77%	0.11
Transplant history				
Pre-emptive transplantation, %	21%	8%	31%	0.27
Hemodialysis, %	38%	38%	38%	0.27
Hemodialysis, time in months	28 ± 19	25 ± 17	36.0 ± 20	0.55
Peritoneal dialysis, %	42%	54%	31%	0.27
Peritoneal dialysis, time in months	25 ± 26	23 ± 31	30 ± 22	0.41
Donor type (LR / LUR / HB / NHB) (%)	11/9/6/3	4/5/2/2	6/3/4/0	a

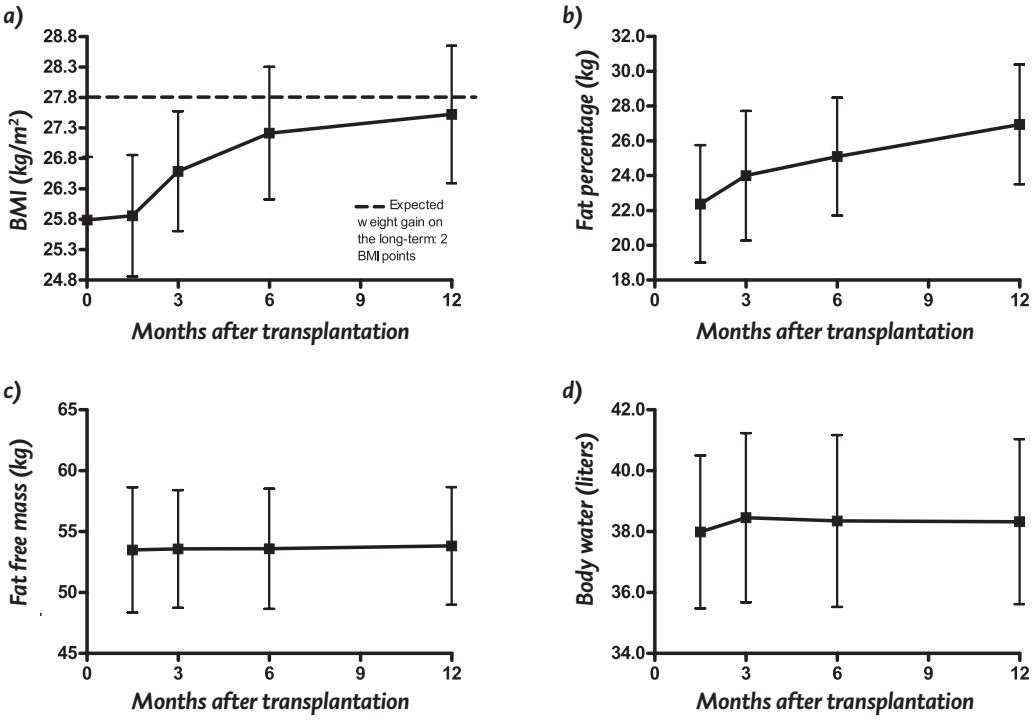
Data are presented as mean ± SD or as % (N). BF, body fat.

a. group sizes not fit for statistical Chi^2 testing.

In the year after transplantation, body weight gain ranged from -2.4 kg to +19.5 kg. After one year, 38% of participants had a BMI > 25 kg/m^2, most of whom were obese (26%, BMI > 30 kg/m2). On average, weight gain after transplantation was 5.7 ± 5.0 kg, and BMI increased on average with 7.9% (2 kg/m^2) in the first year after transplantation (Figure 1a). The dotted line in figure 1 indicates the estimated final BMI after transplantation based on the total transplant population in our hospital. Importantly, most of the weight gain is due to an increase in body fat mass (Figure 1b), whereas fat free mass (Figure 1c) and whole body water content (Figure 1d) remain relatively stable. Since the change in weight varied strongly among individuals, and is also dependent on variation in muscle mass and hydration, the group was divided into those who gain body fat versus those who remain relatively body fat stable based on the median in body fat (BF) gain (≤3% or >3% BF

gain). The groups were comparable with respect to age, gender, BMI at the time of transplantation, BMI at hospital discharge, and dialysis characteristics. Gain in body fat was strongly related to total cholesterol (r=0.46, P=0.017) and triglyceride levels (r=0.511, P=0.011) at one year after transplantation. No differences were observed with regard to blood pressure, glucose homeostasis or renal function between the groups at 1 year after transplantation. If we compare the measurements over time we see significant changes in various clinical parameters (Table 2). BMI and fat percentage were significantly increased at 1 year after transplantation in both groups. Fat free mass increased during the first year in the body fat stable group and did not change in the body fat gain group. Blood pressure increased in the body fat stable group, similar non significant trends were seen in the body fat gain group. Triglycerides increased significantly in the first year in the group who gained body fat. ERPF decreased in both groups, one year after transplantation. FF increased only in the body fat gain group.

Figure 1. Changes in body weight and body composition (BMI, body fat percentage, fat free mass and whole body water content) after renal transplantation.



With regard to the dietary factors, no differences in energy intake, fat intake, fibre intake, protein intake, or salt intake were found between the groups. Interestingly, the intake of mono- en disaccharides was 20% higher in the group that gained body fat (Table 3). Further investigation of

Table 2. Body composition and metabolic assessments at 6 weeks and one year after transplantation.

	6 weeks BF stable (N=13)	1 year BF stable (N=13)	P-value	6 weeks BF gain (N=13)	1 year BF gain (N=13)	P-value
Body composition						
BMI (kg/m^2)	26.0 ± 4.1	27.3 ± 4.1	**0.02**	24.6 ± 3.0	27.3 ± 4.0	**0.02**
Fat free mass (kg)	52.4 ± 8.5	53 ± 9.4	**0.04**	52.1 ± 11.7	51.1 ± 10.7	0.4
Fat %	32.3 ± 7.7	33.9 ± 7.1	**0.004**	29.6 ± 9.5	35.0 ± 9.2	**<0.001**
Blood pressure						
Systolic blood pressure (mmHg)	127 ± 14	142 ± 18	**0.05**	141 ± 16	150 ± 14	0.08
Diastolic blood pressure (mmHg)	75 ± 8	85 ± 14	**0.02**	84 ± 12	90 ± 11	0.07
Lipids						
Total cholesterol, (mmol/l)	5.6 ± 0.9	4.9 ± 0.7	0.06	5.5 ± 1.2	6.1 ± 1.2*	0.1
HDL-cholesterol (mmol/l) ~	1.6 ± 0.5	1.6 ± 0.6	0.5	1.5 ± 0.4	1.4 ± 0.3	0.3
LDL-cholesterol (mmol/l) ~	3.3 ± 0.9	2.9 ± 0.7	0.1	3.4 ± 1.4	3.7 ± 1.1*	0.1
Triglycerides (mmol/l) ~	2.3 ± 1.0	2.0 ± 0.9	0.16	2.3 ± 0.6	3.0 ± 1.1*	**0.04**
Glucose homeostasis						
Glucose, (mmol/l)	6.1 ± 2.0	6.4 ± 1.7	0.7	5.4 ± 1.1	5.5 ± 1.5	0.6
HbA1c (%) #	6.3 ± 0.8	6.1 ± 0.7	0.3	6.0 ± 0.5	5.9 ± 0.7	0.8
Renal allograft function						
Urinary creatinine excretion§	10.8 ± 2.4	10.8 ± 3.6	0.9	11.6 ± 3.9	11.6 ± 2.9	0.9
Serum creatinine (μmol/l)	119 ± 29	123 ± 31	0.5	116 ± 36	137 ± 51	0.09
Creatinine clearance (ml/min)	60.6 ± 23.4	69 ± 25	0.4	71.5 ± 24.9	63 ± 17	0.2
Urinary protein excretion (g/24h) §	0.3 (0.1-0.3)	0.15 (0.0-0.2)	0.1	0.3 (0.1-0.5)	0.20 (0.05-0.30)	0.6
Proteinuria ≥0.5 g/24h (%)	15%	8%	0.2	23%	16%	0.4
GFR (ml/min)	67 ± 17	63 ± 14	0.2	55 ± 11	56 ± 16	0.7
ERPF (ml/min)	229 ± 51	204 ± 48	**0.04**	225 ± 47	201 ± 29	**0.003**
FF (%)	30 ± 3	31 ± 4	0.2	25 ± 4	28 ± 6	**0.01**

Data are presented as mean ± SD or as % (N) or as median (25th percentile; 75th percentile). ERPF, effective renal plasma flow; FF, filtration fraction; GFR, glomerular filtration fraction; HbA1c, glycated haemoglobin; * BF gain group significantly different from BF stable group, P <0.05; ~ N=13 for body fat stable and N=11 for body fat gain group; # N=13 for body fat stable and N=12 for body fat gain group; § N=12 for body fat stable and N=13 for body fat gain group

dietary patterns showed that this could be attributed to a 30% increased intake of energy from drinks and dairy. Energy from drinks and dairy were a strong determinant of total energy intake (r = 0.49, P = 0.01). Vegetable intake was 30% lower in the fat gain group, whereas there were no significant differences in fruit intake. Vegetable intake can be regarded as a marker for a healthy eating pattern, and high vegetable intake was related to a more beneficial plasma lipid profile at 1 year after transplantation (higher HDL-cholesterol, r = 0.48, P = 0.017; lower triglyceride levels, r = -0.34, P = 0.011). When comparing dietary intakes to the guidelines, sufficient vegetable intake was achieved by only 19% of patients. Fruit intake was relatively high: 58% consumed more than 2 pieces

per day. Appliance to all recommendations was achieved by one individual. All others exceeded the recommendations for saturated fat intake, fibre intake was generally low and 92% of patients had a high sodium intake.

Table 3. Comparing lifestyle factors between renal transplant recipients who remain relatively stable in body fat (change ≤3%) or who gain body fat (gain >3%).

	BF stable	BF gain	P-value
Nutrient intake			
Energy intake (kcal/day)	2221 ± 409	2135 ± 257	0.61
Total fat intake (E%)	38.0 ± 3.5	36.1 ± 4.5	0.45
Saturated fat intake (E%)	13.0 ± 2.0	12.9 ± 2.0	0.92
Cholesterol intake (mg/day)	192 ± 26	215 ± 72	0.72
Carbohydrate intake (E%	43.1 ± 4.4	46.5 ± 5.2	0.10
Mono- and disaccharides (sugars, E%)	20.4 ± 4.5	24.5 ± 3.9	**0.01**
Fiber intake (g/day)	26.9 ± 6.9	23.5 ± 5.1	0.24
Protein intake (E%)*	18.0 ± 4.3	16.8 ± 4.7	0.34
NaCl –intake (g/24h)*	9.5 ± 2.8	9.0 ± 2.2	0.72
Alcohol consumption (g/day)	6.5 ± 13.5	3.8 ± 6.1	0.76
Dietary pattern			
Intake of energy-rich drinks/dairy (E%)	15.7 ± 6.9	20.4 ± 5.5	**0.06**
Fruit intake (g/day)	217 ± 128	219 ± 136	0.88
Vegetable intake (g/day)	194 ± 110	135 ± 37	**0.04**
Salty/fatty snacks (E%)	7.2 ± 4.2	5.9 ± 4.4	0.36
Sweet snacks (E%)	8.8 ± 3.5	10.0 ± 4.6	0.55
Physical activity (SenseWear) [1]			
Steps per day (n)	9158 ± 3496	5963 ± 3488	**0.02**
Time spent sedentary (h/day)	21.5 ± 1.7	21.5 ± 1.5	0.61
Moderate-to-vigorous intense activity (min/day)	100 ± 53	86 ± 59	0.63
Physical activity (SQUASH)			
Activity score (AU, time x intensity)	3662 ± 1812	2309 ± 1138	**0.04**
Active days/week	3.8 ± 2.7	3.0 ± 2.3	0.51
Smoking			
Smoker yes/no (%)	0% (N=0)	8% (N=1)	0.31

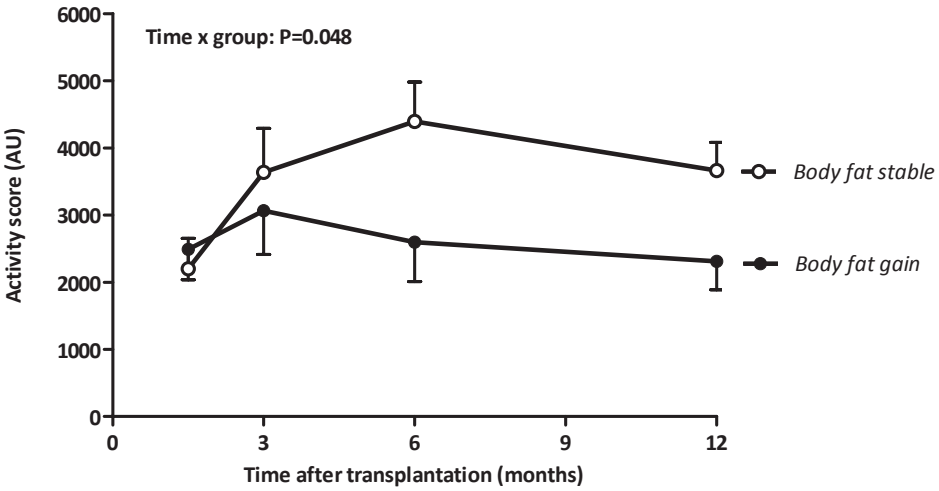
Diet parameters were regarded as multiple measurements of the same variable, since for diet no time trends were observed. For comparison between groups the mean intake or activity over four time points, i.e. 6 weeks, 3 months, 6 months and 12 months after renal transplantation was used. Physical activity is at 12 months after transplantation.

* Protein and sodium intake were estimated using 24h urine. [1] N=12 for SenseWear data in both groups. AU, arbitrary units; BF, body fat; E%, energy percent;

With regard to physical activity, the number of steps per day as well as the activity level assessed by questionnaires was 33-37% higher in those who remained relatively body fat stable

after transplantation (Table 3). Figure 2 shows that those who remained relatively stable in weight had increased their physical activity level after transplantation. At one year after transplantation, only 19% of all patients achieved to take more than 10,000 steps per day.

Figure 2. Increase in physical activity score after transplantation is related to a stable body fat percentage. Data were acquired by SQUASH questionnaire; AU, arbitrary units based on time x intensity.



DISCUSSION

RTR gained substantial weight in the first year after transplantation. Gain in adiposity after renal transplantation is related to cardiovascular risk factors. This weight gain may partly be the result of pre-transplant dietary habits such as low consumption of vegetables and high consumption of energy-rich drinks, and partly due to lack of rehabilitation of daily physical activities.

After transplantation, the restoration of a catabolic state may lead to feelings of hunger, an alleviation of the dietary constrains before transplantation may lead to unrestrained eating behaviour, and pre-transplant habits such as the need to avoid fibre-rich grains, fruits and vegetables, and the need to consume energy-dense liquids may persist (11). All these dietary factors may contribute to weight gain. Comparing patients who gained in fat mass in the first year after transplantation to those remained relatively body fat stable, the most important difference in dietary intake was the consumption energy from drinks and dairy, reflected by an increased energy intake from mono- and disaccharides in those who gained weight. Energy from drinks and dairy included relatively large portions of sugar and fat milk in coffee and tea, sugared beverages such as soda drinks,

energy drinks, chocolate milk, yoghurt drinks and fruit juice. During the dialysis period, patients are encouraged to combine their liquid intake with the intake of calories to counteract weight loss due to their catabolic state. After renal transplantation this habit may enhance the risk for gain in adiposity, and thereby contribute to cardiovascular risk. Another dietary factor that seems to contribute in this respect is vegetable intake. Vegetables may affect eating patterns by enhancing satiety, or can be regarded as a marker for a healthy eating pattern (17). Vegetable intake was higher in those who remained relatively body fat stable, and was strongly related to a more beneficial lipid profile (lower triglycerides, higher HDL-cholesterol). Something of concern was the relatively high salt intake in these patients, although not a determinant of weight gain. Regarding their increased cardiovascular risk, elevated blood pressure and a vulnerable kidney, an appropriate sodium intake would also be a target for lifestyle intervention (18), especially in combination with weight gain and obesity (19). Taken together, both vegetable intake and consumption of energy dense drinks and dairy may provide targets for post-transplant care to prevent weight gain and reduce cardiovascular risk on the long term.

Post-transplant gain in body fat was also related to a lower recovery in physical activity level. Only 19% of patients reached an activity level of 10,000 steps per day at one year after transplantation, which is very low when compared to the 56% who achieve this level in the general population (20). A recent paper shows that low physical activity is strongly related with increased incidence of cardiovascular and all-cause mortality in RTR (12). Zelle et al. showed a dose-dependent effect which was not limited to those who were extremely inactive. Several reasons may explain a lack of regain in daily physical activity in RTR. In the pretransplant condition, i.e. with chronic kidney disease or dialysis, physical activity levels are low due to low muscle mass, low cardiorespiratory fitness (21), and a feeling of tiredness. Post-transplant, this low cardiorespiratory fitness level persists. It is also possible that fear of kidney damage during exercise and a loss of exercise habit are barriers in the regain of daily physical activity (22).

An important factor that is often considered to explain post-transplant weight gain is the use of medication, in particular corticosteroids (9-11). In the present study, all patients follow the same antisuppressant regimen, and all received a maintenance dose of 10 mg corticosteroids. Although we cannot exclude that differences in response to the drugs may play a role in the differences in body fat gain between the groups, we suggest that on top of a possible effect of medication, lifestyle factors may play an important role.

Previous studies have convincingly shown an association between post-transplant BMI, metabolic syndrome and decline in renal function (6,23,24). However, most studies excluded patients who were transplanted for less than one year. It is likely that the effects of post-transplant BMI and weight gain on renal function may develop over the longer term, explaining why at one year after transplantation, no difference in renal function was found. Other studies showed that

obesity associated with an unfavorable renal hemodynamic profile, including lower ERPF and higher FF. Although we did not find any differences for renal function between groups we did find a lower ERPF and a higher FF after one year, demonstrating the first signs of kidney damage.

The study has several strengths and limitations. One of its strengths is the use of the SenseWear®, and objective device to assess daily physical activity, and the detailed phenotyping regarding body composition and dietary intake which was partly derived from analysis of 24h urine collections. However, the number of individuals who participate is limited, and more profound analysis of the data, for example the influence of dialysis type, dietary regimen before transplantation or gender differences could not be studied. Nevertheless, despite the low number of participants, the outcomes provide strong evidence for a role of lifestyle factors in post-transplant weight gain and cardiovascular risk, and these findings will provide targets for intervention. For the future, RCT's are needed to investigate the potential benefit of lifestyle intervention on long-term morbidity and mortality.

In conclusion, specific lifestyle factors such as the rehabilitation of daily physical activity, intake of vegetables and intake of energy-dense drinks are important potential targets for lifestyle intervention in RTR.

Acknowledgments

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6

Low physical activity is associated with increased risk for cardiovascular and all cause mortality in renal transplant recipients

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Abstract

Background: *Low physical activity (PA) is a risk factor for mortality in the general population. This is largely unexplored in renal transplant recipients (RTR). We investigated whether PA is associated with cardiovascular and all cause mortality in a prospective cohort of RTR.*

Methods: *Between 2001-2003, 540 RTR were studied (age 51±12 years, 54% male). PA was assessed using validated questionnaires (TOAQ, MLTPAQ). Cardiovascular and all cause mortality were recorded until August 2007.*

Results: *Independent of age, PA was inversely associated with metabolic syndrome, history of CVD, fasting insulin and triglyceride concentration, and positively associated with kidney function and 24-hr urinary creatinine excretion (i.e. muscle mass). During follow-up for 5.3 [4.7–5.7] years, 81 RTR died, with 37 cardiovascular deaths. Cardiovascular mortality was 11.7%, 7.2% and 1.7%, respectively, according to sex-stratified tertiles of PA (P=0.001). All cause mortality was 24.4%, 15.0% and 5.6% according to these tertiles (P<0.001). In Cox regression analyses, adjustment for potential confounders including history of CVD, muscle mass and traditional risk factors for CVD did not materially change these associations.*

Conclusions: *Low PA is strongly associated with increased risk for cardiovascular and all cause mortality in RTR. Intervention studies are necessary to investigate whether PA improves long-term survival after renal transplantation.*

INTRODUCTION

The incidence and prevalence of cardiovascular disease (CVD) are estimated to be 4-6 times higher in renal transplant recipients (RTR) than in the general population (1,2). Classical CVD risk factors such as dyslipidemia, hypertension and obesity, which commonly coexist as the metabolic syndrome (MS), contribute to this excess in CVD (3,4). Other factors more specific for transplantation, such as use of immunosuppressive drugs and muscle wasting might also be relevant to this high risk profile. However, the influence of physical activity (PA) on CVD and mortality is largely unexplored.

In the general population, PA is associated with a lower prevalence and incidence of cardiovascular (CV) risk factors, including hypertension, type 2 diabetes mellitus, obesity and dyslipidemia (5,6). It has also been established that low PA predicts CVD and mortality in the general population (5,7,8). In advanced chronic kidney disease (CKD), PA levels are low and remain low after renal transplantation (9,10). Most RTR enhance their level of PA slightly immediately after transplantation, although compared with the general population, cardiorespiratory fitness in RTR remains low (11). These low levels of PA can be explained by several factors. Muscle strength is impaired in RTR, compared with controls (10). Use of corticosteroids as immunosuppressive therapy and impaired renal function may contribute to an altered body composition, characterised by higher fat and lower muscle mass (12). Additionally, disuse of muscle by lack of PA can result in muscle atrophy (13), which is a risk factor for mortality in both RTR and general population (12,14).

It is not known whether low levels of PA are related to risk factors for CVD in RTR. Likewise it is not known whether low PA in RTR translates into increased risk for CV and all cause mortality.

We aimed to investigate cross-sectionally whether PA level is associated with CV risk factors and MS in RTR. We furthermore aimed to prospectively investigate in these patients whether PA is associated with CV and all cause mortality. Finally we sought to analyze whether these associations are independent of muscle mass as apparent from 24-hour urinary creatinine excretion.

MATERIALS AND METHODS

Design and subjects

In this prospective cohort study all RTR who visited the outpatient clinic at the University Medical Centre Groningen (UMCG) between August 2001 and July 2003 were invited to participate. Eligible for participation were all adult RTR with transplant duration longer than one year and with functioning allograft at moment of invitation. The group that did sign informed consent was comparable with the group that did not sign informed consent with respect to age, sex, body mass index (BMI), serum creatinine, creatinine clearance, and proteinuria. In patients with fever or other

signs of infection (e.g. complaints of upper respiratory tract infection or urinary tract infection), baseline visits were postponed until symptoms had resolved. Patients with overt congestive heart failure and patients diagnosed with cancer other than cured skin cancer were not considered eligible for the study. Data on PA were available in 547 patients, 7 patients with amputations were excluded because of the possible incapability of being physically active. Full details on the study design have been previously reported (15). The Institutional Review Board approved the study protocol (METc 2001/039).

Endpoints of the study

The primary endpoints of the study were recipient CV and all cause mortality. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of death. CV and all cause mortality were recorded until August 2007. We contacted general practitioners or referring nephrologists in case the status of a patient was unknown. Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9) (16). CV death was defined as deaths in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 410-447.

Renal Transplant Characteristics

The Groningen Renal Transplant Database contains information about all renal transplantations that have been performed at the University Medical Centre Groningen since 1986, including dialysis history. Relevant transplant characteristics, such as age and gender were taken from this database. Current medication was extracted from the medical record. Presence of amputations, smoking status, and CVD history were obtained using a self-report questionnaire. CVD history was considered positive if participants had a myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA).

Physical activity

PA data were estimated using the Tecumseh Occupational Activity Questionnaire (TOAQ) and the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ). These questionnaires, taken by interview with trained research assistants, estimate the total amount of PA over the past 12 months. The TOAQ measures frequency, intensity and duration of a maximum of three occupation-related activities within the previous 12 months. Physical activities associated with transportation to work are also included. The TOAQ is an acceptable measure of occupational PA energy expenditure and has been widely used (17). The MLTPAQ measures leisure time physical activities, including household activities, over the previous 12 months. Both questionnaires have been extensively

validated, against the Caltrac accelerometer, a PA record, and the doubly labeled water method (18,19).

A combination of these two questionnaires was used to estimate PA levels by using metabolic equivalents of task (MET) (20,21). These questionnaires can be combined because they measure both intensity and duration of the activity, which allows to calculate summary scores in MET-minutes per day (MET-min/day). MET-minutes are calculated by multiplying the intensity (indicated by the MET-score) and the duration spent on that activity (measured in minutes). The MET-score can be derived from tables (the Compendium of Physical Activities) (22), that indicate the intensity of the activity relative to resting. MET-minutes spent on PA refer to the energy that is spend on activities, over and above existing levels of resting energy expenditure. Thus, if no notable PA is performed, PA will be scored as 0 MET-min/day. This does not imply that there is no energy expenditure, because resting energy expenditure is not taken into account in the calculation of PA. The combination of these questionnaires covers the whole of physical activities during the day. A MET is a multiple of daytime resting energy expenditure expressed in multiples of 3.5 ml O_2/kg/min consumed, approximately equaling the energy expenditure of an average adult at rest, sitting quietly in a chair (18,22). For example, 1 MET is the rate of energy expenditure while at rest, while normal walking, corresponds with a MET-score of 3.5 and brisk walking with a MET-score of 5. If one would perform 1 hour of brisk walking per day as single activity, the total MET-min/day for PA would be 60 *5 = 300 MET-min/day. This means that during one hour of brisk walking, 5 times more energy is spent than during one hour of resting.

In addition, it was assessed how many RTR fulfilled the most recent PA guideline (23). According to this guideline, adults should do 2 hours and 30 minutes a week of moderate-intensity PA, which equals 30 minutes of moderate PA a day, for 5 days per week. Because moderate PA corresponds to a MET-score of 5 (brisk walking or mid-tempo cycling are typical examples), the guidelines correspond to 30*5 = 150 MET-min/day for 5 days a week.

Measurements and definitions

BMI was determined as a measure of overall obesity. Waist circumference was measured on bare skin midway between the iliac crest and the 10th rib. Muscle mass was estimated by 24-hr urinary creatinine excretion as described earlier (14). 24-hr urinary creatinine excretion is considered a reliable measure of muscle mass even in patients with advanced renal failure, in elderly people, and in patients with wasting conditions (24-26). Blood was drawn after an overnight fasting period, which included no intake of medication, including anti-hypertensive drugs and blood-glucose lowering medication. Blood pressure was measured after a 6-minute rest in supine position as the average of three automated measurements at 1-minute intervals (Omron M4; Omron Europe B.V., the Netherlands).

Total cholesterol was determined using the cholesterol oxidase-phenol aminophenazone method method (MEGA AU 510; Merck Diagnostica, Darmstadt, Germany) and serum triglycerides were determined with the glycerol-3-phosphate oxidase-phenol aminophenazone method. High-density lipoprotein (HDL) cholesterol was determined with the cholesterol oxidase-phenol aminophenazone method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, the Netherlands) and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula (21). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH, USA). Serum high sensitive C-reactive protein (hsCRP) was assessed with a high sensitivity CRP ELISA assay. Serum and urine creatinine concentration were analyzed with a photometric modification with the Jaffé method. Creatinine clearance was calculated from 24-hour urinary creatinine excretion and serum creatinine. Total urinary protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany) and proteinuria was defined as urinary protein excretion ≥0.5 g per 24 hours.

In this study MS was defined by the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII)(27). Recently the American Diabetes Association (ADA) lowered the cut-off point for impaired fasting glucose to ≥ 5.6 mmol/L (28). For our analysis of the prevalence of the MS we used this ADA cut-off point. Diabetes was defined according to the guidelines of the American Diabetes Association as a fasting plasma glucose ≥7.0mmol/l or the use of antidiabetic medication (29).

Statistical analyses

Data were analyzed with SPSS version 16.0 (SPSS Inc., Chicago, IL) and GraphPad Prism version 4.03 (GraphPad Software Inc.,San Diego, CA). Normally distributed variables are expressed as mean ± standard deviation, whereas skewed distributed variables are given as median (25^{th}-75^{th} percentile), percentages were used to summarize categorical variables. Log transformation was used for variables with a skewed distribution. Hazard ratio's (HR) are reported with 95% confidence interval [95% CI].

Recipient-related characteristics were analyzed separately for tertiles of PA level; predicted values are shown adjusted for age. Student's *t*-test or Kruskal Wallis test was used to compare means for continuous variables and with Chi- square for categorical variables. To analyze whether PA is associated with mortality, we first performed a Kaplan-Meier analyses with a Log-rank test.

Multivariate Cox-regression analyses were performed to investigate whether PA is independently associated with CV and all cause mortality. For these analyses MET-min/day were first log transformed to achieve a normal distribution and entered in the model as a continuous variable. In multivariate Cox-regression analyses, we first adjusted for recipient age, sex (Model 2) and history of CV events (Model 3). In addition to the adjustments for age, sex and history of

CV events, we performed adjustment for insulin concentration, systolic blood pressure, waist circumference, triglycerides, smoking and hsCRP (Model 4). Similarly, we adjusted for Framingham risk score factors (Model 5), creatinine clearance and urinary protein excretion (Model 6) and 24-hr urinary creatinine excretion (Model 7). We also investigated potential interactions between PA and 24-hr urinary creatinine excretion as measure for muscle mass.

RESULTS

A total of 540 RTR were studied (mean age 51 ± 12 years, 54% men). Baseline characteristics according to sex stratified tertiles of PA of the RTR are shown in table 1. Because PA was strongly inversely associated with age, we also adjusted the sex-stratified baseline characteristics for age. Median values of PA over the tertiles were 4 [0-27], 115 [75-218] and 378 [234-514] MET-min/day respectively. Median PA levels for women and men separately were 69 [4-171] MET-min/day in women and 204 [56-396] MET-min/day (P<0.001) in men. With regard to the guidelines for minimum requirements of PA, 260 (48%) of RTR were not meeting the criteria and 79 (14.6%) were completely inactive with a PA score of 0 MET-min/day.

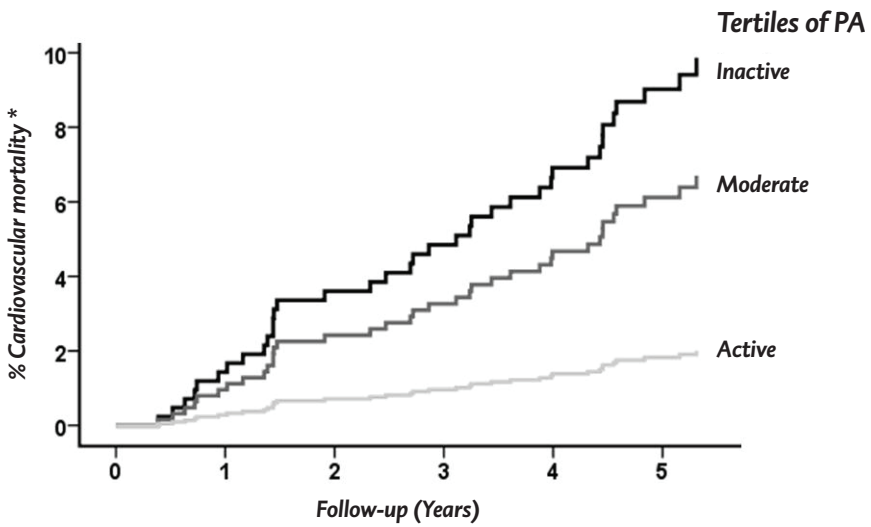
Table1. Baseline characteristics according to tertiles of physical activity

	Tertiles of physical activity*			
	Inactive (N= 180)	Moderate (N=180)	Active (N=180)	P
General characteristics				
Age (yrs)	56.1 ± 11.0	51.6 ± 11.8	46.0 ± 11.5	<0.001
Smoking, n (%)	48 (26)	33 (18)	38 (21)	0.2
MS, n (%)	135 (75)	118 (70)	111 (62)	0.007
Cardiovascular disease				
Myocardial infarction, n (%)	21 (12)	13 (7)	9 (5)	0.06
TIA / CVA, n (%)	17 (10)	9 (5)	6 (3)	0.05
Body composition				
Body mass index (kg/m^2)	26.3 ± 4	25.8 ± 4	26.0 ± 4	0.7
Waist circumference (cm) women	96.6 ± 14	93.2 ± 14	92.4 ± 14	0.1
Waist circumference (cm) men	100.4 ± 12	98.2 ± 12	100.3 ± 12	0.4
Urinary creatinine excretion (mmol/24hr)	10.7 [10.3-11.2]	11.4 [10.9-11.9]	12.5 [11.9-13.1]	<0.001
Blood pressure				
Systolic blood pressure (mmHg)	150 ± 22	157 ± 22	151 ± 23	0.003
Diastolic blood pressure (mmHg)	89 ± 10	92 ± 10	90 ± 10	0.04
Antihypertensive medication, n (%)	164 (91)	149 (83)	158 (88)	0.08
Use of ACE-inhibitor, n (%)	65 (36)	50 (28)	70 (39)	0.09
Use of β-blocker, n (%)	119 (66)	108 (60)	108 (60)	0.5

	Tertiles of physical activity*			
	Inactive (N= 180)	Moderate (N=180)	Active (N=180)	P
Lipids and inflammation				
Total cholesterol (mmol/l)	5.65 ± 1.1	5.59 ± 1.1	5.61 ± 1.1	0.89
HDL-cholesterol (mmol/l)	1.06 ± 0.34	1.12 ± 0.32	1.12 ± 0.34	0.13
LDL-cholesterol (mmol/l)	3.6 ± 1.0	3.5 ± 1.0	3.6 ± 1.0	0.7
Triglycerides (mmol/l)	2.0 [1.9-2.1]	2.0 [1.8-2.1]	1.8 [1.6-1.9]	0.05
hsCRP (mg/L)	2.5 [2.2-2.8]	1.9 [1.7-2.1]	1.8 [1.6-2.0]	0.05
Glucose homeostasis				
Glucose (mmol/l)	5.0 ± 1.4	4.8 ± 1.4	4.8 ± 1. 4	0.4
Insulin (μmol/L)	12.7 [11.7-13.8]	11.2 [10.3-12.1]	10.2 [9.4-11.1]	0.001
Diabetes mellitus n (%)	36 (20)	31 (17)	27 (15)	0.4
Renal function				
Serum creatinine (μmol/l)	141.6 [135-149]	137.1 [131-144]	136.5 [130-143]	0.6
Creatinine clearance (ml/min)	53.1 [50-56]	57.8 [55-61]	63.4 [60-67]	<0.001
Urinary protein excretion (g/24h)	0.36 [0.3-0.4]	0.35 [0.3-0.4]	0.32 [0.3-0.4]	0.8
Proteinuria ≥0.5 g/24h n (%)	50 (28)	52 (29)	50 (28)	0.9

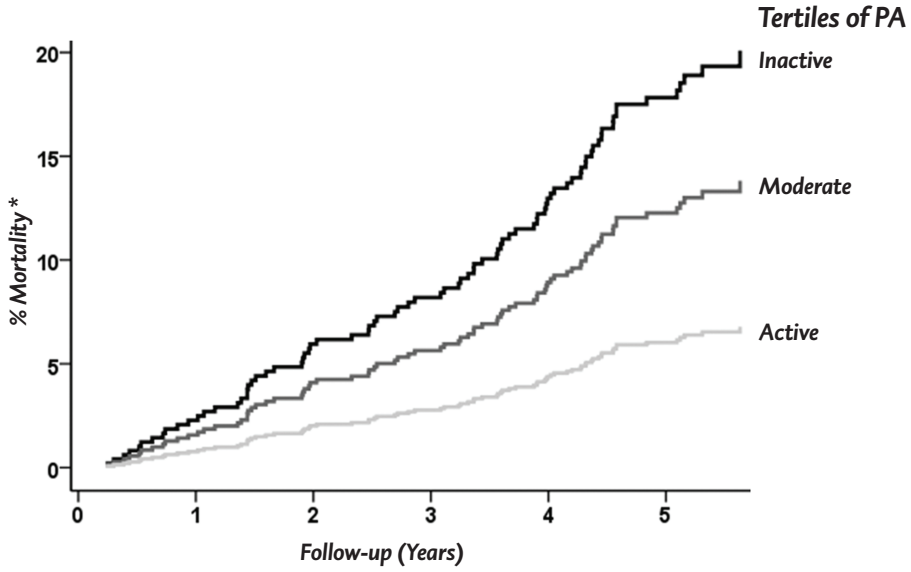
Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t-test or Kruskal Wallis test for continuous variables and with Chi- square for categorical variables. MS, metabolic syndrome; TIA/CVA, Transient Ischemic Attack/ Cerebo Vasculair Accident; ACE-inhibitor, Angiotensin Converting Enzyme inhibitor; LDL-cholesterol, low density lipoprotein cholesterol; HDLcholesterol., high density lipoprotein cholesterol; hsCRP, high sensitive C-reactive protein * tertiles of PA are stratified for gender and values are adjusted for age.

*Figure 1a. Kaplan-Meier curves of cardiovascular mortality according to sex-stratified tertiles of physical activity. * adjusted for age, (P< 0.001).*



Independent of its relation to age, PA was inversely associated with history of CVD, insulin concentration, triglycerides and hsCRP. Also independent of age, PA was positively associated with creatinine clearance and urinary creatinine excretion. Out of the total of 540 patients, 364 (64.0%) fulfilled the criteria for MS. Prevalence of MS decreased from 135 (75%) to 118 (65.5%) and 111 (61.7%) according to increasing tertiles of PA (P= 0.007).

Figure 1b. Kaplan-Meier curves of mortality according to sex-stratified tertiles of physical activity.
** adjusted for age, (P< 0.001).*



During follow-up for 5.3 [4.7 – 5.7] years, 81 recipients died, with 37 deaths CV in origin. Incidence of CV death significantly decreased according to increasing tertiles of PA, with respective numbers of 21 (11.7%), 13 (7.2%) and 3 (1.7%) (P <0.001, Figure 1a). A similar association was present for all cause mortality with numbers of 50 (27.8%), 39 (21.7%) and 30 (16.7%) according to increasing tertiles respectively (P <0.001, Figure 1b). RTR with moderate PA levels (second tertile) already had a lower risk for CV and all cause mortality than inactive RTR (first tertile).

Results of univariate and multivariate Cox-regression analyses for associations of PA as a log-transformed continuous variable with CV and all cause mortality are presented in table 2. In univariate analyses, low PA strongly predicted CV and all cause mortality in RTR (Model 1). These associations slightly weakened after adjustment for age and sex (Model 2). The same was true for adjustment for history of CVD (Model 3) and components of MS, smoking, fasting insulin concentration and hsCRP (Model 4). In Model 5 adjustments were made for Framingham risk score factors (30) (blood pressure, LDL-cholesterol, HDL-cholesterol, smoking and presence of diabetes).

Further adjustment for renal function, urinary protein excretion and 24-hr urinary creatinine excretion as measure of muscle mass (Model 6 and 7) did not materially change hazard ratios. If analyses were repeated with either censoring at the moment of return to dialysis of all RTR who returned to dialysis during follow-up (n=38), or exclusion of these RTR, results of univariate and multivariate analyses remained materially unchanged.

In multivariate Cox regression analyses no interaction between urinary creatinine excretion and PA was found.

Table 2. Hazard ratios for cardiovascular and all cause mortality by PA as a log-transformed continuous variable.

	Cardiovascular Mortality N=37		All-cause Mortality N=81	
Model	**HR [95% CI]**	**P**	**HR [95% CI]**	**P**
1	0.51 [0.39-0.67]	<0.001	0.58 [0.48-0.70]	<0.001
2	0.56 [0.41-0.76]	<0.001	0.67 [0.54-0.83]	<0.001
3	0.58 [0.42-0.79]	0.001	0.69 [0.55-0.85]	0.001
4	0.61 [0.44-0.84]	0.003	0.70 [0.57-0.88]	0.002
5	0.58 [0.42-0.80]	0.001	0.70 [0.56-0.87]	0.001
6	0.62 [0.45-0.85]	0.003	0.76 [0.61-0.95]	0.02
7	0.62 [0.45-0.86]	0.004	0.75 [0.60-0.94]	0.01

Model 1: Crude model of Physical activity as a continuous variable (log-MET-min/day)

Model 2: Model 1 + adjustment for age and sex

Model 3: Model 2 + adjustment for history of cardiovascular events

Model 4: Model 3 + adjustment for insulin concentration, systolic blood pressure, waist circumference, triglycerides, smoking and hsCRP

Model 5: Model 3 + adjustment for Framingham risk score factors (blood pressure, LDL-cholesterol, HDL-cholesterol, smoking, diabetes)

Model 6: Model 5 + adjustment for creatinine clearance and urinary protein excretion

Model 7: Model 5 + adjustment for 24-hr urinary creatinine excretion

DISCUSSION

This study shows that low PA levels are strongly associated with CV and all cause mortality in RTR. To our knowledge, this is the first study to report on PA level and CV and all cause mortality in these patients. This is consistent with earlier findings in dialysis, CKD and the general population (8,9,31). Furthermore, it shows that low levels of PA are positively associated with CVD risk factors in RTR. Independent of age, PA was inversely associated with history of CVD, presence of MS, fasting insulin concentration and triglycerides and positively associated with kidney function and 24-hr urinary creatinine excretion (as measure of muscle mass).

We anticipated that a potential association of PA with mortality could depend on risk factors for CVD, MS or smoking status. We found, however, that the association of PA with mortality was not substantially affected by adjustments for history of CV events, insulin concentrations, components of the MS or Framingham risk score factors. The same holds for adjustments for renal function, despite differences in creatinine clearance over the tertiles. Another anticipated confounder was the effect of muscle mass, as reflected by 24-hr creatinine excretion, on the association between PA and mortality. In a previous study we showed that muscle mass (as measured by 24-hr creatinine excretion) is associated with mortality in RTR (12). There, we speculated that low PA is associated with low muscle mass and thereby with higher mortality. Indeed, we now found that RTR with higher PA also had higher 24-hr creatinine excretion (i.e., larger muscle mass). However, upon multivariate analyses PA was associated with CV and all cause mortality independent of 24-hr creatinine excretion. Based on the association between PA and mortality, it is tempting to speculate that improvement of PA levels could contribute to improved survival in RTR.

PA may modulate CVD risk in several ways. Several clinical studies in the general population demonstrated that PA lowers blood pressure (32), improves body composition (33), lowers triglycerides (34) and improves glucose tolerance and insulin sensitivity (35,36). PA could modulate components of the MS in RTR in the same way as in the general population, which is supported by our data. Most RTR gain substantial weight after transplantation, mainly because of an increase in fat mass (37). Inactivity is strongly associated with obesity and consequently with the accumulation of visceral fat (38). Both visceral fat and physical inactivity itself are associated with the activation of inflammatory pathways (39), which in turn is involved in the cascade of atherosclerosis and insulin resistance (40,41). The factors above could partially explain the modulating effect of PA on CVD risk.

Another mechanism that could elucidate the cardio protective effects of exercise is vascular adaptation. Physical inactivity could lead to endothelial dysfunction in part by reduced activity of vascular nitric oxide (NO), an established precursor to the atherosclerosis process (42,43). Exercise training has been shown to increase NO production, angiogenesis and arteriogenesis, and reduce vascular oxidative stress; this could represent the response to exercise that protects against CVD (43,44).

In our study median energy expenditure attributable to PA was 115 MET-min/day with values of 69 MET-min/day for women and 204 MET-min/day for men. Richardson et al. (18) measured a mean energy expenditure of about 410 MET-min/day, in healthy subjects using the MLTPAQ, which only measures leisure time physical activity. Thus, compared to healthy subjects, RTR have low PA levels in our study, which is in line with two other studies in RTR had a lower level of PA compared to healthy subjects (45,46). A 5 year follow up study showed that most RTR spontaneously became more active after transplantation, which was associated with a better quality of life (46). Although

PA levels increase after transplantation, they often remain relatively low (45,47).

There could be several reasons for the low levels of PA in RTR. Exercise capacity is approximately 30% lower in RTR than in controls (10). The main determinants of exercise capacity in RTR are skeletal muscle strength and PA level (47). It is difficult to determine whether low PA levels are caused by low muscle mass or the other way around. Pre-transplant, muscle mass is frequently low as a consequence of muscle wasting associated with end-stage renal disease and chronic hemodialysis (48). Metabolic acidosis and chronic inflammation contribute to the process of muscle protein degradation (48). After transplantation, chronic exposure to corticosteroids can contribute to decreased muscle mass. In line with this, we found in an earlier study that cumulative prednisolone dose was inversely related to muscle mass as reflected by 24h urinary creatinine excretion (12). Thus, a primary lack in muscle mass may impair RTR in their capacity to exert PA. However, use of corticosteroids have been demonstrated to not per sé limit the potential to build up muscle mass and exercise capacity (47). It has also been shown that disuse of muscle due to a lack of physical activity can adversely affect muscle mass (13). It is therefore obvious that to restore and maintain muscle mass after transplantation, regular PA is required. Other reasons for inactivity may also be uncertainty about being capable and allowed of being physically active – with fear of injuring the graft – can also play a role in RTR.

Exercise studies in RTR have indicated that exercise training results in an improved exercise capacity, increased muscle strength, improved blood pressure control and bone remodeling and higher levels of self-reported physical functioning (11,47). These results show that exercise training in RTR can be effective and feasible. Our results suggest that exercise training may benefit RTR in terms of better survival. In regular care after transplantation, exercise training is not yet incorporated. Further research is needed to study the role of PA intervention after renal transplantation in the prevention and treatment of CV risk factors, physical functioning and long term outcome.

The strength of our study is its prospective design. RTR in this study were closely monitored by regular check-up in our clinic, which allows for extensive information gathering on patient status. A limitation of our study is that we used questionnaires to measure PA. However, the questionnaires have been validated (17-19) and the RTR were interviewed by trained research-assistants. Recall bias and social desirability bias where the participants could tend to report high volumes of PA are unfortunately unavoidable and could influence internal validation. Validation studies have shown that questionnaires may be effective in classifying people into broad categories of PA (e.g. inactive, moderate and active), but less appropriate for quantifying absolute energy expenditure (49). Therefore the focus in this study is not on absolute MET scores of PA but on the ranking based on these scores. It is tempting to speculate that stimulation of physical activity will improve outcome after transplantation. This study is, however, observational in design and not an intervention study, thus hard conclusions on causality can not been drawn. We can not exclude a more general effect

of a more healthy lifestyle, but our data support that PA contributes to a healthy lifestyle pattern in RTR.

In summary, low PA is an independent risk factor for CV and all cause mortality in RTR. Our data suggest that increased PA might have a CV survival benefit for RTR. Given the 4-6 times increased CV mortality in RTR, there is great potential for mortality reduction by exercise participation in these patients.

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7

Kinesiophobia and low physical activity after renal transplantation

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Abstract

Background: *Regular physical activity is a preventive measure for cardiovascular disease in the general population and probably has the same benefits in RTR. It is unknown if kinesiophobia (fear of movement) plays a role in the low physical activity levels after renal transplantation. Therefore we investigated the prevalence of kinesiophobia and its relationship with physical activity levels in RTR.*

Methods: *RTR were investigated between 2001-2003. The Tampa Score of Kinesiophobia – Dutch Version (TSK-DV) was used to asses kinesiophobia.*

Results: *A total of 527 RTR (age 51±12 years, 55% men) were studied. 28% of the RTR were indicated with high level of kinesiophobia. Kinesiophobia was strongly associated with low physical activity level. Kinesiophobia was positively associated with age, medically unfit for work, depression score and anxiety score, history of cardiovascular disease, diabetes and dialysis duration. We found a negative association between kinesiophobia paid employment, urinary creatinine extretion, creatinine clearance and living donor. Independent determinants of kinesiophobia were history of myocardial infarction, anxiety score and lack of paid employment. Fear was the component of TSK-DV that contributed strongest to physical activity level.*

Conclusions: *This study was the first to examine kinesiophobia and its relation with physical activity in RTR. Important determinants of kinesiophobia were history of myocardial infarction, anxiety score and lack of paid employment. Kinesiophobia was strongly associated with low physical activity level. Kinesiophobia need to be considered as a barrier for rehabilitation programs for RTR and important target for intervention.*

INTRODUCTION

The beneficial effects of regular physical activity (PA) in the general population are clearly known. PA can positively influence blood pressure, lipid profile and insulin sensitivity (1,2). These health benefits are relevant to the general health of renal transplant recipients (RTR). After transplantation RTR develop various cardiovascular risk factors such as new onset of diabetes after transplantation, hypertension, and overweight (3-5). The incidence and prevalence of cardiovascular disease (CVD) in RTR is four to six times higher than in the general population (6,7). Regular PA is a preventive measure for CVD in the general population and probably has the same effects in RTR (1). In a recent study we showed that regular PA after transplantation was strongly associated with a lower risk for cardiovascular and all-cause mortality (8). Although RTR should engage in PA, relatively few RTR meet the exercise guidelines (8).

The main determinant of exercise capacity in RTR is skeletal muscle strength (9). A primary lack in muscle mass before transplantation might impair RTR in their capacity to implement PA in their daily life after transplantation. Another reason for a low PA level might also be insecurity about being able to be physically active (10). Fear of injuring the kidney could play a role in this uncertainty.

Fear of movement, or kinesiophobia, refers to the anxiety that individuals with persistent pain can experience, with regard to engaging in activities or physical movements. The cognitive behavioral model of fear of movement supposes that patients with kinesiophobia tend to avoid PA because it might cause pain or harm the patient (11). This false assumption will lead to avoidance behavior, muscle disuse and de-conditioning of the patient. Although kinesiophobia was originally defined as the fear of movement in relation to patients with musculoskeletal pain it might also apply to other patients groups (12). The occurrence of kinesiophobia in RTR is unknown. Therefore we aimed to asses the prevalence of kinesiophobia in RTR and to determine whether kinesiophobia is related to the low PA levels in RTR. Subsequently, the determinants of kinesiophobia will be studied.

MATERIALS AND METHODS

Design and Subjects

Study design and inclusion/exclusion criteria have been described previously (13). In brief, all patients eligible for participation were RTR with transplant duration longer than one year with a functioning allograft at moment of invitation. A total of 606 RTR signed written informed consent, from a total of 847 eligible patients. The Institutional Review Board approved the study protocol.

Renal Transplant Characteristics

The Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. Current medication was taken from the medical record. Standard immunosuppressive treatment was described previously (14). Information on working situation, living situation, smoking and alcohol consumption, and cardiovascular history were obtained by self-report questionnaire.

Daily physical activity

PA data were estimated using the Tecumseh Occupational Activity Questionnaire (TOAQ) and the Minnesota Leisure Time Physical Activity Questionnaire (MLTPAQ). These questionnaires, taken by interview with trained research assistants, estimate the total amount of PA over the past 12 months. The TOAQ measures frequency, intensity and duration of a maximum of three occupation-related activities within the previous 12 months. Physical activities associated with transportation to work are also included. The TOAQ is an acceptable measure of occupational PA energy expenditure and has been widely used (15). The MLTPAQ measures leisure time physical activities, including household activities, over the previous 12 months. Both questionnaires have been extensively validated, against the Caltrac accelerometer, a PA record, and the doubly labeled water method (16,17). A combination of these two questionnaires was used to estimate daily PA levels by using metabolic equivalents of task (MET)(18,19). More concise information on measurement of PA was described previously (8).

Fear of movement

Fear of movement was measured with the Tampa Score for Kinesiophobia (TSK), a 17-item questionnaire (20). Each question is answered on a four-point Likert type scale, ranging from "strongly disagree" to "strongly agree." The total score is calculated after inversion of the individual scores of item numbers 4, 8, 12, and 16. We used the four-factor solution of Vlaeyen et al. In this factor analysis they found four factors labeled harm, fear of (re)injury, importance of exercise, and avoidance of activity. This four-factor solution explained 36.2% of the total variance. The higher the score on a factor of the TSK, the greater the role that factor has in the fear of movement. A score of > 37 points indicates kinesiophobia (21,22). The original TSK was translated into Dutch (TSK-DV) whereby the same scoring format was maintained (23). The TSK-DV was found to be valid and sufficiently reliable (23,24).Cronbach's alphas for the total scale varied from [alpha] = 0.68 to [alpha] = 0.80 (22,23,25).

Depression and anxiety

Quantitative information on depression and anxiety was obtained by self-report questionnaire, using the subscales of the Symptom Checklist (SCL-90)(26). The SCL-90 is designed to measure a broad range of psychological problems and symptoms of psychopathology. In the depression subscale, patients are asked to indicate on a 5-point scale how much hindrance they experienced from psychiatric complaints in the last week. Recently we used the SCL-90 to determine depression after renal transplantation (27).

Body composition

Muscle mass was estimated by 24-hr urinary creatinine excretion as described earlier (28). 24-hr urinary creatinine excretion is considered a reliable measure of muscle mass even in patients with advanced renal failure, in elderly people, and in patients with wasting (29-31). Body mass index was determined as a measure of overall obesity. Waist circumference was measured at the level midway between the lowest rib and the iliac crest.

Clinical measurements and Definitions

Blood was drawn after an overnight fasting period, which included no intake of medication. Total cholesterol, low-density lipoproteins (LDL) cholesterol, high-density lipoproteins (HDL) cholesterol, serum triglycerides, glucose, insulin, serum and urine creatinine concentration were measured as described previously (8) . Proteinuria was defined as urinary protein excretion ≥0.5 g/24h. In this study, metabolic syndrome (MS) was defined according to the definition of the National Cholesterol Education Program Expert Panel (NCEP-ATPIII) (32). New onset of diabetes after transplatation (NODAT) was defined by fasting plasma glucose concentration ≥ 7.0 mmol/l or use of anti-diabetic medication.

Statistical Analyses

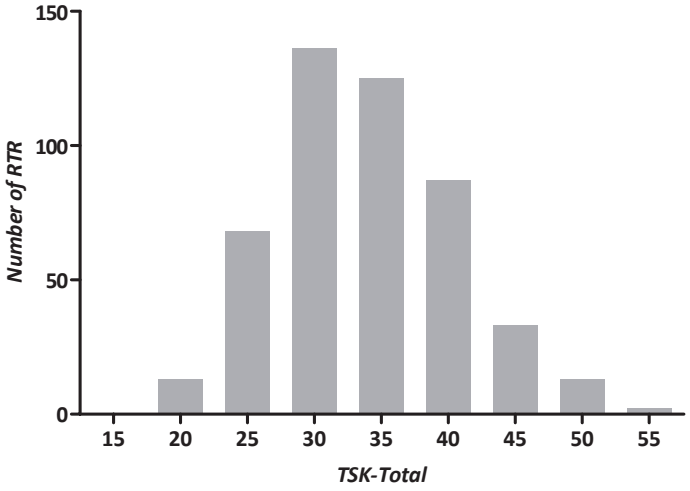
Data were analyzed with SPSS version 19.0 (SPSS Inc.,Chigago, IL). Normally distributed variables were expressed as mean ± SD, whereas skewed distributed variables are given as median (25^{th}-75^{th} percentile); percentages were used to summarize categorical variables. Recipient-related characteristics were analyzed separately for the group group with a score ≤37 for kinesiophobia and the group above this score (>37 points). Differences between groups were tested for statistical significance with Student's t-test for normally distributed variables, Mann–Whitney test for skewed distributed variables, and chi-squared test for categorical variables.

Forward logistic regression analysis was performed including all variables with a $p<0.1$ to determine independent determinants of kinesiophobia. All co-variates from table 1 with a p-value <0.2 were used in the logistic regression. Univariate linear regression analyses were performed to investigate the association between components of TSK-DV and TSK-DV-total score and PA.

RESULTS

A total of 477 RTR were studied (mean age 50.9 ± 12.0; 57% men). The frequency distribution of TSK-DV-total is shown in figure 1, with a higher total score representing a higher degree of kinesiophobia. The median TSK-DV-score on kinesiophobia in RTR was 33 [25th-75th percentile; 29-38]. 28% (n = 135) of the RTR scored above the cut-off score of 37 and were categorized as having kinesiophobia (median TSK-DV-score 41 [39-43]). Differences in baseline characteristics according to low and high levels of kinesiophobia are shown in table 1. RTR with high level kinesiophobia had lower PA level compared with RTR without kinesiophobia, accordingly muscle mass measured by urinary creatinine excretion was also lower. High levels of kinesiophobia were related to a lower creatinine clearance and to longer dialysis duration. RTR with kinesiophobia had less often a living donor and less often paid employment. Kinesiophobia was related to higher co-morbidity. RTR with kinesiophobia had a higher prevalence of NODAT, history of CVD and were more often medically unfit for work. Depression and anxiety score were significantly higher in RTR with kinesiophobia. We found no differences between the groups in BMI and waist circumference.

Figure 1. Frequency distribution of TSK-DV-total. The higher the TSK-DV total score the higher the degree of kinesiophobia. Kinesiophobia was present in 135 (28%) of the RTR.



Multivariate analysis for kinesiophobia

Independent variables associated with kinesiophobia in RTR are shown in table 2. History of myocardial infarction was the strongest factor associated with kinesiophobia in multivariate logistic regression analysis. Anxiety score and lack of paid employment were also independent determinants of kinesiophobia.

Table 1. Baseline characteristics according to groups of Kinesiophobia

	Tampa Scale for Kinesiophobia		
	No-kinesiophobia: score ≤37 (n = 366)	**Kinesiophobia: score >37 (n = 161)**	**P-value**
General characteristics			
Age (yrs)	50.2 ± 11.8	52.7 ± 12.2	0.041
Gender (Male), n (%)	200 (58.5)	72 (53.3)	0.31
Employment status			
Paid employment, n (%)	105 (35)	27 (16)	<0.001
Medically unfit for work, n (%)	82 (24)	50 (39)	0.001
Body composition			
Body mass index (kg/m²)	26.1 ± 4.4	26.2 ± 3.6	0.88
Waist circumference (cm) women	94.4 ± 14.9	94.0 ± 13.1	0.85
Waist circumference (cm) men	99.2 ± 12.6	101.3 ± 11.7	0.2
Urinary creatinine excretion* (mmol/24hr)	12.2 [10.0-14.6]	11.3 [8.8-13.5]	0.002
Psychological condition			
Depression score	20 [18-25]	23 [19-31]	<0.001
Anxiety score	12 [10-14]	12 [11-18]	<0.001
Physical activity level			
Daily PA (METs per day)	162.1 [37 -337]	66.1 [4 to 217]	<0.001
History of cardiovascular disease			
Myocardial infarction, n (%)	21 (6.1)	18 (13.4)	0.009
TIA/CVA, n (%)	10 (2.9)	12 (9.0)	0.005
Blood pressure			
Systolic blood pressure (mmHg)	151.9 ± 21.7	154.6 ± 24.5	0.23
Diastolic blood pressure (mmHg)	89.9 ± 9.8	90.0 ± 10.3	0.98
Lipids			
Total cholesterol (mmol/l)	5.6 ± 1.1	5.8 ± 1.1	0.12
HDL-cholesterol (mmol/l)	1.1 ± 0.3	1.1 ± 0.3	0.89
LDL-cholesterol (mmol/l)	3.6 ± 1.0	3.7 ± 1.0	0.33
Triglycerides (mmol/l)	1.93 [1.4-2.7]	1.92 [1.5-2.8]	0.25
Glucose homeostasis			
Glucose (mmol/l)	4.8 ± 1.2	4.9 ± 1.4	0.64
Insulin (μmol/L)	11.2 [8.2-16.3]	11.2 [7.8-15.9]	0.59
NODAT, n (%)	53 (15.5)	31 (23.0)	0.059
Renal function			
Serum creatinine (μmol/l)	134 [114-164]	131 [107-170]	0.37
Creatinine clearance (ml/min)	60 [50-80]	57 [44-74]	0.059
Proteinuria ≥0.5 g/24h, n (%)	103 (30)	36 (27)	0.43
Transplantation and history			
Dialysis duration (months)	25 [12-45.25]	31 [14-51]	0.061
Living donor, n (%)	57 (16.7)	10 (7.4)	0.005

	Tampa Scale for Kinesiophobia		
	No-kinesiophobia: score ≤37 (n = 366)	Kinesiophobia: score >37 (n = 161)	P-value
Number of previous transplants			
- 0, n (%)	310 (91)	116 (86)	0.092
- 1 or more, n (%)	32 (9)	19 (14)	
Acute rejection, n (%)	160 (47)	58 (43)	0.45
Immunosupression			
Prednisolone dose, mg/d	9.3 ± 1.2	9.1 ± 1.5	0.26
Calcineurine inhibitor (CNI), n (%)	271 (79)	105 (78)	0.73
Proliferation inhibitor, n (%)	254 (74)	91 (67)	0.13

Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t test or Kruskal Wallis test for continuous variables and with Chi- square for categorical variables. * Urinary creatinine excretion was measured as a proxy for muscle mass.

Linear regression analysis for PA

Results of linear regression analysis for TSK-DV score and TSK-DV components with PA as dependent variable are shown in table 3. TSK-DV total score was strongly associated with PA. Fear was the component of TSK-DV that contributed strongest to PA. The components harm and activity avoidance were also strongly related to PA. Figure 2 shows the inverse association between tertiles of PA and kinesiophobia. RTR with kinesiophobia had significantly lower PA levels, compared to RTR without kinesiophobia.

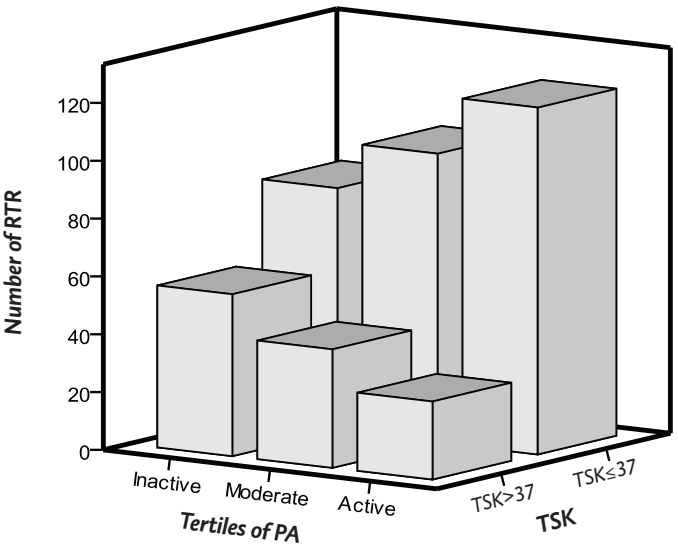
Table 2. Independent determinants of kinesiophobia

Multivariate	Exp(B) [95% CI]	P-value
Myocardial infarction	2.46 [1.20-5.02]	0.009
Paid employment	0.42 [0.25-0.69]	0.001
Anxiety score	1.09 [1.04-1.14]	<0.001

Table 3. Fear is the strongest component of the TSK-DV related to PA level

Univariate	Standardized Coefficients (B) [95% CI]	P-value
TSK-DV-total	-0.22 [-0.12; -0.05]	<0.001
Fear	-0.20 [-0.84; -0.32]	<0.001
Harm	-0.17 [-1.06; -0.34]	0.001
Activity avoidance	-0.14 [-1.08; -0.21]	0.04
Importance of exercise	-0.11 [-0.81; -0.09]	0.02

Figure 2. Physical activity level in RTR with (cut-off>37) and without kinesiophobia



DISCUSSION

The presence of kinesiophobia in RTR is an important finding which can help to identify barriers to PA in RTR. Kinesiophobia was strongly associated with PA level after renal transplantation. Important determinants of kinesiophobia were history of myocardial infarction, anxiety score and lack of paid employment.

Kinesiophobia was present in 28 % of the RTR; this is lower than in patients with chronic musculoskeletal pain, whereby kinesiophobia was found in 50 percent of the patients (33). Levels of kinesiophobia in RTR are more comparable to patients with coronary artery disease in which a high level of kinesiophobia was found in 20% of the patients (21). The nature of experiencing fear of movement can differ across the various patient groups. The TSK-DV provides no specific information on the nature of the fear, it can be presumed that RTR differ from the other populations in which the TSK-DV was previously used. Although renal transplantation offers patients significant survival advantage it also introduces new fear and distress. The most important source of distress is fear of losing the graft. Insecurity about being able to be physically active after transplantation and fear of losing the kidney may contribute to the development of kinesiophobia. This is confirmed by our results where the factors fear and harm were strongly associated with PA level. In addition, the low levels of PA and the muscle weakness in RTR might contribute to a low self-regulation level,

resulting in a vicious cycle of avoidance behavior and inactivity. The component importance of exercise was associated with PA level. This indicates that RTR are not aware of the importance of exercise after renal transplantation. Good information on this topic might help to overcome this.

We found a strong association between kinesiophobia and low PA levels after renal transplantation. RTR with kinesiophobia moved significantly less compared to RTR without kinesiophobia. Although RTR should engage in PA relatively few RTR meet the guidelines for exercise (8). A moderately active lifestyle corresponds to a MET-score of 150 MET-minutes a day for 5 days a week (34). RTR without kinesiophobia did on average meet these guidelines (median 162 METs [37-336]). In contrast, RTR with a high level of kinesiophobia were far below this recommendation (66 METs [4-217]). Regular PA is a preventive measure for CVD in the general population (1,35). At the same time, CVD is the primary cause of death among kidney recipients and almost 50% of post-transplant deaths are attributable to CVD (6). We previously found a strong relationship between low PA levels in RTR and increased risk for cardiovascular and all-cause mortality. Risk for cardiovascular mortality was 6.5 times lower in active RTR when compared with the inactive RTR (8).

To target PA interventions after transplantation, it is important to identify barriers of PA. We previously showed that history of CVD and risk factors for CVD were strongly associated with low PA as well as low muscle mass (measured by 24-hour urinary creatinine excretion) in RTR. Together with kinesiophobia, these are important barriers that need to be addressed in development of a rehabilitation intervention program for RTR.

Targeting kinesiophobia in lifestyle intervention programs could improve the success of these interventions. Transplant healthcare providers should focus on the self-care practice predictors such as: patients their health beliefs and removing barriers to self-care . The Social Cognitive Theory supposes that people learn strategies to manage their chronic illness through self-regulation processes of observations, judgments and reactions (36). Self-regulation refers to the capacity of individuals to effectively perform a behavior to achieve a desired goal or outcome (37). Self-regulation is a critical element in renal transplant self-care. Dispose of self-care requires the believe that patients perceive self-efficacy, to perform self-care practices. RTR will engage the PA guidelines, if they observe that their practices successfully achieve the outcomes they expected before.

In summary this study was the first to examine kinesiophobia and its relation with PA in RTR. Kinesiophobia was strongly associated with PA level. Our study shows that kinesiophobia is a psychological factor that is common in RTR and acts as a barrier to engage in PA. To target this fear of movement among RTR sufficient information about PA should be provided. Self-care practice predictors such as kinesiophobia are important targets for rehabilitation intervention programs in RTR.

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8

Impact of depression on long-term outcome after renal transplantation: a prospective cohort study

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Abstract

Background: *Renal transplantation is the treatment of choice for end stage renal disease. Although there is more depression in wait-listed versus transplant patients, depression persists after transplantation. We investigated the determinants of depression in renal transplantation recipients (RTR) and the association with cardiovascular (CV) and all-cause-mortality and graft failure.*

Methods: *RTR were investigated between 2001-2003. Depression was assessed using the Depression Subscale of the Symptom Checklist (SCL-90). Mortality and graft failure were recorded until May 2009.*

Results: *A total of 527 RTR (age 51±12 years, 55% men) were studied. 31% of the RTR were indicated with depression. Independent variables associated with depression were medically unfit for work, proteinuria, lower physical activity level and longer dialysis duration. During follow-up for 7.0 [6.2-7.5] years, 114 RTR (59 CV) died. In Cox-regression analyses, depression was strongly associated with increased risk for CV (HR=2.12 [1.27-3.53], p=0.004) and all-cause mortality (HR=1.96 [1.36-2.84], p<0.001). Adjustments for confounders did not materially change these associations. The association with graft failure (HR=1.77 [1.01-3.10], p=0.047), disappeared after adjustment for kidney function (p =0.6).*

Conclusion: *Although our study has several limitations, including the lack of pre-transplant depression status, we identified medically unfit for work, proteinuria, lower physical activity level and longer dialysis duration as independent variables associated with depression. We furthermore found that depression is associated with CV and all cause-mortality in RTR.*

INTRODUCTION

Transplantation is the preferred treatment in end-stage renal disease (ESRD). Compared with maintenance dialysis, it offers significant survival advantage and brings emotional and psychological benefits to patients. On the other hand, it also introduces new concerns such as fear of losing the new kidney and complications that may lead to emotional distress (1-3) . Although there is more depression in wait-listed versus transplant patients, depression seems to be a persistent problem after transplantation (4).

Research among individuals with chronic medical conditions, such as type 2 diabetes and coronary artery disease, shows that depressed patients have increased mortality compared with their non-depressed counterparts (5-9). Various studies have shown that depression is common among patients on dialysis, with a prevalence of depression ranging from 5 to as high as 71% (10), depending on the study population and method of diagnosis. We also know that depression has been associated with increased morbidity and mortality in ESRD (11,12).

There is only limited data about the prevalence of depression after renal transplantation, and few studies were done on the relation of depression with long-term outcome. Dobbels et al. (13) show that depression is associated with a 2-fold greater risk of graft failure and death. Novak et al. (14) show that mortality in patients with depression after kidney transplantation was higher than in patients without depression. No data are available about the risk of cardiovascular (CV) mortality in renal transplant recipients (RTR) in relation to depression. Early identification of patients at risk is hampered by the lack of knowledge about the determinants of depression in RTR. Therefore, recipient-related factors such as proteinuria, blood pressure and lifestyle factors need to be explored for their potential association with depression (12-14).

We hypothesize, first, that there is a high prevalence of depression in RTR. Second, that symptoms of depression may lead to a worse survival and graft failure, the former possibly attributable to CV disease. To investigate these hypotheses we investigated several recipient-related factors for their association with depression in RTR in a large single-center cohort. We furthermore aimed to investigate whether symptoms of depression are associated with CV and all-cause mortality and graft survival.

MATERIALS AND METHODS

Design and Subjects

Study design and inclusion/exclusion criteria have been described previously (15). In brief, for this prospective cohort study all adult allograft recipients between August 2001 and July 2003 who

survived with a functioning allograft beyond the first year after transplantation were eligible to participate at their next visit to the outpatient clinic. A total of 606 RTR signed written informed consent, from an eligible 847. Data on depression were available in 527 RTR. Baseline data were collected between August 2001 and July 2003 and RTR were followed for several years. The Institutional Review Board approved the study protocol.

Endpoints of the study

The primary endpoints of this study were recipient mortality and graft failure. Graft failure was defined as a return to dialysis or re-transplantation. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status and cause of death. We contacted general practitioners or referring nephrologists if the status of a patient was unknown. For this study, we used follow-up data for mortality and graft loss, recorded until May 2009.

We also collected follow-up data on percutaneous transluminal angioplasty (PTA), percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass grafting (CABG). Cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by a physician from the Central Bureau of Statistics. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9) (16).

Renal Transplant Characteristics

The Groningen Renal Transplant Database contains information on all renal transplantations performed at our center since 1968. Relevant transplant characteristics such as age, gender and date of transplantation were extracted from this database. Current medication was taken from the medical record. Standard immunosuppressive treatment was described previously (17). Information on employment status, living situation, smoking and alcohol consumption, and CV history were obtained by self-report questionnaire.

Depression

Quantitative information on depression was obtained by the Depression Subscales of the Symptom Checklist (SCL-90). The SCL-90 is the screening instrument most commonly used by psychologists in clinical practice as well as in research settings in the Netherlands (18). The SCL-90 is widely used as an outcome measure in intervention studies and as a screening instrument in both psychiatric and medical patients for clinical and research purposes (19-21). SCL-90 is designed to measure a broad range of psychological problems and symptoms of psychopathology and has shown to have good psychometric properties (22,23). The SCL-90 depression subscale measures the typical symptoms of depression; dysphoric mood, signs of withdrawal of life interest, lack of motivation, and loss of vital energy. Feelings of hopelessness and thoughts of suicide are also included in the

depression subscale. The SCL-90 depression subscale was administered once in all RTR between August 2001 and July 2003. In the depression subscale, patients are asked to indicate on a 5-point scale how much hindrance they experienced from psychiatric complaints in the last week. The depression subscale measures only psychological depressive symptoms and no somatic symptoms of depression. The depression subscale was validated in patients who experienced their first-ever ischemic stroke. The C-statistic of the SCL-90 depression subscale was 0.81 (24). For the indication of possible depression, we used a cut-off score of ≥25 on the depression subscale. Sensitivity and specificity of this threshold were 88.5 and 60.7 respectively (24).

Measurements and Definitions

Physical activity was assessed using validated questionnaires (15). Single imputation was used to obtain more complete data on physical activity. Body mass index, waist circumference and blood pressure were measured as described previously (15).

Blood was drawn after an overnight fasting period, which included no intake of medication. Total cholesterol, low-density lipoproteins (LDL) cholesterol, high-density lipoproteins (HDL) cholesterol, serum triglycerides, glucose, insulin, 24-hour creatinine excretion as a measure for muscle mass (25), serum and urine creatinine concentration were measured as described previously (15). 25-hydroxyvitamin D3 levels were determined using isotopedilution–onlinesolidphaseextractionliquid chromatography–tandem massspectrometry. Proteinuria was defined as urinary protein excretion ≥0.5 g/24h. Metabolic Syndrome (MS) and diabetes were defined according to the guidelines as described previously (16). Medically unfit for work applies to both the physical and mental status of a patient.

Statistical Analyses

Data were analyzed with SPSS version 16.0 (SPSS Inc.,Chigago, CA). Normally distributed variables were expressed as mean ±SD, whereas skewed distributed variables are given as median (25th-75th percentile); percentages were used to summarize categorical variables. Log transformation was used for variables with a skewed distribution. Hazard ratios are reported with 95% confidence interval.

Recipient-related characteristics were analyzed separately for the group below the cut-off score of 25 and the group above this score. Differences between groups were tested for statistical significance with Student's t-test for normally distributed variables, Mann–Whitney test for skewed distributed variables, and chi-squared test for categorical variables. Forward logistic regression analysis was performed including all variables with a $p<0.1$ to determine independent determinants of depression. All co-variates from table 1 with a p-value <0.2 were used in the logistic regression. To analyze whether symptoms of depression are associated with CV and all-cause mortality,

and graft failure, we performed Kaplan-Meier analysis with log rank test to assess significance of difference between groups. Cox regression analyses were performed to study whether depression was independently associated with CV and all-cause mortality and graft failure. In these analyses we considered several potential covariates known to be associated with mortality and graft failure in RTR as well as covariates that were independently associated with depression (15,26-29).

RESULTS

A total 527 RTRs (mean age, 51±12 years; 55% male) participated at median time of 6.0 [2.6-11.4] years post-transplant. The median SCL-90 score on depression in the RTR was 21 [25th-75th percentile; 18-26]. 31% (n = 161) of the RTR scored ≥25 and were categorized as depressed (median SCL-90 score 30 [27-39]). Baseline characteristics of the RTR according to the two groups of depression score are shown in table 1. Depressed RTR had significantly lower physical activity level, urinary creatinine excretion, hemoglobin levels, ACE-inhibitor intake, and creatinine clearance, had less often paid employment, and had less often a living donor. Depressed patients also were more often medically unfit for work, had more often proteinuria, had a longer history of dialysis and used more often antidepressant medication. Use of SSRIs or TCAs in the total population was 4 %.

Table 1. Baseline characteristics according to groups of depression score

	Depression score (SCL-90)		
	No-depression: score <25 (n = 366)	**Possible depression: score ≥25 (n = 161)**	**P-value**
General characteristics			
Age (yrs)	51.16 ± 11.85	51.38 ± 12.03	0.84
Gender (Male), n (%)	209 (57)	81 (50)	0.15
Living alone, n (%)	56 (15)	32 (20)	0.21
Employment status			
Paid employment	139 (39)	126 (21)	<0.001
Medically unfit for work, n (%)	80 (22)	68 (42)	<0.001
Unemployed, n (%)	70 (20)	23 (14)	0.16
Retired, n (%)	30 (8)	15 (9)	0.7
Unspecified, n (%)	34 (9)	16 (10)	0.85
Lifestyle			
Metabolic Syndrome, n (%)	242 (66)	101 (63)	0.45
Physical activity (METS)	147.82 [37.08-345.17]	90.15 [8.36-227.30]	<0.001
Smoking			
- In the past, n (%)	152 (42)	74 (46)	0.34
- Currently, n (%)	79 (22)	41 (26)	0.33

	Depression score (SCL-90)		
	No-depression: score <25 (n = 366)	Possible depression: score ≥25 (n = 161)	P-value
Alcohol consumption			
- Abstainers, n (%)	170 (46)	75 (47)	0.96
- <10 g/d, n (%)	140 (38)	64 (40)	0.80
- 10-30 g/d, n (%)	51 (14)	18 (11)	0.37
- >30 g/d, n (%)	2 (1)	4 (3)	0.06
Body composition			
Body mass index (kg/m²)	26.23 ± 4.29	25.81 ± 4.40	0.31
Waist circumference (cm) women	94.23 ± 14.21	93.35 ± 15.68	0.67
Waist circumference (cm) men	100.49 ± 12.74	98.92 ± 12.20	0.34
Urinary creatinine excretion (mmol/24hr)	11.85 [9.73-14.58]	11.70 [8.85-13.95]	0.05
History of cardiovascular disease			
Myocardial infarction, n (%)	27 (7)	12 (8)	0.22
CVD event, n (%)	54 (15)	28 (17)	0.44
Antidepressant use			
Selective serotonin re-uptake inhibitors (SSRI), n (%)	7 (2)	12 (7)	0.002
Tricyclic antidepressants (TCA), n (%)	3 (1)	1 (1)	0.8
Benzodiazepines, n (%)	14 (4)	25 (16)	<0.001
Vitamin D status			
25(OH)D (nmol/l)	51.4 [37.5-68.9]	47.4 [35.4-67.2]	0.21
Hemoglobin level			
Hemoglobin (mmol/l)	8.7 [8.1-9.3]	8.4 [7.7-9.2]	0.03
Blood pressure			
Systolic blood pressure (mmHg)	152.75 ± 22.52	154.70 ± 23.62	0.36
Diastolic blood pressure (mmHg)	90.14 ± 9.81	89.94 ± 9.81	0.84
Antihypertensive medication, n (%)	339 (93)	149 (93)	0.98
Use of ACE-inhibitor, n (%)	139 (38)	46 (29)	0.04
Use of _-blocker, n (%)	230 (63)	99 (62)	0.77
Lipids and inflammation			
Total cholesterol (mmol/l)	5.64 ± 1.14	5.61 ± 1.04	0.76
HDL-cholesterol (mmol/l)	1.09 ± 0.33	1.11 ± 0.31	0.55
LDL-cholesterol (mmol/l)	3.57 ± 1.07	3.56 ± 0.88	0.91
Triglycerides (mmol/l)	1.92 [1.42-2.61]	1.89 [1.39-2.64]	0.59
hsCRP (mg/L)	2.13 [0.93-4.60]	2.64 [0.77-7.46]	0.30
Glucose homeostasis			
Glucose (mmol/l)	4.88 ± 1.38	4.83 ± 1.42	0.71
Insulin (μmol/L)	11.4 [4.80-16.33]	10.9 [7.90-14.75]	0.61
Diabetes mellitus, n (%)	66 (18)	28 (17)	0.86
Renal function			
Serum creatinine (μmol/l)	132 [111.75-167.00]	137 [114-169.5]	0.24
Creatinine clearance (ml/min)	62 [49-79]	57 [42-75]	<0.01
Urinary protein excretion (g/24h)	0.2 [0.00-0.50]	0.3 [0.00-0.60]	0.11
Proteinuria ≥0.5 g/24h, n (%)	92 (25)	60 (37)	0.01

	Depression score (SCL-90)		
	No-depression: score <25 (n = 366)	Possible depression: score ≥25 (n = 161)	P-value
Transplantation and history			
Dialysis duration (months)	25 [12-45.25]	31 [17-56]	<0.01
Living donor, n (%)	61 (17)	14 (9)	0.02
Time since transplantation (years)	5.81 [2.51-11.10]	6.52 [2.82-12.20]	0.53
Number of previous transplants			
- 0, n (%)	331 (90)	137 (85)	0.07
- 1 or more, n (%)	35 (10)	24 (15)	
Acute rejection, n (%)	163 (45)	74 (46)	0.76
Immunosupression			
Prednisolone dose, mg/d	9.17 ± 1.33	9.29 ± 1.21	0.59
Calcineurine inhibitor (CNI), n (%)	287 (78)	125 (78)	0.84
Proliferation inhibitor, n (%)	270 (74)	121 (75)	0.74
Tacrolimus (trough level,ug/l)	8.64 ± 2.76	8.64 ± 5.31	0.99
Ciclosporine (trough level,ug/l)	114.10 ± 47.98	112.99 ± 46.42	0.85

Data are represented as mean ± SD, or median [95% CI]. Differences were tested by t test or Kruskal Wallis test for continuous variables and with Chi- square for categorical variables.

Independent variables associated with depression in RTR are shown in table 2. Medically unfit for work, proteinuria, low physical activity level and longer prior dialysis duration were the most important independent determinants of depression in a logistic regression analysis.

Table 2. Independent determinants of depression

Multivariable	Exp(B) [95% CI]	P-value
Medically unfit for work	2.48 [1.52-4.04]	<0.001
Proteinuria (≥0.5 g/24h)	2.18 [1.34-3.53]	0.002
Physical activity (200 METS)	0.65 [0.44-0.98]	0.039
Dialysis duration (years)	1.08 [1.01-1.15]	0.028

During median follow-up for 7.0 [6.2 – 7.5] years, 114 recipients died, with 59 deaths being CV in origin. PTA was performed twice (3 %), PTCA was performed once (2%) and CABG was performed in 3 (5%) RTR. CV mortality was significantly increased in the depression group as compared to the no-depression group (27 (17%) versus 32 (9%), respectively p=0.004; Figure 1a). The same was found for all-cause mortality, with respective numbers of 64 (18%) and 50 (31%) (p <0.001, Figure 1b).

Results of the Cox regression analyses for the association of depression with CV and all-cause mortality are shown in table 3. Depression was strongly associated with increased risk for

CV mortality and all-cause mortality (Model 1). These associations were independent of age and gender (Model 2). Adjustment for dialysis duration (Model 3), creatinine clearance and proteinuria (Model 4), hemoglobin levels (Model 5) physical activity level (Model 6) and medically unfit for work (Model 7) did not materially change the association.

Figure 1a: Kaplan-Meier curve of cardiovascular mortality according to depression score groups tested with Log-rank test (P< 0.001).

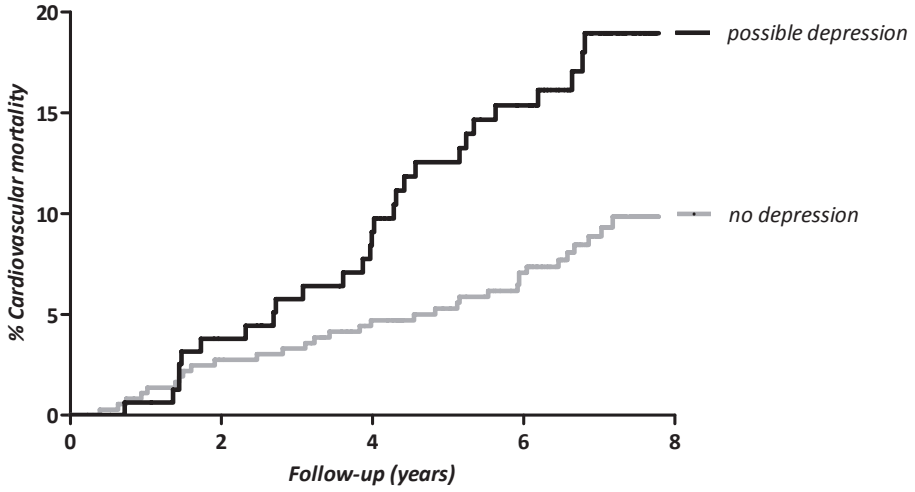

Figure 1b: Kaplan-Meier curve of all-cause mortality according to depression score groups tested with Log-rank test (P= 0.004)

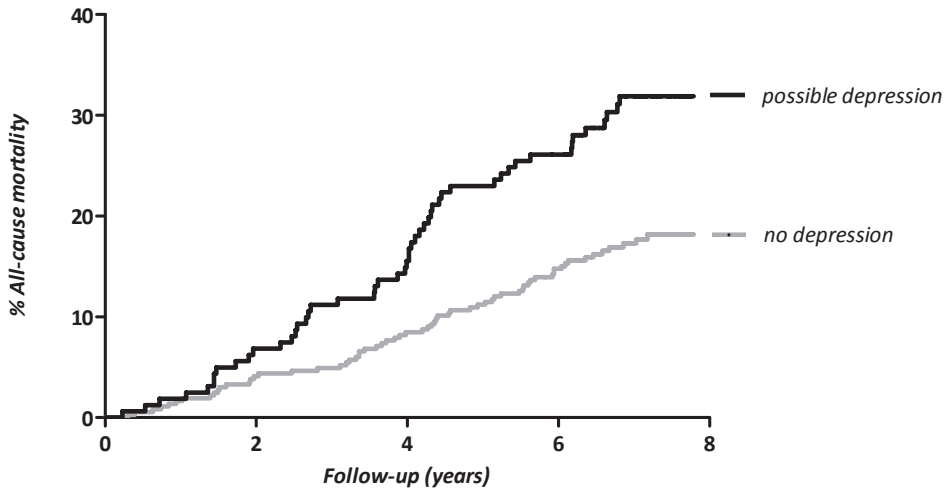

Figure 1c: Kaplan-Meier curve of graft failure according to depression score groups tested with Log-rank test (P= 0.04)

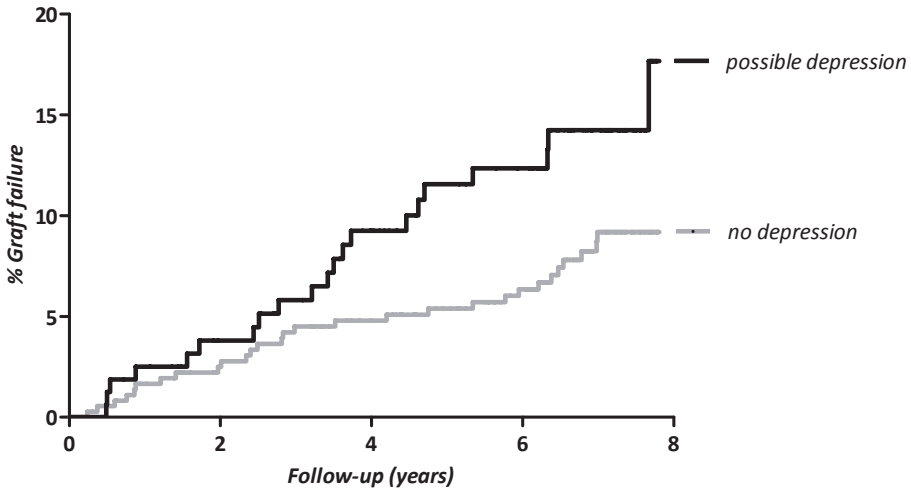

Table 3. Depression is associated with cardiovascular and all-cause mortality

	Cardiovascular Mortality			All-Cause Mortality		
Model	No depression	(n = 59) Possible depression		No depression	(n = 134) Possible depression	
	Reference	HR [95% CI]	P	Reference	HR [95% CI]	P
1	1.0	2.12 [1.27-3.53]	0.004	1.0	1.96 [1.36-2.84]	<0.001
2	1.0	2.06 [1.23-3.45]	0.006	1.0	1.92 [1.33-2.79]	0.001
3	1.0	1.94 [1.16-3.26]	0.012	1.0	1.89 [1.30-2.74]	0.001
4	1.0	1.84 [1.09-3.11]	0.022	1.0	1.74 [1.19-2.55]	0.004
5	1.0	1.85 [1.09-3.12]	0.022	1.0	1.74 [1.19-2.54]	0.005
6	1.0	1.82 [1.07-3.09]	0.026	1.0	1.73 [1.18-2.54]	0.005
7	1.0	1.73 [1.01-2.94]	0.045	1.0	1.61 [1.09-2.37]	0.016

Model 1: crude model.

Model 2: Model 1 + adjustment for age and gender.

Model 3: Model 2 + adjustment for dialysis duration (months)

Model 4: Model 3 + adjustment for creatinine clearance and for proteinuria (≥0.5 g/24h)

Model 5: Model 4 + adjustment for hemoglobin.

Model 6: Model 4 + adjustment for physical activity.

Model 7: Model 4 + adjustment for medically unfit for work.

Table 4. Association between depression and graft failure disappears after adjustment for kidney function

	Graft failure		
		(n=50)	
Model	No depression	Possible depression	
	Reference	HR [95% CI]	P
1	1.0	1.77 [1.01-3.10]	0.047
2	1.0	1.82 [1.04-3.20]	0.037
3	1.0	1.93 [1.09-3.41]	0.024
4	1.0	1.19 [0.66-2.11]	0.6

Model 1: crude model.

Model 2: Model 1 + adjustment for age and gender.

Model 3: Model 2 + adjustment for dialysis duration (months).

Model 4: Model 3 + adjustment for proteinuria (≥0.5 g/24h) and creatinine clearance

During median follow-up for 7.0 [6.2-7.5] years, a total of 50 (9%) RTR suffered graft failure necessitating return to dialysis. In the no-depression group, 29 (8%) of the RTR suffered from graft failure compared to 21 (13%) in the possible depression group (p=0.04, Figure 1c). Results of Cox regression for depression and graft failure are shown in table 4. Depression was associated with a higher risk for graft failure. After adjustments for proteinuria and creatinine clearance the relationship disappeared.

DISCUSSION

In this study we showed that the prevalence of depression after transplantation is high. Important independent variables associated with depression were medically unfit for work, proteinuria, low physical activity and longer dialysis duration. Furthermore we showed that depression was associated with CV and all cause mortality, and graft failure in RTR.

In our study, 161 (31%) RTR were categorized as depressed based on the depression cut-off score of the SCL-90 questionnaire. This number of occurrence is higher than in the general population (15.9%) (30), but comparable with other studies which were also based on self-reported depressive symptoms (14,31,32). The large study by Dobbels et al in patients from the United States Renal Data System, reported a much lower prevalence of 9-13 %, 3 years after transplantation (13). In that study, diagnoses of depression were based on Medicare claims which, as the authors say, probably underestimate the real prevalence of depression after renal transplantation (13).

We identified several important independent variables associated with depression after renal transplantation. In our study, medically unfit for work is the strongest independent variable

associated with depression. Medically unfit for work can be the consequence of a bad physical health, leading to more depressive symptoms. The other way around, depression can lead to a bad physical health, which can lead to being unable to work. Proteinuria was the second strongest independent variable associated with depression. The burden of disease in these patients is higher which gives more psychological distress and a higher risk for depression. Twenty four hour protein excretion was only retained in the final model of determinants of depression if it was included as a dichotomized variable with proteinuria defined as ≥0.5 g/24h, indicating that risk is particularly present in the high end of the distribution of 24h protein excretion. Physical activity is also a strongly associated with depression. Depression can negatively influence lifestyle behaviors like physical activity, which would add to risk consequences of low physical activity that is already present in RTR (15). We found that longer dialysis duration is a factor that is related to possible depression. RTR with a longer duration of dialysis have a longer history of chronic kidney disease, increasing the risk for co-morbidity and mortality (26). These findings are in line with the notion that depression can be caused by psychological distress from a higher burden of disease.

Besides these important variables described above we know from the literature that immunosuppressive medication could also influence depression (33). In the general population, use of corticosteroids is associated with depression (33). Brown (34) shows that the risk for depression appears to increase with higher corticosteroid doses. It is however difficult to determine whether depression is caused by steroidal treatment or by a higher burden of disease. In our study however, we didn't find a relationship between corticosteroids and depression. This may be the consequence of low variation in steroid doses in the population we investigated, which did not allow for us finding such a relationship. Tryptophan metabolism may provide a link between renal function and depression. Russo et al hypothesized about the role of tryptophan in psychopathology and somatic states (35). Even mild degrees of renal insufficiency as often encountered in RTR are associated with chronic low-grade inflammation. Chronic inflammation in turn leads to a high activity of Indoleamine 2,3-dioxygenase (IDO), resulting in low tryptophan levels, with insufficient amounts for the formation of serotonine, resulting in depression (36). In line with this, Pawlak et al. showed that dialyzed patients have significantly lower tryptophan than controls (37). Further research is needed to explore whether this mechanism plays a role in RTR.

The association of depression with mortality and graft failure could have been confounded by co-morbidity or a complex recovery after transplantation. Several chronic illnesses are associated with psychiatric co-morbidity. Noohi et al. (38) found an increased morbidity among depressed RTR, compared to non-depressed RTR. RTR with proteinuria are at higher risk for graft failure, co-morbidity and mortality (27). Patients with low GFR may have an increased physical symptom burden (39). In our Cox regression analyses for CV and all-cause mortality we therefore adjusted for creatinine clearance and for proteinuria. Up-on these analysis Hazard ratio for all-cause mortality

decreased from 1.89 [1.30-2.74] to 1.74 [1.19-2.55], remaining significant. Showing that creatinine clearance and proteinuria only explain a very small part of the association. The relationship between depression and graft failure was confounded by a deterioration of kidney function. Another potential confounder of the association of depression with mortality in RTR could be dialysis duration. Longer dialysis duration means that patients are longer exposed to the chronic effects of end-stage renal failure and dialysis treatment. Dialysis treatment is associated with altered inflammatory state, altered immunologic function and acceleration of atherosclerosis (40-42). Previous studies showed that dialysis duration was associated with risk for mortality and graft failure in RTR (26,43). In our study dialysis duration was strongly associated with symptoms of depression. This is in line with the study of Dobbels et al. (13), in which patients with high depressive symptoms had significant longer dialysis duration, compared to patients with moderate or low depressive symptoms. Although we found that dialysis duration was related to depression, it was not significant in the Cox regression analysis. As haemoglobin level is an important risk factor for outcome in RTR we additionally adjusted for hemoglobin levels (28), this adjustment did not change the results. We previously showed that low physical activity is a risk factor for mortality in RTR (15) therefore we adjusted for physical acticvity in our analysis. Upon this analysis physical activity level did explain a small part of the association. Unfit for work refers to the status that RTR are physically or mentally unfit for work, which can be a result of a long history of chronic kidney disease and transplantation with possible complications, together representing a substantial overall burden of disease. Adjustment for this confounder showed that part of the association was explained by medically unfit for work. Although we adjusted for al potential confounders residual confounding could not be ruled out.

There are several pathways that could explain the relationship between symptoms of depression and mortality. Depression could be a result of a long history of chronic kidney disease and transplantation with possible complications, together representing a substantial overall burden of disease. We will distinguish between biological, lifestyle and psychosocial factors to describe the possible mechanisms that could explain the relationship between depression and outcome after renal transplantation. Results from a large systematic-review showed that there is a link between depression and increased risk for CV disease (44). This is in line with our study, were CVmortality is strongly associated with depression. Research into possible mechanisms of depression in CV disease shows that much of the association remains unclear due to the complexity of the network of systems involved (45). Lifestyle factors are important factors in the association between depression and mortality. In our study depressive symptoms were associated with an unhealthy lifestyle. RTR with more depressive symptoms had significantly lower physical activity levels, and were more likely to consume more than 3 alcoholic drinks per day. These unhealthy lifestyle behaviors may directly or indirectly lead to mortality. We previously showed that low physical activity was a strong independent predictor for CV and all-cause mortality in RTR (15)(18). Whereas moderate alcohol

consumption is protective in RTR, alcohol dependence before transplantation is related to mortality and graft failure (46). Lifestyle factors are modifiable, which offers an opportunity for intervention towards a healthier lifestyle in RTR.

Psychosocial factors might modulate the relationship between depressive symptoms and mortality. A potential cause of death in patients with high depressive symptoms relates to compliance with therapy. DiMatteo et al showed that depressed patients had a 3-fold greater risk of nonadherence behavior (47). Depressive symptoms like feelings of hopelessness, difficulties with memory, may result in behaviors that include forgetting to take pills or missing regular follow-up appointments (48). In kidney and pancreas transplantation patients and patients with coronary artery disease, noncompliance with therapy adversely affects the recovery and subsequent life (48-50). In our study however we did not find any differences in trough levels of tacrolimus and cyclosporine.

In most clinics there is no active screening program for depression after transplantation. Based on the low antidepressant use in our study (4 %), depression after renal transplantation is presumably under diagnosed and under treated. Screening for depression in RTR could therefore be useful in this high risk population. Further research is needed to develop and evaluate these screening programs and consequently apply existing interventions studies targeting depression. Perceived control could be an important target for intervention, in managing the high stress levels in the recovery after transplantation. Cukor et al. found that transplantation recipients who had better perceived control over their outcome, had decreased levels of depression, compared with patients who attributed their health outcome to chance (51). In this respect, self-management is important. Self-management strategies should be stimulated and guided by the healthcare professionals and can be incorporated in the healthcare treatment plan. Self-management strategies can be used to target behaviors like: exercise, smoking and adherence to medication.

A strength of our study is its prospective design. RTR in this study were closely monitored by regular check-up in our clinic, which allows for extensive information gathering on patients status. We furthermore assessed depression using the Depression Subscales of SCL-90. This subscale measures only psychological depressive symptoms and no somatic symptoms of depression. This makes the depression subscale more suitable for measuring depression in RTR. Some depression questionnaires don't distinguish between depressive and somatic symptoms, which can lead to unreliable results in patients with chronic disease.

Some methodological topics warrant consideration. First, we used a self report questionnaire for measuring depression, which is informative but does not replace diagnosis of depression by psychiatric clinical assessment according to the DSM-IV criteria for major or minor depression. Nevertheless, questionnaires are valuable screening instruments for depressive symptoms in large epidemiological studies and the questionnaire was validated against the DSM-IV criteria for major

or minor depression, in stroke patients (24). It remains unclear, however, whether the validation of the cutoff values in stroke patients, 1 month after they experienced their first-ever ischemic stroke, may be of influence on the classification of depression in RTR (24). In a sensitivity analysis (data not published), the use of the depression score as a continuous variable were comparable to the findings of depression assessed with cutoff values. Next, this study is based on a single measurement design; it is possible that depressive symptoms could have changed over time. Pre-transplant information on depression is not available in this study. Multiple measurements of depression and pre-transplant information on depressive symptoms would have strengthened our results. Information on psychotherapy was unavailable in this study. Moreover this study is, observational in design and thus, conclusions on causality cannot been drawn.

In summary, depression is a condition that is very common among RTR. Independent risk factors for depression were medically unfit for work, proteinuria, lower physical activity level and longer dialysis duration. Our data show that depression after kidney transplantation is a serious condition associated with reduced patient and graft survival. Based on the association between symptoms of depression and mortality, it is tempting to speculate that reduction of depressive symptoms could contribute to improved survival in RTR. Additional studies on detection and treatment of depression after renal transplantation are needed.

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9

Discussion, future perspectives and conclusion

DISCUSSION

Prelude

The first successful kidney transplantation was performed by the team of Joseph Murray, on December 23, 1954, at Brigham Hospital in Boston (1). The transplantation was performed in identical twins. Ronald Lee Herrick donated his kidney to his brother Richard. This operation kept Richard alive for 8 years. The donor Ronald died at the age of 79, following complications from heart surgery. This landmark surgery was performed despite the warnings and scepticism of the experienced clinicians and scientists. Joseph Murray said the following about the ethical difficulties that they experienced:

> ***"This list of potential risks posed an ethical dilemma for us. While we routinely asked patients to incur some risk in order to achieve a benefit for themselves, none of us had ever asked a healthy person to accept this magnitude of risk solely for the sake of someone else. We consulted with experienced physicians within and outside of the Brigham, clergy of all denominations, and legal counsel before offering the option of transplantation. The team met several times with the family to describe in detail what was involved for Ronald and Richard. We advised neither for nor against the operation, and we stated the obvious: We could not know if it would work (2)."***

This successful kidney transplantation was the beginning of the modern era of transplant medicine. Doyle et al. gave an historic overview of renal transplantation, telling the success story of renal transplantation and addressed the most important problems (3). The major barriers to successful transplantation in the early years were technical requirements, acute rejection and infectious complications. An editorial by Kaplan and Meier-Kriesche with the appealing title: "Renal transplantation: A half century of success and the long road ahead", draws attention to the complexity of reaching improvement in long-term survival (4). Although renal transplantation increases life expectancy compared to dialysis treatment, survival of renal transplant recipients (RTR) is shortened by the high risk for cardiovascular death when compared to the general population (5).

RTR are at risk

Cardiovascular disease (CVD) is the main cause of death in RTR. Incidence and prevalence of CVD is estimated to be 4-6 times higher in RTR than in the general population (6,7). A number of traditional risk factors such as smoking, dyslipidemia, obesity and hypertension are known to be associated

with an increased risk for CVD in the general population but also in RTR (8,9). We showed that RTR have increases of various traditional risk factors that cluster in the metabolic syndrome (MS). Besides these traditional risk factors, transplant specific factors such as infections and side effects of immunosuppressants contribute to this increased risk (10,11). Moreover, the history of chronic kidney disease (CKD) and risk associated with dialysis treatment contribute to the adverse risk profile. Immunosuppresive medication is atherogenic and diabetogenic, it can deteriorate pre-existing medical conditions and cause post transplantation diseases, such as diabetes (12,13).

Biological Risk Factors: Metabolic syndrome and beta-cell dysfunction

RTR are at increased risk for new onset of diabetes after transplantation (NODAT) and CVD but not all RTR develop these co-morbidities. By early identification of high risk patients, interventions or treatment can be individualized to target the risk factors. Clinicians often use classical risk factors like obesity, dyslipidemia and hypertension to compose the risk profile of an individual patient. Beside these classical risk factors some new risk factors emerge in the literature. Non-alcoholic fatty liver disease (NAFLD) is one of those new risk factors. NAFLD is considered to be the new hepatic component of the MS and is associated with increased risk of CVD in the general population (14,15). In **chapter 2** we investigated the liver enzymes ALT, GGT and AP and their relationship with the MS. We hypothesized that the hepatic manifestation of the MS could be a new risk factor for cardiovascular related death in RTR. Indeed we found that ALT, GGT and AP were positively associated with prevalence of MS. Associations with abdominal obesity, hypertriglyceridemia and impaired fasting glucose were the strongest contributors in these relationships. Furthermore, we showed that increased levels of GGT and AP are associated with increasing cardiovascular and all-cause mortality. These associations were independent of age, sex, creatinine clearance, urinary protein excretion, and cardiovascular risk factors. In **chapter 3** we assessed the relationship between beta-cell dysfunction and development of NODAT. Increased circulating levels of proinsulin are seen as a marker of beta-cell stress when insulin demands required for maintenance of glycaemic control are relatively high for the prevailing beta-cell capacity, resulting in a 'spillover' of proinsulin. Both insulin resistance and beta-cell dysfunction are involved in the development of NODAT. We found that proinsulin, as a marker for beta-cell dysfunction was strongly associated with the development of NODAT in RTR, independent of known risk factors for NODAT. From previous studies we know that use of calcineurin inhibitors contribute to an increased risk for NODAT. This was confirmed in our study where the use of tacrolimus increased the risk for NODAT almost by three-fold. We furthermore showed that proinsulin could be a good biomarker for identification of RTR at risk for NODAT. Identification of RTR at risk for NODAT could allow for early intervention and treatment strategies to preserve beta-cell function.

Behavioural Factors: Alcohol consumption

Alcoholism and alcohol abuse can lead to various health problems such as malnutrition, depression, liver cirrhosis and cancer in the general population. Pre-transplant alcohol addiction is associated with early graft failure (16). However, research in the general population showed that moderate alcohol consumption is associated with reduced risk of diabetes, mortality and CVD when compared to abstainers and sporadic users of alcohol (17,18). In **chapter 4** we showed that moderate alcohol consumption has protective effects in RTR similar to the general population. We found no association between alcohol consumption and graft loss. The protective effect of moderate alcohol consumption can be explained by several mechanisms. Alcohol consumption can modulate the cardiovascular risk by changes in lipid profile, platelet aggregation, plasma fibrinogen, and insulin resistance (17,19-21). An important message is that in contrast to common belief, moderate alcohol consumption after transplantation does not appear to be detrimental and may be protective against diabetes and mortality.

Behavioural Factors: Diet and Physical activity

Obesity is a medical condition in which excessive body fat accumulates. After transplantation large changes occur in body composition (22). The use of corticosteroids, nutritional status, and low physical activity level can lead to muscle atrophy and accumulation of fat (22-24). These unfavourable changes in body composition are associated with an increased risk for CVD (25,26). To adequately measure body composition in RTR, water content should also be estimated. Although kidney function is restored in most patients, the hydration status after transplantation is often not constant. In **chapter 5** we showed that RTR gain substantial weight in the first year after transplantation. This weight gain could be completely explained by a gain in fat mass, no changes in muscle mass and water content were observed. Part of this weight gain may be related to dietary factors such as high consumption of energy-rich drinks and low intake of vegetables. This pattern could be a historical reflection of the diet that many patients consumed during their years on dialysis treatment, as patients on dialysis are advised to reduce the intake of potassium, phosphorus, sodium and liquid in their diet. While this diet was an advised choice during dialysis treatment, after restoring renal function these eating patterns might be unhealthy. We furthermore showed that low daily physical activity measured by an accelerometer (an objective device to measure physical activity) was a good predictor of post transplant weight gain.

In **chapter 6** we found that physical activity level is very low after renal transplantation. Moreover, physical activity was associated with an increased risk for cardiovascular and all cause mortality. The main determinant of exercise capacity in RTR is skeletal muscle strength. A primary lack in muscle mass before transplantation might impair RTR in their capacity to implement physical activity in their daily life after transplantation. Another reason for a low physical activity level might

be insecurity about being able to be physically active. Fear of injuring the kidney could play a role in this uncertainty. Patients with fear of movement, also called kinesiophobia, tend to avoid physical activity because it might cause pain or harm the patient. In **chapter 7** we showed that 28% of the RTR could be categorized as having kinesiophobia. Kinesiophobia was strongly associated with low physical activity level after renal transplantation. We identified important determinants of kinesiophobia: history of myocardial infarction, high anxiety score and lack of paid employment. The presence of kinesiophobia in RTR is an important finding, which can help us to identify barriers to physical activity in RTR.

In this thesis we identified multiple risk factors that, each on their own, increase the risk for a worse outcome after transplantation. Of course we should account for potential confounders and intermediates that could have influenced these relationships. For example we hypothesized that the association of physical activity with mortality could be confounded by risk factors for CVD, MS or smoking status. However, we found that the association between physical activity and outcome was not materially influenced by these adjustments, making the relationship between physical activity and mortality a very complex one. Physical activity has direct effects on better survival by cardio and vascular protective effects. On the other hand, physical activity can modulate several 'single' risk factors such as: obesity, blood pressure, triglyceride, glucose tolerance and insulin sensitivity. As physical activity acts on various risk factors simultaneously it could be very powerful in the prevention of CVD after renal transplantation. The classical dilemma of reversed causality remains. Is low physical activity causing a worse cardiovascular risk profile and thereby predisposing patients to a higher chance of premature death? Or is it the other way around, with a poor cardiac performance giving rise to low physical activity? To discuss this vicious circle I would like to give more insight in physical inactivity and its relation to chronic disease.

Low physical activity is physiologically abnormal

If we compare the Western society today with our ancestors, we lead a lifestyle that is much more sedentary (27). In the time of the hunter-gatherer society, physical labour was crucial for survival (28). For the preparation of food, shelter, clothing and tools, physical labour was needed (28). After the industrial revolution, labour became less physical, and occupation demanding much physical effort became rare. The average energy expenditure (per unit body size) of contemporary humans is only 40% of that of our human ancestors (27). Our human population is progressively becoming inactive. According to the World Health Organization in 2008, 31% of the adults of the world population were inactive, meaning that they perform less than 30 minutes of moderate physical activity per day (29). Inactivity was highest in the region of the Americas and the Eastern Mediterranean Region, with almost 50% of women being inactive, while for men this was 40% in the Americas and 36% in Eastern Mediterranean countries. It is estimated that approximately 42% of the

Dutch population fail to achieve this minimum recommendation of 30 minutes daily (30). Inactivity levels in our renal transplant population are somewhat higher, with 48% of RTR not meeting the criteria for minimal physical activity and 15% of RTR being completely inactive. Physical inactivity is an important trigger for the development of several chronic diseases. Inactivity is underlying 17 unhealthy conditions and it causes 1.9 million deaths each year (31) (Table 1).

Table 1: Unhealthy conditions precipitated by physical inactivity

Unhealthy Condition
Hypertriglyceridemia
Hypercholesterolemia
Hyperglycemia
Insulin resistance
Increased thrombosis
Increased resting blood pressure
Increased risk of myocardial ischemia
Increased incidence of lethal ventricular arrhythmias
Decreased cardiac stroke volume and maximal cardiac output
Obesity
Type 2 diabetes
Breast and colon cancer
Osteoporosis
Sarcopenia
Back pain
Gallstone disease
Decreased psychological well-being

The genetic makeup of the modern Homo sapiens changed only very little during the past 10,000 years (32). It is speculated that our human body has a biological requirement for a certain amount of physical activity (31,32). Our modern inactive lifestyle does not provide the necessary metabolic fluxes and muscle loading needed and thereby disturbs the normal homeostatic mechanisms to maintain health. Physical inactivity can be seen as physiologically abnormal and contributes to chronic health conditions such as CVD, hypertension and type 2 diabetes (31,32).
In 1988, Eaton et al (33). wrote

> ***"From a genetic standpoint, humans living today are Stone Age hunter-gatherers displaced through time to a world that differs from that for which our genetic constitution was selected. Unlike evolutionary maladaptation, our current discordance has little effect on reproductive success; rather it acts as a potent promoter of chronic illnesses: atherosclerosis, essential***

hypertension, many cancers, diabetes mellitus, and obesity among others. These diseases are the results of interaction between genetically controlled biochemical processes and a myriad of biocultural infuences – lifestyle factors – that include nutrition, exercise, and exposure to noxious substances."

Psychosocial Factors

Besides behavioural factors, we should pay attention to the social and psychological aspects after renal transplantation. The final goal after renal transplantation is not only to restore health, but also to return to ´normal´ life. The change from dialysis treatment to transplantation demands adaptation and new coping strategies. Although survival perspectives and quality of life often improve after renal transplantation, successful outcomes are not ensured and patients have to face new challenges. RTR have to comply to a strict immunosuppressive regime with associated side effects, have many hospital visits, are at risk for co-morbidities and often experiences feelings of uncertainty about possible rejection or disease. In **chapter 8** we found that prevalence of depression was high, 31% of the RTR were categorized as depressed according to our questionnaire. Medically unfit for work, proteinuria, low physical activity level and longer prior dialysis duration were the most important independent determinants of depression. Furthermore, we found that depression was associated with cardiovascular and all cause mortality, and graft failure in RTR. It is hard to determine the causality in this relationship. The association of depression with mortality and graft failure could have been confounded by co-morbidity or a complex recovery after transplantation. In our analysis we have tried to correct for these confounders but adjustment did not materially change these associations. The main message of our study, however, should not be focussed on the causality of the relationship, rather we want to show that depression is a condition that is very common among RTR, and moreover that depression is a serious condition associated with reduced patient and graft survival. Depression after transplantation is probably under diagnosed and under treated. Therefore additional studies on detection and treatment of depression after renal transplantation are needed.

Pharmacological interventions

As RTR are vulnerable to CVD, the management of CVD should be incorporated in long-term transplantation care. Although it is obvious that good management of cardiovascular risk factors is warranted for patient and graft survival, unfortunately there is a lack of scientific evidence regarding prevention and management of CVD in RTR. Blood pressure is one of the main modifiable risk factors targeted with pharmacotherapy. The National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines have recommended blood pressure goals of <130/80 mmHg for all RTR. Most RTR need a combination of drugs to control blood pressure. The choice

of initial antihypertensive agent in our hospital often consists of calcium-channel blockers, beta-blocker or diuretics in patients with oedema. Unfortunately there are no randomized controlled trials that investigated the effects of blood pressure control on graft survival or CVD. Dylipidemia is another important modifiable cardiovascular risk factor. The treatment of dyslipidemia is based on the recommendations of the National Cholesterol Education Program (NCEP) III and the NKF K/DOQI guidelines. Recommended target goals are low-density lipoproteins (LDL) cholesterol <100 mg/dl, non- high-density lipoproteins (HDL) cholesterol (calculated as: total cholesterol—HDL cholesterol) <130 mg/dl and a triglyceride concentration <150 mg/dl. The only randomized controlled trial that was done in RTR found that treatment of LDL-cholesterol with fluvastatin did not significantly reduce the primary end point, however a 35% risk reduction in the incidence of fatal and non-fatal myocardial infarction was seen in the treatment group (34). Controlling glucose levels is also an important part of cardiovascular risk management. Early detection of NODAT is essential because timely and adequate treatment may prevent the serious complications of diabetes. RTR should be regularly screened for NODAT, starting directly after transplantation, since tacrolimus, cyclosporin, and corticosteroids can cause NODAT (12,35,36). The choice of immunosuppressive medication could be individualized to minimize the risk for NODAT. Once NODAT is diagnosed HbA1c and glucose should be measured regularly. Preferred pharmacological agents in our hospital are Metformin, Sulfonylureas, Meglitinides and Insulin.

Integrated approach

Despite the efforts to manage cardiovascular risk profile with pharmacological interventions, classical cardiovascular risk factors are generally not fully controlled. Therefore, lifestyle modification may be the next target in cardiovascular risk management after transplantation. Ideally treatment of cardiovascular risk factors like hypertension, dyslipidemia and NODAT should consist of a combination of drug therapy and lifestyle modification. Figure 1 shows the complex relationship between medication, physical activity, diet, and associated risk factors with outcome after transplantation.

For instance, reduction of sodium intake to recommended amounts could reduce SBP substantially in RTR (37). Weight-loss, limited alcohol intake and regular exercise are other lifestyle factors that can positively influence cardiovascular risk factors in RTR. Although lifestyle modification could be an important tool in management of cardiovascular risk, transplant centres unfortunately often do not have the tools to offer such programs to their patients.

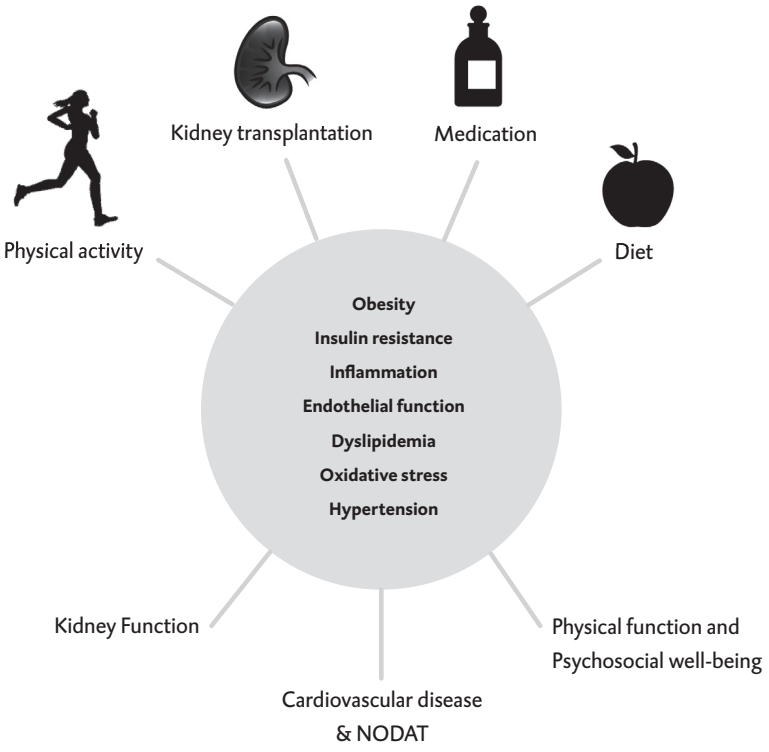
Lifestyle modification

There is decisive evidence that regular physical activity is effective in the primary and secondary prevention of several chronic diseases (e.g., CVD, diabetes, cancer, hypertension, obesity,

depression and osteoporosis) and premature death (28,31). Various studies investigated the effects of lifestyle interventions including physical activity in the prevention of diabetes mellitus type 2 (38,39) in the general population. The Finnish Diabetes Prevention Study was the first randomized controlled trial to investigate the feasibility and efficacy of lifestyle intervention in high-risk subjects (40). The intervention group received individualized counselling aimed at reducing weight and intake of total and saturated fat, and increasing intake of fiber and physical activity level. Results of this study showed that risk of diabetes was substantially reduced, by 58% in the intervention group compared with the control group. There are only few studies that investigated the effects of lifestyle interventions in RTR. Sharif et al. showed that lifestyle modification is beneficial for RTR with glucose intolerance. Intensive lifestyle modification (dietician, exercise program and weight loss advice) resulted in 15% improvement in 2-hr postprandial glucose (41).

Recent evidence also suggests that physical activity is associated with a slower progression of renal function decline (42). It can be concluded that the beneficial effects of regular physical activity are clearly known and that many of these health benefits can contribute to a better health and well-being of RTR, and therefore deserve more attention after renal transplantation.

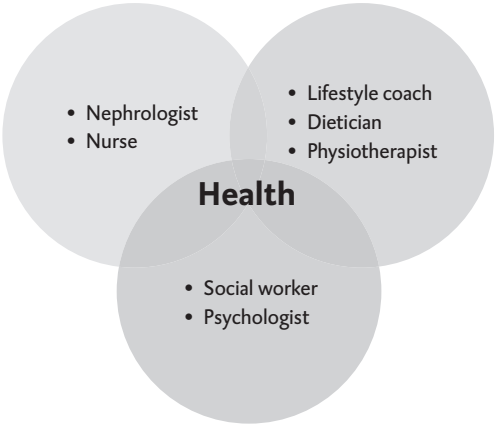
Figure 1. Role of physical activity and diet, risk factors and health



From other patient groups we know that lifestyle interventions are feasible and effective. For example, rehabilitation is very successful after a lung transplantation or cardiac surgery (43,44). Throughout the Netherlands specialized rehabilitation programs exist to target needs of various patient groups. For renal patients such a program is still not available. We should therefore aim to develop such programs in the near future. Lifestyle interventions could also be used to target NODAT, MS and NAFLD after transplantation. Studies in the general population showed that NAFLD can be effectively treated with weight loss interventions by increasing physical activity and restricting energy intake (45,46). Besides improvements of the risk profile, physical activity intervention studies should aim at increasing fitness level and muscle strength. From previous studies we know that the maximal aerobic capacity after renal transplantation is not different from dialysis patients and significantly lower than aerobic capacity in the general population (47,48). Maximal aerobic capacity of many RTR is comparable to aerobic capacity of heart failure patients. This has major consequences for the level of physical functioning of the patient; every day household work, climbing the stairs or doing groceries are demanding the patients' maximal effort. To conclude: both physical activity and diet are important targets for lifestyle intervention programs after renal transplantation. Before lifestyle programs can be implemented in regular care, a randomized controlled trial is needed. A randomized controlled trial can give an objective evaluation of the effects of a lifestyle intervention. Health care and insurance companies should be involved in evaluation of cost effectiveness, to ensure reimbursement of the rehabilitation costs for the patients.

To make large intervention studies targeting both physical activity and diet as well as psychosocial aspects a success, a multi disciplinary approach is required. Nephrologists, lifestyle coaches, social workers, dieticians and physiotherapists should work together as a team to achieve these goals (Figure 2).

Figure 2. Model for multi disciplinary approach in renal transplant health care.



This model is illustrating the multi disciplinary approach for management of general health, physical activity and diet and psychosocial well-being after renal transplantation. The nephrologist can refer a RTR to the lifestyle coach and to the social worker or psychologist. The lifestyle coach can fulfill a central role in the management of the integrated health care approach. By a general intake assessing body composition, dietary patterns and physical activity level, the lifestyle coach can compose a specific lifestyle program with personal goals. To successfully achieve these goals, self management strategies are important and can be guided and stimulated by all healthcare professionals. The lifestyle coach can refer the RTR to a dietician or physiotherapist for more specialized care. It is important that the nephrologist informs the lifestyle coach on general health parameters as well as specific lifestyle factors such as urinary sodium excretion. For this model to be successful, all disciplines should work together, feedback should be provided on patients' health, lifestyle changes and psychosocial well-being to the healthcare workers that are involved.

FUTURE PERSPECTIVES

Prediction

Forecasting is the process of making statements about events whose actual outcomes have not yet been observed. Attempting to forecast the future has been practised by humans for ages. Astrology is one of the oldest examples of predictions of the future. The broad definition of astrology is the search for meaning in the sky. This concept dates back to the time when humans first started to measure, record, and predict seasonal changes by reference to astronomical cycles (46). Astrology in our modern time, also known as horoscope, uses positions of the sun, moon, and other planetary objects at the time of their birth to predict future events. Another well known type of prediction is weather forecasting. Weather forecasting is a different way of making predictions than astrology. Weather forecasts are based on quantitative data about the atmosphere and scientific models are used to predict how the atmosphere will evolve over a certain time period. The principles of predictions of weather are quite similar to the prediction of disease in humans – although the prediction of disease is much more complex. A similarity to weather forecasts is that prediction of disease is not always correct. The accuracy of disease prediction is often evaluated by testing the discrimination of a prediction model. In general discrimination refers to the ability of a model to distinguish well between individuals with and without the incident disease. Emerging biomarker studies show us that individual novel biomarkers have good predictive properties but often have limited additive value on top of existing prediction models containing robust and classical risk factors like: age, sex, BMI and blood pressure. The actual value of these novel biomarkers should be carefully evaluated, as measurement costs of these biomarkers are often very high. Another major consideration in evaluating biomarkers is their value for clinical practice. Many novel biomarkers are a surrogate marker for disease or damage in the human body but don't have any role in the pathophysiological process of the disease and could therefore not be targeted by interventions. Should we continue to search for just another new biomarker which has no additive value in prediction and which is too expensive to measure in every day clinical practice? Or should medical

research focus more on biomarkers that could be target for intervention? The last option is more in line with my perspective of modern research. In this thesis we use prediction as a tool to identify modifiable risk factors, that can be incorporated in future intervention studies. Hereby we took the first step in the direction of applied lifestyle research in RTR.

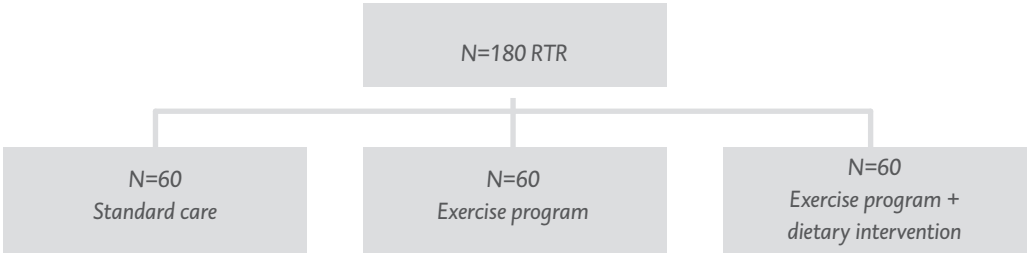
Psychosocial

"Mens sana in corpore sano" often translated as, "A healthy mind in a healthy body". It is an ancient Latin quote, which already showed the importance of both a good mental and physical health. It is derived from the Satire X by the poet Juvenal (50). The vision of this poem fits within the modern holistic view on health. The physical part of transplantation, the surgery and co-morbidities has not only impact on the patient's body but just as much on the mind. As our results highlight, the population of RTR is at high risk for depression, making the need for good detection methods more urgent. Besides early detection of depression, prevention of depression could also be a target of intervention. Transplant health care should provide sufficient support on personal psychological health but also on social participation, reintegration and rehabilitation. Therefore, research should also focus on the needs for psychological support after transplantation. By the use of focus groups one could determine what the most common problems are where RTR would need professional help. From our research in **chapter 6** we learned that kinesiophobia is a psychological factor that is common in RTR and acts as a barrier to engage in physical activity. To target this fear of movement among RTR, sufficient information about physical activity should be provided. Together with the Dutch Kidney Foundation we developed a brochure on physical activity after renal transplantation. This brochure contains information about the importance of exercise and physical activity after renal transplantation. It also includes practical tips. Besides this practical information, we also share some stories of patients for which physical activity plays an important role in their everyday life. This brochure should help RTR to engage in physical activity in daily life. The brochure will be available at the offices of nephrologists, general practitioners, physiotherapy practices and kidney patient associations.

Group Rehabilitation for kidney patients, Active Care after Transplantation

As our data in **chapter 4** shows, RTR gain substantial weight in the first year after transplantation. Moreover, we showed that diet and physical activity are both valuable targets for lifestyle intervention. In cooperation with Maastricht University Medical Centre we developed a randomized controlled intervention trial with a combined diet-and- physical activity approach: Group Rehabilitation for kidney patients, Active Care after Transplantation (GRN: ACT). For this study 180 RTR will be randomized over 3 groups: standard care, exercise program and exercise program + dietary intervention (Figure 3). Participants in this study will be monitored for 15 months.

Figure 3. Randomisation scheme GRN:ACT



GRN: ACT has two primary aims:

1. To study the effects of an exercise program and combined exercise and dietary intervention on exercise capacity, muscle strength, dietary habits, quality of life and social participation.
2. To study the effects of an exercise program and combined exercise and dietary intervention on the development of adiposity and its adverse cardio metabolic effects.

Furthermore, we will study success factors and barriers of the intervention such as kinesiophobia, to optimize efficacy of the intervention and facilitate future implementation.

Standard care

The standard care group will receive standard care after transplantation. Participants will be advised to be active according to the Dutch guidelines for physical activity.

The exercise program

The exercise program will be carried out in a rehabilitation clinic by an experienced and trained physiotherapist or movement specialist. Participants will visit the rehabilitation clinic 2 times per week for an exercise session of one and an half hour. The exercise program starts with endurance exercises on bicycle and treadmill, followed by an individualized dynamic strength training program. After a social break the program continues with 30 minutes sport games or activities in the swimming pool. During the program a movement diary will be used to stimulate the participant to log physical activity and helps to overcome barriers. After 3 months participants will receive an individual sport and movement advice by the physiotherapist as well as individual counselling by a lifestyle coach. This counselling is based on theories of behavioural change and motivational interviewing and helps to consolidate achieved healthy dietary habits and levels of physical activity in daily life.

Exercise program and dietary intervention

The combined exercise and diet group will receive the same exercise program completed with a dietary intervention. During the first period of the program participants are consulted by their dietician every 2 weeks. In these consults participants are coached to achieve a healthy diet. Advises

are based on the results of a three-day nutritional diary that the participant kept in the days before the consult. The main goal of the dietary intervention is prevention of weight gain by a balanced energy intake. Intervention topics are energy intake from high energy drinks, salt intake, intake of fat and consumption of fruit and vegetables according to the Dutch guidelines.

Measurements

To correctly capture the effects of a lifestyle intervention, good measurements are inevitable. A battery of tests will be performed at baseline, 3 months, 6 months and 15 months after inclusion. Sophisticated measures of physical fitness and physical activity will be used to evaluate the effects of our intervention. Physical fitness is composed of two components: aerobic fitness and muscle strength. VO2max will be used as a measure of aerobic fitness. VO2max is the maximum capacity for oxygen consumption by the body during maximum exercise. Muscle strength will be measured with a maximal strength test for various muscle groups. Daily physical activity will be evaluated by the Baecke questionnaire and will be quantitatively measured with an accelerometer. Furthermore, blood and urine samples will be collected for storage at all time points. To evaluate the social and psychological aspects of the intervention, participants will receive various questionnaires containing i.a. questions about quality of life, depression, kinesiophobia, coping strategies and employment status. Dietary habits are monitored by a nutritional diary and measured by urinary parameters like salt excretion. Weight, hip- and waist circumference will be measured to monitor post transplant obesity. A multi frequency bioelectrical impedance analysis will be used to determine body composition.

GRN: ACT is the first randomized controlled intervention study that examines the effects of a combined approach to improve the overall health of RTR. The well considered design of the study, together with the exceptional dedication of our multidisciplinary team is expected to make GRN:ACT to a great success. Although the program is quite demanding patients are very enthusiastic about this new experience. The ultimate goal of GRN: ACT is to implement a lifestyle rehabilitation program for RTR in regular care, thereby making it available for all RTR.

CONCLUSION

As illustrated throughout the different chapters in the thesis there is a great opportunity to reduce cardiovascular risk in RTR by lifestyle modification. Modifying diet and physical activity has multiple positive spin-offs, by influencing several cardiovascular risk factors as well as direct positive effects. Future results from our randomized controlled intervention study will provide formal evidence for the role of lifestyle management in long-term graft and patient survival. The setting of renal transplantation provides excellent opportunities for prevention of cardio metabolic and lifestyle related complications, as an area where large health benefits can be gained. Lifestyle management should be a primary target in current transplantation medicine.

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Nederlandse samenvatting en discussie

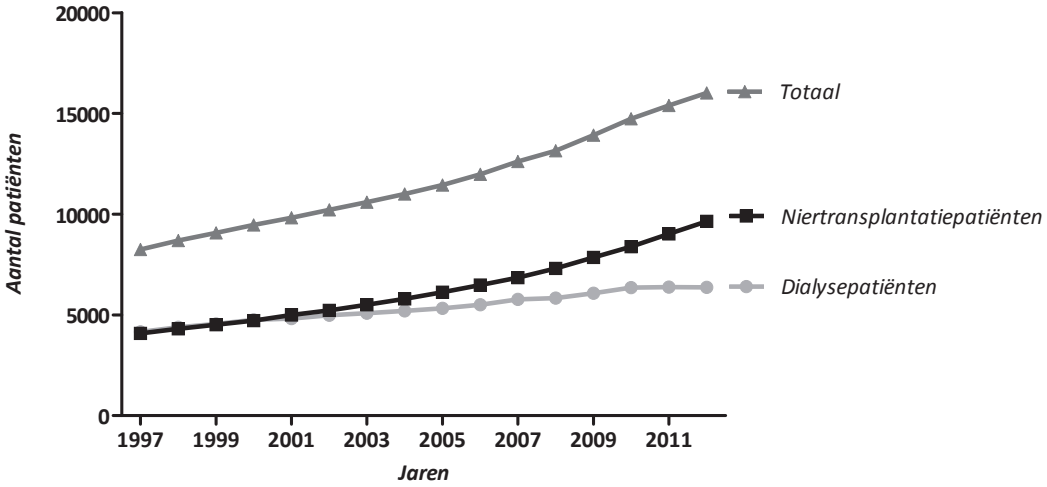
SAMENVATTING

Chronische Nierziekte

Het aantal patiënten met een chronische nierziekte stijgt wereldwijd. De belangrijkste risicofactoren voor het ontstaan van nierziekte zijn: leeftijd, diabetes mellitus, hypertensie (hoge bloeddruk), roken, overgewicht en cardiovasculaire ziekten. Patiënten met chronische nierziekte hebben een verhoogd risico op cardiovasculaire ziekten, nierfalen en vroegtijdig overlijden.

Het aantal patiënten met chronische nierziekte is het afgelopen decennium bijna verdubbeld. Volgens het bureau Registratie Nierfunctie Vervangende therapie Nederland (RENINE) waren er in 1997 8,243 patiënten met ernstige chronische nierziekte terwijl dit is opgelopen tot 16,018 in 2012 (Figuur 1). Deze stijging kan niet volledig verklaard worden door de vergrijzing. Waarschijnlijk is dit ook een weerspiegeling van de stijging in overgewicht en diabetes, belangrijke risicofactoren voor nierschade.

Figuur 1. Verdubbeling van het aantal patiënten met chronische nierziekte.

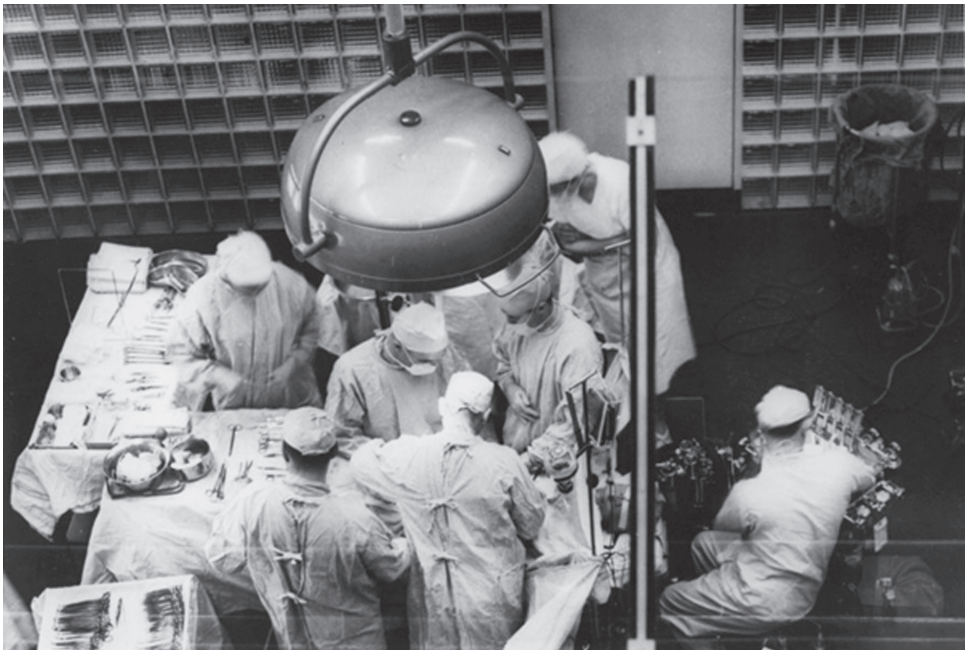


Transplantatie

Voor patiënten in het eindstadium nierfalen is niertransplantatie in veel gevallen de beste behandeling. De eerste succesvolle niertransplantatie werd uitgevoerd in 1954 door het transplantatie team van Joseph Murray, in het Brigham ziekenhuis in Boston. De transplantatie werd uitgevoerd bij een identieke tweeling. Ronald Lee Herrick doneerde zijn nier aan zijn broer Richard. De operatie was succesvol en Richard leefde nog 8 jaar met zijn nieuwe nier. De donor Ronald stierf op de leeftijd van 79 aan de complicaties van een hartoperatie. Ten tijde van de operatie was de medische wereld erg sceptisch over de uit te voeren transplantatie.

Joseph Murray zei het volgende over de ethische dilemma's die werden ervaren:

> ***"De vele potentiële risico's stelden ons voor een ethisch dilemma. Normaal gesproken maken we een afweging van de risico's van een operatie en de beoogde gezondheidswinst voor de patiënt. Niemand van ons had ooit aan een gezond persoon gevraagd om een groot risico te accepteren om het leven van iemand te redden. Voordat we de operatie aan de patiënt aanboden hebben we om advies gevraagd aan ervaren artsen, dominees en een medische raad. Het operatieteam heeft de risico's en procedures van de operatie veelvuldig doorgesproken met de familie. Uiteindelijk adviseerden we niet voor of tegen de operatie, we zeiden het volgende: 'We weten niet of de operatie succesvol zal zijn'."***



De eerste niertransplantatie op 23 december 1954. Tweede van links plastisch chirurg J. Murray.
Foto: Center for the History of Medicine

Na een niertransplantatie is acceptatie en goed functioneren van het nieuwe orgaan de belangrijkste prioriteit. Door de komst van nieuwe afweeronderdrukkende medicijnen is de korte termijn overleving van het donororgaan sterk verbeterd. Op de lange termijn gaan er echter nog veel nieren verloren. De helft van alle getransplanteerde nieren gaat verloren binnen 10 tot 12 jaar

na transplantatie. De belangrijkste oorzaken hiervan zijn chronische transplantaat dysfunctie en verhoogde cardiovasculaire sterfte.

Niertransplantatiepatiënten hebben verhoogd risico op sterfte

Cardiovasculaire ziekten zijn de primaire doodsoorzaak na niertransplantatie. Cardiovasculaire ziekten komen na een niertransplantatie 4-6 keer vaker voor dan in de algemene populatie. Traditionele risicofactoren zoals roken, hoog cholesterol en hypertensie dragen bij aan dit hoge risico. Naast deze traditionele factoren zijn er ook factoren specifiek voor transplantatiepatiënten; langdurig dialyseren, het gebruik van afweeronderdrukkende medicijnen en virusinfecties vergroten ook het risico op cardiovasculaire ziekten.

Doel thesis

Dit proefschrift beschrijft onderzoek naar de risicofactoren voor diabetes en sterfte na niertransplantatie. Daarbij hebben we gekeken naar de invloed van psychologische factoren en de rol van leefstijl na niertransplantatie.

Biologische Risicofactoren: Metabool syndroom en beta-cel dysfunctie

Niertransplantatiepatiënten hebben een verhoogd risico om diabetes te ontwikkelen. Het is van groot belang patiënten met een hoog risico op diabetes vroegtijdig te herkennen, zodat een passende behandeling ingezet kan worden. Artsen gebruiken vaak klassieke risicofactoren zoals hypertensie en overgewicht voor het bepalen van het individuele risico van de patiënt. Naast deze klassieke risicofactoren worden er ook steeds nieuwe risicofactoren ontdekt. Een van deze nieuwe risicofactoren is een vette lever. Risicofactoren voor het ontstaan van een vette lever zijn obesitas, hypertensie, verminderde gevoeligheid voor insuline en een ongunstige samenstelling van de vetten in het bloed. Wanneer drie van deze factoren tegelijkertijd aanwezig zijn spreekt men van het metabool syndroom. In **hoofdstuk 2** hebben we onderzoek gedaan naar de leverenzymen die verhoogd zijn bij een vette lever en hun relatie met het metabool syndroom. Onze hypothese is dat een vette lever als onderdeel van het metabool syndroom een nieuwe risico factor is voor cardiovasculaire sterfte na niertransplantatie. Uit ons onderzoek blijkt dat de leverenzymen sterk gerelateerd zijn aan het metabool syndroom. De sterkste relatie werd gevonden met buikomvang, hoge triglyceriden en de verminderde gevoeligheid voor insuline. Daarnaast gaven hoge concentraties van de leverenzymen GGT en AP een aanzienlijker hogere kans op sterfte. Deze relaties werden niet beïnvloed door leeftijd, geslacht, nierfunctie of andere risicofactoren.

In **hoofdstuk 3** onderzochten we de relatie tussen beta-cel dysfunctie en het ontstaan van posttransplantatie diabetes. Beta-cellen produceren insuline voor het reguleren van de

bloedsuikerspiegel. Een verhoogde concentratie pro-insuline in het bloed duidt vaak op schade aan de beta-cellen. Verminderde gevoeligheid voor insuline en beta-cel dysfunctie zijn de belangrijkste oorzaken van het ontstaan van posttransplantatie diabetes. Uit eerder onderzoek is gebleken dat het gebruik van specifieke afweeronderdrukkende medicatie (Tacrolimus) de beta-cellen beschadigt en het risico op diabetes verhoogt. Onze resultaten bevestigen dat het gebruik van Tacrolimus het risico op posttransplantatie diabetes verdrievoudigt. Daarnaast kan de pro-insuline concentratie in het bloed gebruikt worden om te bepalen welke patiënten het hoogste risico hebben op diabetes. Het tijdig herkennen van patiënten met een hoog risico is belangrijk om zo vroegtijdig een behandeling in te zetten en hiermee de beta-cel functie te sparen.

Gedragsgerelateerde Factoren: Alcohol consumptie

Onderzoek in de algemene populatie laat zien dat matig alcohol gebruik een lagere kans geeft op het ontstaan van diabetes en tevens de kans op cardiovasculaire aandoeningen verlaagt.
In **hoofdstuk 4** hebben we aangetoond dat matig alcohol gebruik bij niertransplantatiepatiënten dezelfde gunstige effecten heeft als in de algemene populatie. We vonden geen relatie tussen alcohol consumptie en verlies van de transplantaatnier. Het beschermende effect van matige alcohol consumptie kan verklaard worden door verschillende mechanismen. Matige alcohol consumptie zorgt voor verbetering van de insulinegevoeligheid, verhoging van het HDL-cholesterol en heeft gunstige antistollingseffecten. Tot nu toe zijn de adviezen over alcoholconsumptie voor patiënten met een niertransplantatie zeer terughoudend. Amerikaanse adviezen op het internet zijn veelal: drink geen alcohol en overleg met je arts. Een belangrijke boodschap uit ons onderzoek is dat matige alcohol consumptie niet schadelijk is voor de nier en misschien zelfs beschermend werkt tegen diabetes en sterfte.

Gedragsgerelateerde Factoren: Voeding en inactiviteit

Na transplantatie verandert de lichaamssamenstelling. Het gebruik van afweeronderdrukkende medicatie, inactiviteit en voedingstoestand leiden tot spierafbraak en een toename in vetmassa na transplantatie. Deze ongunstige veranderingen in lichaamssamenstelling verhogen het risico op cardiovasculaire ziekten. Voor het nauwkeurig meten van de lichaamssamenstelling bij niertransplantatiepatiënten, is het belangrijk om rekening te houden met de vochttoestand. Hoewel de volume regulatie na transplantatie in de meeste patiënten voor een groot deel is hersteld, is de water- en zouthuishouding soms toch niet optimaal, zodat patiënten teveel vocht vasthouden. Overgewicht is een bekend probleem na transplantatie. In **hoofdstuk 5** laten we zien dat niertransplantatiepatiënten flink aankomen in het eerste jaar na transplantatie. Deze gewichtstoename kon geheel verklaard worden door een toename in vetmassa, er werden geen veranderingen in spiermassa of vocht waargenomen. Tevens zijn de oorzaken van deze

gewichtstoename onderzocht. Hieruit bleek dat een deel van de gewichtstoename gerelateerd was aan voedingsgewoonten zoals hoge inname van energierijke dranken en lage inname van groenten. Deze patronen zouden een reflectie kunnen zijn van het dieet dat de patiënten gebruikten tijdens de dialysebehandeling, voorafgaand aan de transplantatie. Dialysepatiënten worden geadviseerd om de inname van vocht en bepaalde groenten en fruit te beperken, en voor hun energiebehoefte calorierijke dranken te gebruiken. Tijdens dialyse is dit dieet de beste optie, maar na transplantatie zou dit dieet ongezond kunnen zijn. Daarnaast hebben we lichamelijke activiteit gemeten met een accelerometer (objectief instrument om beweging te meten). Te weinig lichaamsbeweging was een belangrijke voorspeller voor gewichtstoename na transplantatie.

In **hoofdstuk 6** hebben we onderzoek gedaan naar bewegen na transplantatie. Uit onze resultaten blijkt dat niertransplantatiepatiënten een zeer lage lichamelijke activiteit hebben in vergelijking met de algemene populatie. Deze lage lichamelijke activiteit was een sterke voorspeller van een hogere kans om vroegtijdig te overlijden aan een cardiovasculaire aandoening of andere doodsoorzaak. Uit eerder onderzoek bleek spiermassa de belangrijkste determinant van inspanningscapaciteit bij niertransplantatiepatiënten. De lage spiermassa, veroorzaakt door een lange voorafgaande periode van nierziekte en door de behandeling daarvan, kan niertransplantatiepatiënten beperken in het implementeren van dagelijkse activiteit na transplantatie. Mogelijk speelt ook angst en onzekerheid over bewegen een rol in de lage lichamelijke activiteit. Angst om de nieuwe nier te beschadigen tijdens bewegen zou een rol kunnen spelen in deze onzekerheid. Patiënten met zogenaamde bewegingsangst vermijden bewegen omdat zij denken dat dit pijn kan veroorzaken of schadelijk is. Wij laten in **hoofdstuk 7** zien dat 28% van de niertransplantatiepatiënten bewegingsangst heeft. De mate van bewegingsangst was sterk gerelateerd aan lichamelijk activiteit na transplantatie. Uit dit onderzoek kwamen belangrijke determinanten van bewegingsangst naar voren. Wanneer een patiënt in het verleden een hartinfarct heeft gehad verhoogt dit de kans op bewegingsangst. Ook angstige patiënten en patiënten zonder betaalde baan hadden meer kans op bewegingsangst na transplantatie. Hiermee laten we zien dat bewegingsangst na transplantatie waarschijnlijk een belangrijke factor is bij het gebrek aan lichaamsbeweging na transplantatie. Met de uitkomsten van dit onderzoek hopen we in de toekomst de barrières voor een actievere leefstijl te verkleinen.

Psychosociale factoren

Naast gedragsgerelateerde factoren is het belangrijk om aandacht te geven aan de psychosociale factoren na transplantatie. Niertransplantatie herstelt de nierfunctie van de patiënt, en legt daarmee een basis voor herstel van de gezondheid. De patiënt krijgt hierdoor de kans om weer aan het alledaagse leven deel te nemen. De periode na niertransplantatie brengt vele veranderingen met zich mee, dit vereist nieuwe coping strategieën. Ondanks het verbeterde overlevingsperspectief en kwaliteit van leven na transplantatie is een succesvolle uitkomst niet gegarandeerd. Het traject na

niertransplantatie is vaak zwaar met de vele ziekenhuisbezoeken, afweeronderdrukkende medicatie met bijwerkingen, kans op afstoting en een verhoogd risico op andere aandoeningen. Dit brengt voor sommige patiënten een hoge psychologische druk met zich mee. In **hoofdstuk 8** hebben we onderzoek gedaan naar depressie in relatie tot overleving na niertransplantatie. Uit ons onderzoek bleek dat 31% van de niertransplantatiepatiënten een mogelijke depressie had. Dit werd gemeten door middel van een specifieke depressie vragenlijst. Patiënten met een mogelijke depressie waren vaker medisch afgekeurd om te werken, hadden een slechte nierfunctie, bewogen weinig en waren voor hun transplantatie voor een lange periode gedialyseerd. Daarnaast bleek depressie gerelateerd te zijn aan cardiovasculaire sterfte, sterfte door alle oorzaken en transplantaat falen. Het is moeilijk om de causaliteit van deze relatie aan te tonen. De relatie tussen depressie en sterfte kan beïnvloed worden door een hoge comorbiditeit en complex herstel na transplantatie. In de statistische analyses hebben we rekening gehouden met deze factoren maar deze beïnvloeden de relatie niet. De belangrijkste boodschap van dit onderzoek is dat depressie relatief veel voorkomt na niertransplantatie. Depressie na transplantatie wordt waarschijnlijk te weinig gediagnosticeerd en daarmee mogelijk onder behandeld. In de toekomst is meer onderzoek nodig naar herkenning en behandeling van depressie na niertransplantatie.

Interdisciplinaire aanpak

Binnen de transplantatiezorg wordt veel gedaan aan de behandeling van risicofactoren voor cardiovasculaire ziekten. Door middel van medicatie voor hypertensie, cholesterol en diabetes worden de risicofactoren zo goed mogelijk behandeld. Ondanks deze inzet om de risicofactoren in te dammen lukt dit in veel gevallen niet volledig. Een gezonde leefstijl zou kunnen helpen in het terug brengen van deze risicofactoren. De optimale behandeling voor cardiovasculaire risicofactoren zoals hypertensie en diabetes bestaat uit medicatie in combinatie met een gezondere leefstijl. Gewichtsbeheersing, matige alcohol inname en regelmatig bewegen kunnen het risico op cardiovasculaire aandoeningen verlagen. Ondanks dat leefstijl belangrijk is in de behandeling na transplantatie, beschikken veel ziekenhuizen nog niet over de juiste tools om dit de patiënten aan te bieden. Vanuit andere patiëntengroepen weten we dat leefstijl interventies effectief zijn. Revalidatie na een longtransplantatie of hartoperatie is heel succesvol en onderdeel van de standaardzorg. Voor niertransplantatiepatiënten is er nog geen revalidatieprogramma beschikbaar in de reguliere zorg. Het is van groot belang dat in de toekomst revalidatie toegankelijk wordt voor deze patiëntengroep.

BLIK OP DE TOEKOMST

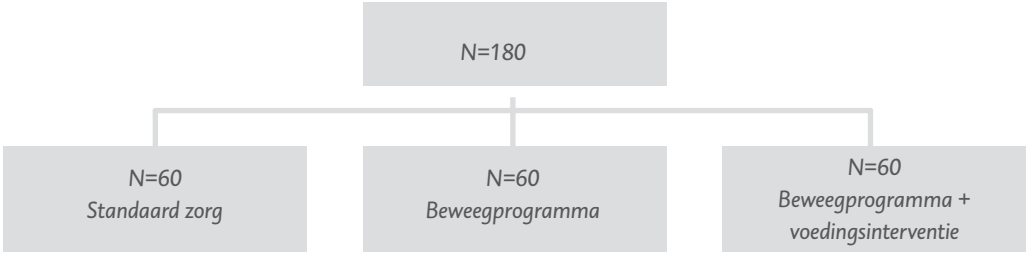
Uit het onderzoek in **hoofdstuk 6** bleek dat bewegingsangst een psychologische barrière is voor bewegen na transplantatie. Informatie over bewegen zou kunnen helpen om deze

bewegingsangst tegen te gaan. Samen met de Nierstichting heb ik een brochure ontwikkeld over bewegen na niertransplantatie. Deze brochure bevat informatie over het belang van bewegen na niertransplantatie. In de brochure staan tevens praktische tips en patiëntenervaringen over bewegen in hun dagelijks leven. De brochure zal verstrekt worden door nefrologen, huisartsen, fysiotherapeuten en patiëntenverenigingen.

Beschrijving GRN:ACT

Data uit dit proefschrift laat zien dat zowel bewegen en voeding belangrijke doelen zijn voor interventie na transplantatie. In samenwerking met het Maastricht Universitair Medisch Centrum hebben we een gerandomiseerd leefstijlinterventie onderzoek opgezet: Groeps Revalidatie Nierpatiënten: ACtieve zorg na Transplantatie (GRN:ACT). Voor dit onderzoek worden 180 niertransplantatiepatiënten door middel van loting, over drie studiearmen verdeeld: standaard zorg, beweegprogramma, beweegprogramma en voedingsinterventie (Figuur 2). De totale duur van het onderzoek is 15 maanden.

Figuur 2: Loting over de 3 studiearmen van GRN:ACT



De belangrijkste twee doelen van GRN:ACT zijn:

1. Het bestuderen van de effecten van een beweegprogramma en voedingsinterventie op inspanningscapaciteit, spierkracht, voedingsgewoonten, kwaliteit van leven en sociale participatie.
2. Het besturen van de effecten van een beweegprogramma en voedingsinterventie op het ontstaan van overgewicht en metabole risicofactoren.

Standaardzorg

De deelnemers in de standaardzorggroep ontvangen geen bewegingsprogramma. Zij ontvangen de standaardzorg. Daarnaast krijgen zij het advies om zich te houden aan de Nederlandse Norm Gezond Bewegen.

Beweegprogramma

Gedurende 12 weken volgen de deelnemers een beweegprogramma in een revalidatiecentrum. De revalidatie wordt uitgevoerd in kleine groepen onder supervisie van ervaren fysiotherapeuten en bewegingsagogen. Het beweegprogramma start met een training van het uithoudingsvermogen op

de fiets en loopband, gevolgd door een individuele dynamische krachttraining. De training wordt afgesloten met sport en spel of zwemactiviteit. Gedurende het programma krijgt de deelnemer een beweeglogboek voor het in kaart brengen van het beweeggedrag. Na 12 weken ontvangt de deelnemer een individueel beweegadvies. In de 12 maanden daarop volgend is er een leefstijlcoach beschikbaar die de deelnemer helpt om de actieve leefstijl thuis vast te houden.

Beweegprogramma en voedingsinterventie

De gecombineerde groep ontvangt het beweegprogramma aangevuld met een voedingsinterventie. In de eerste periode van het programma is er intensief contact tussen de diëtist en de deelnemer. De diëtist coacht de deelnemer om een gezond eetpatroon aan te leren. Om gerichte adviezen te kunnen geven, vult de deelnemer een voedingsdagboek in. Aan de hand van dit voedingsdagboek worden realistische doelen gesteld. Het belangrijkste doel van de voedingsinterventie is het voorkomen van overgewicht door een gebalanceerd dieet. Er is aandacht voor een beperkte inname van energierijke dranken, beperkte inname van zout, goede en slechte vetten en het verbeteren van de inname van groente en fruit.

Metingen

Om de resultaten van de leefstijlinterventie zorgvuldig in kaart te brengen zijn goede metingen noodzakelijk. Op baseline en na 3, 6 en 15 maanden wordt er een testbatterij afgenomen. Voor het meten van inspanningscapaciteit maken we gebruik van een maximale inspanningstest op een fietsergometer. Uit deze test wordt de maximale zuurstofopname bepaald, dit is een betrouwbare maat voor conditie. Spierkracht wordt gemeten met een maximale spierkracht test op fitnessapparatuur. Dagelijkse activiteit wordt gemeten met een vragenlijst en met een geavanceerde bewegingsmeter. Daarnaast wordt er bloed en urine afgenomen voor opslag. Voor evaluatie van de sociale en psychische factoren wordt er gebruik gemaakt van specifieke vragenlijsten. Voedingsgewoonten worden geanalyseerd door middel van een voedingsdagboek en gemeten in de urine (bijv. zoutinname). Voor het monitoren van overgewicht meten we op alle tijdspunten gewicht, vetpercentage en tailleomvang.

CONCLUSIE

In dit proefschrift hebben we laten zien dat leefstijlverandering potentieel een belangrijke bijdrage kan leveren aan het verlagen van het cardiovasculaire risico bij niertransplantatiepatiënten.

Een gezonde voeding en voldoende bewegen heeft vele positieve effecten. Een gezonde leefstijl beïnvloedt meerdere cardiovasculaire risicofactoren tegelijk zoals overgewicht, bloeddruk en cholesterol en verlaagt daarmee indirect het risico op cardiovasculaire aandoeningen. Het heeft daarnaast ook een direct positief effect op hart en bloedvaten. Het is belangrijk om deze kansen juist bij transplantatiepatiënten te benutten.

De toekomstige resultaten van de GRN:ACT studie zullen bijdragen aan de wetenschappelijke onderbouwing voor de rol van leefstijlbegeleiding in het verbeteren van patiënt en transplantaat overleving. Na niertransplantatie zijn er vele mogelijkheden voor preventie van cardiometabole en leefstijl gerelateerde aandoeningen. Op dit vlak kan nog veel gezondheidswinst behaald worden. Leefstijlbegeleiding zou daarom een belangrijk onderdeel moeten worden van de huidige transplantatie zorg.

Dankwoord

Het voor u liggende resultaat was er niet geweest zonder alle medewerking en steun van collega's, vrienden en familie. In dit dankwoord wil ik in het bijzonder een aantal mensen bedanken voor hun waardevolle bijdrage aan mijn promotieperiode.

Allereerst wil ik alle deelnemers bedanken die geparticipeerd hebben in de studies in dit proefschrift. Zonder deelnemers geen onderzoek, dank voor jullie deelname.

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