Insulin resistance, renal dysfunction and cardiovascular disease, studies in a high and a low risk population
Oterdoom, Leendert Harmen

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Urinary creatinine excretion reflecting muscle mass is a predictor of mortality and graft loss in renal transplant recipients

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Transplantation, 2008; 86: 391-398
Abstract

Background
Insulin resistance has been implicated to underlie both excess cardiovascular disease and chronic transplant dysfunction after renal transplantation. Skeletal muscle mainly determines peripheral insulin resistance, and could therefore affect outcome.

Research design and methods
All transplant recipients at our out-patient clinic with a functioning graft >1 year were invited to participate between 2001-2003. Mortality and death censored graft loss were recorded until August 2007. We used 24h urine creatinine excretion as measure of muscle mass. Cox regression was used to analyze the prospective data.

Results
604 renal transplant recipients (age 51 ± 12 yrs, 55% male) were studied. Creatinine excretion was 10.1 ± 2.6 mmol/24h in women and 13.6 ± 3.4 mmol/24h in men. During follow-up of 5.3 [4.7-5.7] years, 95 recipients died and 42 suffered graft loss. Determinants of creatinine excretion were weight, sex, age, height, cumulative prednisolone dosis, and diabetes (R²=0.45). Creatinine excretion was associated with both mortality (3rd vs 1st tertile HR: 0.4 [95%CI 0.2-0.7], P=0.003) and graft loss (3rd vs 1st tertile HR 0.4 [95%CI 0.1-0.9], P=0.03) independent of age, sex, serum creatinine, proteinuria, insulin resistance related factors, time after transplantation and duration of dialysis.

Conclusions
Creatinine excretion as measure of muscle mass is associated with mortality and graft loss after renal transplantation, independent of insulin resistance and its related factors. We speculate that preservation of muscle mass by stimulating exercise, sufficient diet, and less use of corticosteroids may be relevant for improving prognosis in renal transplant recipients.
Introduction

One-year graft survival after renal transplantation has improved impressively over the past decades. However, long term allograft survival has not paralleled this improvement. One main reason for poor long-term outcome is premature death (with a functioning graft) due to cardiovascular disease. Another reason is the gradual decline in graft function owing to chronic transplant dysfunction, ultimately requiring return to dialysis. Insulin resistance has been implicated underlying both excess cardiovascular disease and chronic transplant dysfunction, since insulin resistance is a connecting factor between obesity, type 2 diabetes, hypertension, dyslipidaemia, and other cardiovascular and renal risk factors, including chronic low-grade inflammation and proteinuria.

Skeletal muscles play an important role in the insulin resistance syndrome. In vivo studies indicate that skeletal muscle is the principal site of glucose uptake under insulin-stimulated conditions, accounting for approximately 75% of glucose disposal after glucose infusion. In obese subjects and in patients with type 2 diabetes, there is resistance to insulin-stimulated uptake of glucose. Exercise training can overcome impairment in insulin-stimulated glucose uptake in skeletal muscles, by inducing increases in muscular capillary density, proportion of red fibers, and muscle mass. As such, low muscle mass could underlie excess cardiovascular disease because of the relation to insulin resistance.

The classic method for estimation of total-body skeletal muscle mass is assessment of excretion of creatinine in a collection of 24h urine. 24h urinary excretion of creatinine is considered a reliable measure of muscle mass even in patients with advanced renal failure, in children and adolescents, in elderly people, and in patients with wasting conditions. We aimed to investigate determinants of 24h creatinine excretion in urine, and to prospectively investigate whether 24h creatinine excretion in urine, as measure of muscle mass is associated with mortality and graft loss in renal transplant recipients.

Material and Methods

Research design and subjects
This is a prospective cohort study for which we invited all renal transplant recipients who visited our outpatient clinic between August 2001 and July 2003 to participate if they had a functioning graft for at least one year.
Recipients were asked to participate at a later visit to the outpatient clinic if they were ill or had an infection. A total of 606 renal transplant recipients signed written informed consent, from an eligible 847 (72% consent rate). The group that did not sign informed consent was comparable with the group that signed informed consent with respect to age, sex, body mass index (BMI), serum creatinine, creatinine clearance, and proteinuria. We excluded 2 transplant recipients, in whom no creatinine excretion was assessed, leaving 604 renal transplant recipients for the present analysis. Further study details have been described previously. The Institutional Review Board approved the study protocol (METC 2001/039). Funding sources had neither a role in the collection and analysis of data, nor in publication of the manuscript.

**Outcome events**
The primary endpoints of the study were recipient mortality and death-censored graft loss. Death-censored graft loss was defined as return to dialysis or re-transplantation. The continuous surveillance system of the outpatient program ensures up-to-date information on patient status. In case status of a patient was unknown we contacted general practitioners or referring nephrologists. Mortality and graft loss of all transplant recipients were recorded until August 2007. There was no loss to follow-up.

**Renal transplant characteristics**
Relevant transplant characteristics were taken from the Groningen Renal Transplant Database. This database holds information on all renal transplantations performed at our center since 1968, including dialysis history and creatinine excretion at 6 months after transplantation. Creatinine excretion was also assessed after inclusion of the study after 3.2 [2.5-3.6] years when transplant recipients visited the outpatient clinic. Standard immunosuppressive treatment and current medication were described previously. Cumulative dose of prednisolone was calculated by multiplying the time since transplantation by prednisolone dose at inclusion in the study and adding the dose of prednisolone or methylprednisolone required for treatment of acute rejection (a conversion factor of 1.25 was used to convert methylprednisolone dose to prednisolone dose). For acute rejection different amounts of prednisolone or methylprednisolone were administered, which was taken into account in the calculations. BMI, waist circumference, body surface area (BSA), and blood pressure were measured as described previously. Alcohol consumption, smoking status and cardiovascular history were recorded with a self-report
questionnaire. Cardiovascular disease history was considered positive if there was a previous myocardial infarction (MI), transient ischemic attack (TIA) or cerebrovascular accident (CVA). Insulin resistance was estimated by fasting insulin and HOMA. Both are valid estimates of insulin resistance in renal transplant recipients. Glomerular filtration rate was estimated (eGFR) by using the MDRD formula.

Laboratory measurements
Blood was drawn after an 8-12h overnight fasting period. Creatinine excretion was calculated by multiplying creatinine concentration in the urine with volume of the urine of a single 24 hours urine collection. Creatinine in serum and urine were determined using the Jaffé method on a MEGA AU 510 (Merck Diagnostica, Darmstads, Germany). Plasma glucose, serum total cholesterol, HDL cholesterol, triglycerides, high-sensitivity C-reactive protein (CRP), insulin, and urinary protein excretion were assessed as described previously.

Statistical analysis
Data were analyzed with SPSS version 14.0 (SPSS Inc. Chicago, IL, USA), and GraphPad Prism version 4.03 (GraphPad Software Inc. San Diego, CA, USA). Normally distributed variables are given as means ± standard deviation, whereas variables with a skewed distribution are given as median [interquartile range]. Hazard ratio’s (HR) are reported with 95% confidence interval [95%CI]. Differences between the tertiles were tested for statistical significance with one-way ANOVA for normally distributed variables, a Kruskal-Wallis test for skewed distribution, and Chi-square test for categorical variables. A two-sided P-value less than P<0.05 indicated statistical significance.

Recipient characteristics are shown according to tertiles of creatinine excretion, stratified for sex. We stratified for sex because of the large difference in muscle mass between men and women. We proceeded with an exploration of relevant independent determinants and associates of creatinine excretion. Weight, height and sex are obvious potential determinants, and were first entered in a multivariate linear regression analysis. All characteristics with a P-value <0.1 across tertiles of creatinine excretion were entered into a backward linear regression analysis and removed in successive steps at a threshold of P<0.1. Retained variables were considered independent determinants and associates of creatinine excretion. In multivariate regression analyses, adjustments were performed for serum creatinine and eGFR as measures of renal function, because creatinine
clearance is mathematically dependent on creatinine excretion. Therefore, creatinine clearance can not be included in the multivariate regression models together with creatinine excretion.

To analyse whether creatinine excretion is associated with mortality and graft loss, we first performed Kaplan-Meier analyses with a Log-rank test. Multivariate Cox-regression analyses were performed with the 1st tertile as reference to investigate whether 24h creatinine excretion is independently associated with mortality and graft loss. We also analysed our data with creatinine excretion as a continuous variable, because analyzing the data of creatinine excretion across tertiles, although providing a good comparison between groups, is somewhat arbitrary.

Finally, we investigated possible interactions between 24h creatinine excretion and all investigated characteristics for mortality and graft loss. The interactions were tested by entering 24h creatinine excretion, the investigated characteristic, and their product term in multivariate Cox-regression analyses.

**Results**

Creatinine excretion was $13.6 \pm 3.4$ mmol/24h in men and $10.1 \pm 2.6$ mmol/24h in women (difference $P<0.001$). Baseline characteristics are shown in table 1 according to sex-stratified tertiles of creatinine excretion. With increasing creatinine excretion transplant recipients were younger, had a shorter dialysis time prior to transplantation, and had a shorter time between renal transplantation and inclusion of the study. In the 1st tertile versus the 3rd tertile, there were twice as many previous myocardial infarctions, 3 times more TIA/CVA, and 1.5 times as many diabetic renal transplant recipients. Body weight, BMI, waist circumference, height, and body surface area were higher with higher creatinine excretion. Also, alcohol consumption, HDL-cholesterol, and fasting insulin and serum albumin concentrations were higher with higher creatinine excretion. There were no significant differences in CRP, serum creatinine, and eGFR across tertiles of creatinine excretion.

Determinants and associates of creatinine excretion are shown in table 2. As expected, height, weight and male sex were determinants of creatinine excretion ($R^2=0.36$). In further linear regression analyses, there were independent negative associations with recipient age, cumulative prednisolone dose, and presence of diabetes ($R^2=0.45$). Waist circumference was not entered in the regression models because of co-linearity with weight.
Median follow-up until mortality was 5.3 [4.7-5.7] years. During the follow-up of the 604 renal transplant recipients, 94 died and 42 suffered graft loss necessitating return to dialysis. In the lowest tertile of creatinine excretion 50 (24.5%) subject died during follow-up, whereas these numbers were 28 (14.1%) and 16 (7.9%) for the middle and highest tertile respectively (Log-Rank test: P<0.001, figure 1). Numbers of graft loss were 17 (8.6%), 17 (8.6%), and 8 (4.0%) in increasing tertiles of creatinine excretion respectively (Log-Rank test: P=0.09, figure 1).

Transplant recipients that survived had both at 6 months after transplantation, at inclusion, and at last assessment of creatinine excretion during follow-up a higher creatinine excretion than patients who died (table 3). Transplant recipients that suffered from graft loss had greatest decreases in creatinine excretion from 6 months after transplantation until last assessment of creatinine excretion during follow-up.

Creatinine excretion was associated with mortality and graft loss after adjustment for age, sex (model 2, table 4). The same was true with further adjustment for co-morbidity (model 3), adjustment for serum creatinine, proteinuria, time after transplantation, and duration of dialysis (model 4), and adjustment for fasting insulin and other components of the insulin resistance syndrome (model 5). For mortality, adjustment in model 5 increased the significance, as the 2nd versus the 1st tertile was
Table 1. **Characteristics in sex stratified tertiles of creatinine excretion (median with range is given).**

<table>
<thead>
<tr>
<th>Tertiles of creatinine excretion</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt;</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men, n=</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine excretion, mmol/24h</td>
<td>10.3 (3.1-12.0)</td>
<td>13.6 (12.1-14.6)</td>
<td>16.5 (14.7-28.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>108</td>
<td>109</td>
<td></td>
</tr>
<tr>
<td><strong>Women, n=</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine excretion, mmol/24h</td>
<td>7.7 (3.8-8.7)</td>
<td>9.8 (8.8-10.9)</td>
<td>12.5 (11.0-19.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>90</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

**Recipient demographics**

| Age, yr            | 55±12 | 52±11 | 47±12 | <0.001 |

**(Pre)transplant history**

| Time since transplantation, yr | 8.1 [3.8-13.1] | 5.4 [2.4-10.3] | 5.4 [2.1-9.3] | <0.001 |

**Cardiovascular disease history**

| Myocardial Infarction, n (%) | 21 (10.4) | 16 (8.2) | 11 (5.4) | 0.07       |
| TIA<sup>(1)</sup>/CVA<sup>(2)</sup>, n (%) | 19 (9.4) | 9 (4.6) | 5 (2.5) | 0.002     |
| Amputation, n (%)           | 4 (2.0) | 4 (2.0) | 0 (0) | 0.09     |

**Body composition**

| Weight, kg | 72±13 | 77±13 | 82±13 | <0.001 |
| BMI, kg/m<sup>2</sup>       | 25.1±4.4 | 26.0±3.9 | 27.0±4.4 | 0.03     |
| Waist circumference, cm     | 96±14 | 97±13 | 99±14 | 0.07     |
| Height, m                   | 1.70±0.10 | 1.71±0.10 | 1.75±0.09 | <0.001 |
| Body surface area (BSA), m<sup>2</sup> | 1.83±0.18 | 1.89±0.19 | 1.97±0.17 | <0.001 |

Normally distributed variables are expressed as mean ± SD, whereas variables with a skewed distribution are given as median (interquartile range).
### Substance use

<table>
<thead>
<tr>
<th>Consumption</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>1-3 Units/week</td>
</tr>
<tr>
<td></td>
<td>&gt;4 Units/week</td>
</tr>
<tr>
<td>Current smoking</td>
<td>80 (39)</td>
</tr>
</tbody>
</table>

### Blood pressure (BP)

| BP | Systolic BP, mmHg | 155±25 | 153±23 | 151±20 |
|    | Diastolic BP, mmHg | 89±10 | 90±11 | 90±9 |

### Lipids

| Lipid | HDL cholesterol, mmol/l | 1.06±0.30 | 1.10±0.32 | 1.13±0.35 |
|       | Triglycerides, mmol/l | 1.9 [1.4-2.6] | 2.0 [1.4-2.8] | 1.8 [1.4-2.5] |

### Glucose homeostasis

| Glucose, pmol/l | 4.9±1.3 | 4.9±1.5 | 4.8±1.4 |
| Insulin, mml/ml | 10.3 [7.6-14.7] | 11.4 [7.8-16.2] | 12.1 [8.6-16.7] |
| HOMA | 2.11 [1.55-3.25] | 2.29 [1.53-3.72] | 2.32 [1.70-3.72] |
| Diabetes, n (%) | 43 (21) | 35 (18) | 28 (14) |

### Serum albumin g/l


### Inflammation

| CRP, mg/l | 2.4 [1.0-5.3] | 1.8 [0.8-4.7] | 1.9 [0.7-4.8] |

### Prednisolone use

| Prednisolone dose, mg/dy | 10 [7.5-10] | 10 [7.5-10] | 10 [7.5-10] |

### Acute rejection (3), n (%)

| Acute rejection | 68 (33) | 60 (30) | 59 (29) |

### Renal allograft function

| Creatinine, μmol/l | 134 [107-166] | 129 [111-166] | 137 [120-166] |
| eGFR (4), ml/min/1.73m² | 46±17 | 46±16 | 45±14 |
| Proteinuria, g/24h | 0.2 [0.0-0.5] | 0.2 [0.0-0.5] | 0.2 [0.0-0.5] |

---

(a) TIA: Transient Ischemic Attack
(b) CVA: Cerebrovascular Accident
(c) Acute rejection treatment with high dose corticosteroids
(d) eGFR was estimated by using the extended MDRD formula
Table 2. **Determinants and associates of creatinine excretion (n=604).**

<table>
<thead>
<tr>
<th>Basic determinants, $R^2=0.36$</th>
<th>Standardized Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, m</td>
<td>0.23</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Final result of backward analysis, $R^2=0.45$</th>
<th>Standardized Beta</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male</td>
<td>0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Recipient age, yr</td>
<td>-0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height, m</td>
<td>0.14</td>
<td>0.001</td>
</tr>
<tr>
<td>Cumulative prednisolone dose, gr</td>
<td>-0.08</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes, yes</td>
<td>-0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 3. **Creatinine excretion at 6 months after transplantation, at inclusion, and at last assessment of the study in those that survived, died, and had graft loss.**

<table>
<thead>
<tr>
<th>n=</th>
<th>Survived</th>
<th>Died</th>
<th>Death-censored graft loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine excretion mmol/24h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 6 months after transplantation,</td>
<td>12.4±3.3</td>
<td>11.6±3.5</td>
<td>13.3±4.1</td>
</tr>
<tr>
<td>At inclusion</td>
<td>12.3±3.6</td>
<td>10.6±3.2</td>
<td>11.7±3.4</td>
</tr>
<tr>
<td>At last assessment</td>
<td>11.3±4.3</td>
<td>9.1±3.3</td>
<td>10.2±3.1</td>
</tr>
<tr>
<td>Time between, yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplantation and inclusion</td>
<td>6.0 [2.6-11.6]</td>
<td>6.1 [2.8-11.6]</td>
<td>5.8 [2.7-10.2]</td>
</tr>
<tr>
<td>Inclusion and last assessment</td>
<td>3.3 [2.9-3.7]</td>
<td>2.0 [1.0-3.4]</td>
<td>2.0 [0.9-3.0]</td>
</tr>
<tr>
<td>Change between 6 months and last creatinine excretion, %</td>
<td>-5.8±38.9</td>
<td>-16.6±34.4</td>
<td>-20.4±19.1</td>
</tr>
</tbody>
</table>

* a) $P<0.05$ versus those that survived
* b) $P<0.05$ versus those that died
Table 4. Cox regression analyses for mortality and graft loss in sex stratified tertiles of creatinine excretion.

<table>
<thead>
<tr>
<th>Tertiles of creatinine excretion</th>
<th>1st reference</th>
<th>2nd HR [95% CI]</th>
<th>P-value</th>
<th>3rd HR [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality, n (%)</td>
<td>50 (24.5%)</td>
<td>28 (14.1%)</td>
<td>16 (7.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality ratea</td>
<td>51</td>
<td>28</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0</td>
<td>0.6 [0.4-1.0]</td>
<td>0.03</td>
<td>0.3 [0.2-0.6]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0</td>
<td>0.8 [0.5-1.2]</td>
<td>0.3</td>
<td>0.5 [0.3-0.9]</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0</td>
<td>0.7 [0.5-1.2]</td>
<td>0.2</td>
<td>0.5 [0.3-0.9]</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.0</td>
<td>0.7 [0.4-1.1]</td>
<td>0.1</td>
<td>0.5 [0.3-0.9]</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.0</td>
<td>0.6 [0.4-0.9]</td>
<td>0.03</td>
<td>0.4 [0.2-0.7]</td>
<td>0.003</td>
</tr>
<tr>
<td>Graft Loss, n (%)</td>
<td>17 (8.3%)</td>
<td>17 (8.6%)</td>
<td>8 (4.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft Loss ratea</td>
<td>17</td>
<td>17</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0</td>
<td>1.0 [0.5-1.9]</td>
<td>0.9</td>
<td>0.4 [0.2-1.0]</td>
<td>0.05</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0</td>
<td>0.9 [0.5-1.8]</td>
<td>0.8</td>
<td>0.4 [0.2-0.9]</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0</td>
<td>0.9 [0.5-1.8]</td>
<td>0.8</td>
<td>0.4 [0.2-0.8]</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 4</td>
<td>1.0</td>
<td>0.9 [0.5-2.0]</td>
<td>0.9</td>
<td>0.4 [0.2-0.9]</td>
<td>0.03</td>
</tr>
<tr>
<td>Model 5</td>
<td>1.0</td>
<td>0.9 [0.5-2.2]</td>
<td>0.9</td>
<td>0.4 [0.1-0.9]</td>
<td>0.03</td>
</tr>
</tbody>
</table>

a) Rates are given as incidence per 1000 person years.

Model 1: Crude Model of creatinine excretion
Model 2: Model 1 + adjustments for age, sex
Model 3: Model 2 + cardiovascular disease history (MI and TIA/CVA), and amputation
Model 4: Model 3 + serum creatinine, proteinuria, time after transplantation, and dialysis time
Model 5: Model 4 + insulin resistance syndrome (fasting insulin, waist circumference, systolic blood pressure, glucose, HDL-cholesterol, and triglyceride concentrations)
also associated with mortality. Results were not materially different with HOMA instead of fasting insulin as measure of insulin resistance (data not shown). Finally, results were not materially changed with use of estimated GFR by extended MDRD equation as measure of renal function instead of serum creatinine (data not shown).

In Cox-regression analysis with creatinine excretion we found significant interactions between creatinine excretion and glucose, proteinuria, and serum albumin for mortality (all interaction terms \( P < 0.05 \)). The interaction of glucose for mortality was non-significant in non-diabetic transplant recipients, indicating that the interaction in the whole group was dependent on those subjects with diabetes. Creatinine excretion (per mmol/24h) as a continuous variable predicted mortality with a HR 0.82 [0.75-0.89] \((P < 0.001\)) in a multivariate model including all variables of model 5 (table 4) and the interaction term between creatinine excre-
The interaction between creatinine excretion and proteinuria is shown in Figure 2. The interaction indicated that transplant recipients with high creatinine excretion and low urinary protein excretion had the lowest mortality rate, while recipients with low creatinine excretion but high urinary protein excretion had the highest mortality rate. Among recipients in the highest tertile of creatinine excretion and in the lowest tertile of proteinuria only 2 (3.4%) patients died (incidence rate of 6 per 1000 person years). Among recipients in the lowest tertile of creatinine excretion and the highest tertile of proteinuria 20 (29.4%) patients died (incidence rate of 65 per 1000 person years). The interaction between creatinine excretion and serum albumin became insignificant when the interaction between creatinine excretion and proteinuria was taken into account.

Figure 3
Graft loss rate (death-censored) per 1000 person according to creatinine excretion and proteinuria.

For men cut-off points for creatinine excretion were: 1st tertile: <12.1 mmol/24h, 2nd tertile: 12.1-14.6 mmol/24h, and 3rd tertile: >14.6 mmol/24h. For women cut-off points for creatinine excretion were: 1st tertile: <8.8 mmol/24h, 2nd tertile: 8.8-10.9 mmol/24h, and 3rd tertile: >10.9 mmol/24h. Cut-off points of proteinuria were: 1st tertile: <0.2 g/24h, 2nd tertile 0.2-0.4 g/24h, and 3rd tertile >0.4 g/24h. Death-censored graft loss is reported as incidence rate per 1000 person years.
For graft-loss we found interactions between creatinine excretion and recipient age, serum albumin, and proteinuria (all interaction terms $P<0.05$). The interactions of age and serum albumin became insignificant after the interaction of proteinuria was taken into account. Creatinine excretion (per mmol/24h) as a continuous variable predicted death-censored graft loss with a HR $0.83 [0.73-0.95]$ ($P=0.007$), in a multivariate model including all variables of model 5 (table 4) and the interaction term between creatinine excretion and proteinuria. The interaction between creatinine excretion and proteinuria was dictated by proteinuria, as can be seen in figure 3. Only in the 3rd tertile of proteinuria (urinary protein excretion $>0.4g/24h$) was there a visible difference in the incidence of graft loss between the tertiles of creatinine excretion. Among recipients in the highest tertile of proteinuria and in the highest tertile of creatinine excretion there were only 6 (8.2%) cases of graft loss (incidence rate of 17 per 1000 person years). Among recipients in the highest tertile of proteinuria and lowest tertile of creatinine excretion there were 13 (19.1%) cases of graft loss (incidence rate of 46 per 1000 person years).

**Discussion**

This study shows that low urinary creatinine excretion as measure of low muscle mass is independently associated with mortality and graft loss in renal transplant recipients. The association for both mortality and graft loss was modified by proteinuria. Significant interactions indicated more mortality and graft loss in those patients with less creatinine excretion and more proteinuria. Independent determinants and associates of creatinine excretion were weight, male sex, age, height, cumulative prednisolone dose and diabetes.

Body composition is subject to great changes after renal transplantation. Determinants of greater weight gain after transplantation are female sex, pretransplant obesity, living related kidney donation, younger age, and ethnicity (African-Americans gain more weight than Caucasians).\textsuperscript{26-29} Weight gain after transplantation is predominantly due to an increase in fat mass.\textsuperscript{30} In cross sectional analyses we found independent associations for high creatinine excretion (ie higher muscle mass) with high weight, male sex, young age, higher height, low cumulative prednisolone dose, and absence of diabetes. These determinants are remarkably similar to those found in an earlier smaller retrospective study of 100 renal transplant recipients.\textsuperscript{31}
In the general population low muscle mass and lean body mass are associated with mortality, though impaired muscle strength has a stronger association with mortality than muscle mass.\textsuperscript{32-34} In patients initiating haemodialysis muscle mass, as measured by 24h creatinine excretion, was associated with all-cause mortality and cardiovascular mortality during dialysis.\textsuperscript{18} Intriguingly, the protective effect associated with obesity in dialysis patients was only present in those with normal or high muscle mass.\textsuperscript{18} A similar phenomenon could be observed in our study, as those with the most muscle mass had the greatest BMI and waist circumference. In patients with other chronic diseases such as chronic obstructive pulmonary disease (COPD) or liver cirrhosis, less mid-arm muscle area is associated with mortality.\textsuperscript{35-37} It should be noted that in COPD patients, mid-arm muscle area has stronger association with mortality than BMI.\textsuperscript{35,36}

We hypothesized that insulin resistance could underlie the relation between muscle mass and mortality.\textsuperscript{7,8} Skeletal muscle accounts for about 75\% of all insulin stimulated glucose uptake.\textsuperscript{11,12} Furthermore exercise training can overcome impairment to resistance of insulin-stimulated glucose uptake in skeletal muscles.\textsuperscript{14-17} We found that high muscle mass was independently associated with a decreased prevalence of diabetes. In the first tertile of creatinine excretion (low muscle mass) 21\% had diabetes, whereas in the third tertile (high muscle mass) only 14\% had diabetes. However, our multivariate analyses of mortality and graft loss remained essentially unchanged after adjustment for components of the insulin resistance syndrome and diabetes, indicating that results were not dependent on insulin resistance. Therefore, our data do not provide direct evidence that insulin resistance or the insulin resistance syndrome are key mechanisms linking muscle mass and outcome in renal transplant recipients.

Thus, a more likely explanation for the association between muscle mass and mortality is that 24h urinary excretion of creatinine provides a reflection of chronic disease, disability and nutritional deficiencies. This is substantiated by the fact that low serum albumin was associated with low muscle mass, as has also been shown in elderly subjects.\textsuperscript{38} Quantification of muscle mass by 24h excretion of creatinine has been an important focus in nutrition studies in patients suffering from protein-energy malnutrition.\textsuperscript{39-41} In the short-term, protein restriction does not lead to the development of protein-energy malnutrition, as long as urinary losses of protein are compensated for in renal patients.\textsuperscript{42-44} However, in the long term intermittent illnesses, periods of fasting and use of catabolic drugs increase protein-energy malnutrition and unmask an effect of proteinuria.

\textbf{CHAPTER 4}
on wasting of muscle mass. Day to day variation in diet explains some of
the variation in creatinine excretion, but substantial effects on creatinine
excretion can only be observed if after more prolonged low protein cre-
tinine free (=meat free) diets endogenous creatine pools start to decrease,
resulting in decrease of endogenous creatinine generation. Indeed,
prolonged low and very low protein diets decreased arm muscle area and
24h creatinine excretion compared to a control diet in the Modification of
Diet in Renal Diseases (MDRD) study. Importantly, this decay in muscle
mass was observed despite the fact patients of the MDRD study did not
chronically use glucocorticosteroids.

Chronic exposure to exogenous glucocorticosteroids may further
unmask an adverse effect of proteinuria on muscle mass because chronic
treatment with glucocorticosteroids compromises protein-energy bal-
ance by adversely affecting body nitrogen conservation via various path-
ways. High cumulative prednisolone dose was indeed an independent
determinant of lower muscle mass in our study. Prednisolone may increase
susceptibility to adverse effects of proteinuria on muscle mass, thereby
explaining the interactions found. Indeed, in liver transplant patients, a
higher dose of corticosteroids was associated with greater proteinuria.

We also found 24h urinary creatinine excretion associated with
graft loss, with an interaction with proteinuria. The association between
creatinine excretion and graft loss has been previously reported in an
earlier retrospective study. Decline of 24h creatinine excretion between
3 months after transplantation and follow up after 89 ± 35 months was
on average 27% in those that returned to dialysis. In patients that died,
creatinine excretion decline was on average 7%, compared to 4% in
those patients that did not die or experience graft loss at the end of the
study. However, this retrospective study did not investigate a potential
interaction between creatinine excretion and proteinuria. An obvious
mechanism underlying low creatinine excretion as a risk factor for graft
loss is lacking. It is unlikely that prevention of chronic immunological
rejection by ‘over immunosuppression’ with corticosteroids plays a role.
This would have led to an association with high creatinine excretion as a
risk factor for graft loss, rather than low creatinine excretion.

A limitation of this study could be that we used 24h creatinine excre-
tion as measure of muscle mass. Fat-free body mass has been suggested as
an alternative, but has the disadvantage that it does not always accurately
reflect differences in muscle mass between individuals. Likewise
dual-energy x-ray absorptimetry is also an alternative, but is expensive
and is possibly inaccurate in subjects with altered body water contents,
including those with impaired renal function. In contrast, 24h urinary excretion of creatinine is considered a reliable measure of muscle mass even in patients with advanced renal failure, in children and adolescents, in elderly people, and in patients with wasting conditions. A specific limitation of using 24h urine collection as measurement of muscle mass is that it is prone to errors, even though we carefully instructed each renal transplant patient verbally and in writing. However, if associations between muscle mass and outcome are as we report, collection errors would underestimate instead of overestimate our results. Finally, it could be that low muscle mass represents morbidity and chronic disease burden for which we have not fully adjusted for in our analyses, as indicated by the high prevalence of CVD history and diabetes in those with the low muscle mass. An important strength of the study is that follow-up was complete for all patients.

It is tempting to speculate that preservation of muscle mass may serve to improve outcome after transplantation. It should, however, be realised that our study is longitudinal in design and not an intervention study, thus such conclusions can not be drawn about the effects of perserving muscle mass. Strategies to possibly preserve muscle mass in future intervention studies could include stimulation of exercise, dietary advices and tapering of steroids. Exercise trials in renal transplant recipients have indicated increases in exercise capacity and muscle strength, though not consistently that muscle mass or lean body mass increases. Furthermore, both physical exercise and a proper diet could decrease cardiovascular risk by decreasing insulin resistance and blood pressure.

In conclusion, low urinary creatinine excretion as a measure of low muscle mass is associated with mortality and graft loss, independent of renal function and insulin resistance. Determinants and associates of greater creatinine excretion were greater weight, male sex, younger age, higher height, lower cumulative prednisolone dose and absence of diabetes.
References to Chapter 4


24. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from


48 Jindal RM, Hughes D, Sidner RA, Leapman SB,


