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**LOSS OF RENAL RESERVE CAPACITY IS NOT AN EARLY PHENOMENON IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE**

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**Background:**
In the early stage of Autosomal Dominant Polycystic Kidney Disease (ADPKD) it is assumed that renal function is sustained in the normal range for several decades despite progressive formation of cysts and loss of nephrons. It has been suggested that this sustainment of renal function is due to hyperfiltration of remnant nephrons. In this study we are the first to formally investigate the extent to which ADPKD patients are hyperfiltrating at various stages of the disease by measuring renal reserve capacity (RRC).

**Methods:**
In this cross-sectional study we measured glomerular filtration rate (mGFR) and effective renal plasma flow (ERPF) with continuous infusion of $^{125}$I-lothalamate and $^{131}$I-hippurate, respectively, in ADPKD patients and age- and sex-matched healthy controls. Filtration fraction (FF) was calculated as mGFR/ERPF. RRC was defined as percentage change in GFR from baseline during dopamine infusion (4.5-6 mg/hr). Subjects were divided in 5 age groups (18-29; 30-39; 40-49; 50-59 and ≥60 years) and values were compared between ADPKD patients and healthy controls.

**Results:** A total of 363 subjects were studied (129 ADPKD patients and 234 healthy controls). The comparison between ADPKD and healthy controls for the different age groups is presented in the attached table. Across all age groups, mGFR was lower in ADPKD patients compared to healthy controls, whereas ERPF was more strongly decreased, resulting in a higher FF. Importantly, in the youngest age group of ADPKD patients, RRC was comparable to healthy controls. In the older age groups RRC decreased progressively in ADPKD patients, but not in healthy controls. Multivariable linear regression analysis indicated that results were similar in males vs. females, in subjects using vs. not using RAAS-inhibitors and were independent of sodium and protein intake.

**Conclusion:** ADPKD patients at young adult age, despite some loss of kidney function, are still able to increase their GFR in response to dopamine. Loss of renal reserve capacity is therefore not an early phenomenon in ADPKD.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>18-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>≥60</th>
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<tbody>
<tr>
<td><strong>Pre-dopamine</strong></td>
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<tr>
<td>mGFR (ml/min/1.73m$^2$)</td>
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<tr>
<td>ADPKD</td>
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<td>90</td>
<td>62</td>
<td>52</td>
<td>40</td>
</tr>
<tr>
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<td>115</td>
<td>109</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>FF</td>
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<td>0.35</td>
<td>0.33</td>
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<tr>
<td>ADPKD</td>
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<tr>
<td>Healthy</td>
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**Dopamine**

| ΔmGFR = RRC (%) | ADPKD | 10.7  | 3.7  | 4.0  | 2.1  | -0.4 |
| Healthy       | 7.7   | 10.0  | 8.3  | 9.3  | 7.7  |
| ΔFF (%)       | ADPKD | -3.3  | -4.2 | -4.9 | -6.6 | -4.4 |
| Healthy       | -8.0  | -11.7 | -8.1 | -11.2| -10.4|

Data are given as means.

* p<0.05 and ** p<0.01 vs. healthy controls
BACKGROUND:

Chronic pain affects more than 60% of patients with autosomal dominant polycystic kidney disease (ADPKD) and is refractory in some cases. Treatment of chronic pain in ADPKD is difficult and often unsatisfactory. It is assumed that pain caused by pressure by the enlarged kidney and/or liver on adjacent organs is relayed via the celiac plexus and splanchnic nerves, whereas pain caused by distension of the renal capsule is relayed via sensory nerves around the renal artery to the aorticorenal plexus. We studied the efficacy of a novel multidisciplinary stepwise protocol that applies sequential nerve blocks.

METHODS:

Patients were eligible if pain was present ≥3 months with a VAS-score of ≥50 out of 100 and a large impact on daily activities and social life, and if they had insufficient response to previous therapies, including chronic opioid treatment. In a multidisciplinary setting it was assessed if pain was ADPKD-related. Every patient underwent a MRI to exclude other potential pain causes and to delineate the celiac plexus location. As first step a diagnostic, temporary celiac plexus block with a local anesthetic was performed. In case substantial pain relief was obtained, we assumed that pain was relayed via the celiac plexus and major splanchnic nerves (MSN). When pain recurred, patients were scheduled for an ipsilateral MSN block with radiofrequency ablation. In case no pain relief was obtained, it was assumed that pain was relayed via the aorticorenal plexus, and catheter-based renal denervation was performed.

RESULTS:

We screened 60 patients, that were referred by nephrologists or were self-referrals from all across the volume 1.66 [0.93–2.61] L, liver volume 2.61 [1.94–3.33] L). Pain was present for 7 [4-18] years, and experienced as refractory for 12 [10-24] months. In 36 patients (81.8%) the diagnostic celiac plexus block resulted in substantial pain relief (change in VAS pre-post intervention 50/100 [26-68]; p<0.001). Of these patients, 23 (52.3%) received a MSN block because pain recurred, with a change in VAS pre-post MSN block of 53/100 [23-65]; p<0.001). Out of the 8 patients without pain relief after the diagnostic celiac plexus block, renal denervation was performed in 5 (11.4%), with a change in VAS pre-post intervention 20/100 [0-50]; p=0.07). After a follow-up of 12 [8-17] months, 81.8% of the 44 patients experienced a sustained improvement in pain intensity, leading to cessation of daily opioid use in 63.6%. Adverse events related to the procedures were minimal.

CONCLUSIONS:

These data indicate that our treatment protocol consisting of sequential nerve blocks is effective in obtaining substantial pain relief in most ADPKD patients with refractory chronic pain.
ACTIVIN SIGNALING IN POLYCYSTIC KIDNEY DISEASE

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Background
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by the formation of thousands of kidney cysts, which leads to renal failure. It is caused by PKD1 or PKD2 mutations and affects 4 in 10,000 individuals. Although many signaling pathways have been discovered to be de-regulated in PKD, the contribution of each of these pathways is poorly understood. We recently showed increased mRNA expression of ActivinA and ActivinB in polycystic kidneys of mice, and that sequestering these ligands by treatment with a soluble ligand-trap (sActRIIB-Fc) slowed PKD progression. In this study we aimed to further elucidate the role of Activins in PKD, and whether the therapy can be optimized by selectively targeting Activin-B or by down-regulating the Activin-type I receptor Alk4.

Methods
ELISA’s were performed to detect Activin-A in cyst fluid and urine samples of ADPKD patients, and in kidneys of sActRIIB-Fc treated or untreated mice. We studied injury/repair in vitro by performing wound assays in the presence or absence of Activins. We treated PKD mice with an Activin-B specific Follistatin and we used Antisence Oligonucleotides (ASO’s) to downregulate Alk4.

Results
Activin-A was abundantly present in cyst fluid of ADPKD patients but could not be detected in urine. PKD kidneys of mice had increased protein expression of Activin-A, which was reduced even below WT levels by sActRIIB-Fc treatment. In-vitro: The capacity of cultured proximal epithelial cells to close an existing wound, was increased by Activin-A and Activin-B. In vivo: Neither the treatment with Activin-B specific Follistatin, nor Alk4 down regulation, slowed PKD in mice.

Conclusions
The presence of high Activin-A protein levels in cyst fluid of patients, and in PKD samples of mice confirms a role for Activin-A in PKD. We and others have suggested that excessive activation of repair signaling programs underlies cyst formation. Based on the increased capacity of wound closure under Activin stimulation, we speculate that Activins might contribute to such a ‘repairovershoot’ in ADPKD. However, although sequestering Activins by sActRIIB-Fc was effective in different PKD-models, downregulation of Alk4 or targeting only Activin-B is not sufficient to slow down PKD progression. It remains to be shown if targeting Activin-A might be an alternative route to slow PKD.
FUROSEMIDE DOES NOT CAUSE ENaC-INDEPENDENT URINE ACIDIFICATION IN MAN

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Background:
The furosemide fludrocortisone test (FF test) is advocated as a test to diagnose distal renal tubular acidosis (dRTA). The FF test is based on the assumption that furosemide increases distal tubular sodium delivery and that fludrocortisone, by stimulating sodium re-absorption through the epithelial sodium channel ENaC, induces H⁺ secretion in the collecting duct. In mice however, it was shown that furosemide itself can acidify the urine by stimulating the Na⁺-H⁺ transporter NHE3. If such a mechanism is present in humans as well, the FF test could erroneously suggest normal urinary acidification in patients with dRTA. This would limit the use of the FF test as a diagnostic test for patients with dRTA. We therefore investigated whether furosemide can induce ENaC-independent urine acidification in man.

Methods:
An intervention study in which ten healthy volunteers underwent two FF tests on separate days, either with or without pre-treatment with the ENaC blocker amiloride (20 mg orally), two hours prior to the start of the test.

Results:
In the absence of amiloride, urine pH was reduced to values ≤ 5.3 after FF in all volunteers. In the presence of amiloride, there was an attenuated decrease of urine pH. Still, two subjects had a pH < 5.3 at the end of the test. Urine sodium and potassium excretion indicated that at this time point, blockade of ENaC was incomplete. Indeed, during the time period in which ENaC was optimally blocked, no increase in H⁺ excretion was observed compared to the same time period in the standard FF test (Figure).

Conclusion:
These data show that the urine acidification secondary to FF is ENaC-dependent and, thus, that furosemide does not cause ENaC-independent urine acidification in man. Our data support the applicability of the FF test for diagnosing dRTA.
MAGNESIUM PREVENTS VASCULAR CALCIFICATION INDEPENDENT OF OSTEOGENIC CONVERSION AND APOPTOSIS IN BOVINE VASCULAR SMOOTH MUSCLE CELLS

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Background
Development of vascular calcification is a major cause of cardiovascular complications in patients with chronic kidney disease (CKD). In several patient studies, serum magnesium (Mg 2+) directly correlated with cardiovascular risk and survival. Despite a body of evidence that Mg 2+ prevents calcifications both in vitro and ex vivo, the mechanisms remain unclear. This study aimed to investigate the effect of Mg 2+ on phosphate (Pi) induced vascular calcification and to study the cellular mechanisms by which Mg 2+ counteracts vascular calcification in vascular smooth muscle cells (VSMCs).

Methods
Bovine aortic VSMCs (bVSMCs) were supplemented with 10 mM bèta-glycerophosphate (BGP) for 14 days to induce calcification, in the absence or presence of MgCl2 reaching a final concentration of 2 mM. Medium Pi concentration, cellular calcium (Ca 2+) deposition and alkaline phosphatase (ALP) activity were determined by spectrophotometric assays. VSMC calcification was visualized by Alizarin Red and Von Kossa staining methods. Apoptosis was detected by immunofluorescence using an Annexin V-FITC assay kit. Gene expression was investigated by quantitative polymerase chain reaction (qPCR).

Results
BGP supplementation resulted in pronounced bVSMC calcification and increased medium Pi concentration, reaching 5.0 ± 0.5 mM after 14 days (P<0.05 versus 1.16 ± 0.05 mM in untreated cultures). This effect was even stronger in the presence of 2 mM MgCl 2 (8.3 ± 1.0 mM, P<0.05). Alizarin Red/Von Kossa staining showed marked calcification in BGP, but not in Mg 2+ supplemented cultures. In addition, quantification of cellular Ca 2+ deposits showed that Mg2+ supplementation abrogated the development of calcifications, as observed in cells treated with BGP alone (3.6 ± 0.2 versus 9.9 ± 1.0 µg Ca2+/mg protein, respectively, P<0.05). Despite the high Pi levels, no upregulation of osteogenic transcription factors (RUNX2 and BMP2) was observed in BGP-treated bVSMC. Apoptosis was only detected secondary to VSMC calcification and was not observed in BGP-treated cultures prior to mineral deposition. In addition, ALP activity remained unchanged in all treatments irrespective of calcification severity.

Conclusion
Our results demonstrate the preventive effect of Mg 2+ on Pi-induced VSMC calcification, which is independent of osteogenic conversion or apoptosis in bVSMC. These findings stand in contrast to several previous studies and suggest that cellular responses associated with osteogenic conversion may be secondary to bVSMC mineralization rather than being the primary cause for vascular calcification.
CARDIOVASCULAR RISK FACTORS, LIFESTYLE AND KIDNEY FUNCTION DECLINE IN STABLE OLDER POST-MYOCARDIAL INFARCTION PATIENTS RECEIVING OPTIMAL DRUG THERAPY: A PROSPECTIVE COHORT STUDY

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Background:
The incidence of chronic kidney disease increases worldwide and is mainly driven by classic modifiable cardiovascular risk factors and unhealthy lifestyle. The age-related kidney function decline of about 1.0 ml/min/1.73m2 per year after age 40 is doubled in post-myocardial infarction (MI) patients. Little is known about the impact of cardiovascular risk factors and lifestyle on kidney function in post-MI patients with cardiovascular drug-treatment. We investigated this association in a large cohort of stable post-MI patients.

Methods:
Data were analyzed from 2,426 post-MI patients in the Alpha Omega Trial, aged 60-80 years (79% men). Serum cystatin C (cysC) was measured at baseline and after 41 months. Individual eGFRcysC decline rates were calculated with the CKD-EPI equation. The association of diabetes, hypertension (blood pressure ≥140/90 mmHg), high LDL cholesterol (≥2.5 mmol/l), current smoking, low physical activity (<3 METs <5 days/week) and obesity (body-mass index ≥30.0 kg/m2) with annual kidney function decline was assessed by multivariable ANCOVA.

Results:
Mean time since MI was 4.5 years, 19% of all patients had diabetes, 56% had hypertension, 56% had high LDL, 16% were current smokers, 78% had a low physical activity level, and 23% were obese. Baseline mean (SD) eGFRcysC was 81.5 (19.6) ml/min/1.73m2 and mean (95%-CI) annual eGFRcysC decline was -1.34 (-1.47 to -1.21) ml/min/1.73m2. After multivariable adjustment, the additional annual eGFRcysC decline in patients with diabetes, hypertension and obesity was -0.89 (-1.22 to -0.56), -0.51 (-0.77 to -0.25), and -0.30 (-0.61 to 0.01) ml/min/1.73m2, respectively. Former and current compared to never smokers had an additional annual eGFRcysC decline of -0.18 (-0.54 to 0.18) and -0.35 (-0.80 to -0.11) ml/min/1.73m2. We found no association between kidney function and LDL levels or physical activity. From these results we chose to combine diabetes, hypertension, obesity and current smoking into one composite variable. Patients without these risk factors had an annual eGFRcysC decline of -0.92 (-1.17 to -0.66) ml/min/1.73m2. Compared to absence of risk factors, we found a fivefold increase of the annual eGFRcysC decline when all four risk factors were present: -4.60 (-6.16 to -3.05) ml/min/1.73m2.

Conclusion:
Older stable post-MI patients with optimal cardiovascular parameters and healthy lifestyle had an annual kidney function decline rate comparable to healthy individuals.

Figure: Per risk score the presence (%) of different risk factors (columns) and the mean (95%-CI) annual kidney function decline (black line), adjusted for age, sex, and n-3 fatty acid treatment. Included risk factors are: diabetes, hypertension, obesity and current smoking. A risk score of 0 implies: no risk factors present.
DIETARY APPROACH TO STOP HYPERTENSION DIET IS ASSOCIATED WITH LOWER RISK OF ALL-CAUSE MORTALITY AND GRAFT FAILURE IN RENAL TRANSPLANT RECIPIENTS

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BACKGROUND
The incidence of all-cause mortality and graft failure is high in renal transplant recipients (RTR). We hypothesized that a Dietary Approach to Stop Hypertension (DASH) diet protects against these adverse events in RTR.

METHODS
In this prospective cohort study, we used data of adult RTR with a functioning graft for at least one year. Dietary data were collected using a validated food frequency questionnaire (FFQ) of 177 items. Food items of the FFQ were divided over the components of the DASH diet. Subjects were classified into sex-specific quintiles according to their intake. For the components fruit, vegetables, whole grains, low-fat dairy products, legumes and nuts, a score ranging from 1 to 5 according to sex-specific quintiles of intake was obtained. For the components sodium, red processed meats and sweetened beverages, the scoring was reversed (5 points for the lowest sex-specific quintile and 1 point for the highest sex-specific quintile). The component scores were summed up to obtain an overall DASH score ranging from 8 (lowest adherence) to 40 (highest adherence). Subsequently, we divided all patients in tertiles of the overall DASH score. A total of 632 RTR were eligible for analyses. Cox multivariable regression models were used to study the associations with all-cause mortality and graft failure.

RESULTS
Mean ± SD age was 53.0 ± 13.2 years and 56.5% were males. During median follow-up for 5.4 (interquartile range [IQR], 0.2-6.8) years, 128 (20.3%) RTR died and 75 (11.9%) RTR developed graft failure. The highest two tertiles of the DASH score were associated with both a significantly lower risk of all-cause mortality (hazard ratio [HR]= 0.58; 95%CI, 0.40-0.83, P=0.003) and graft failure (HR= 0.56; 95%CI, 0.35-0.91, P=0.02), compared to the lowest tertile, independent of age and sex. Adjustment for potential confounders, including BMI, blood pressure, smoking and eGFR, did not materially change the results of the analyses.

CONCLUSION
High adherence to the DASH diet is associated with a lower risk of all-cause mortality and graft failure after renal transplantation.
“CLINICAL MANIFESTATIONS OF SYSTEMIC OXALOSIS IN PRIMARY HYPEROXALURIA TYPE 1: WHAT OCCURS AT WHAT DEGREE OF RENAL INSUFFICIENCY”

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Background:
Primary hyperoxaluria type 1 (PH1) is a rare inborn error of glyoxylate metabolism characterised by an increased endogenous oxalate production, which leads to the development of urolithiasis, nephrocalcinosis and ultimately renal failure. Once PH1 patients develop renal failure systemic deposition of oxalate accelerates, resulting in oxalosis, a life-threatening condition affecting multiple organs most notably the skeleton, heart, veins, eyes and skin. Knowledge of the manifestations of systemic oxalosis remains sparse. Despite their clinical significance, it is not known which patients are at risk of a severe course of the disease and what factors (e.g. estimated glomerular filtration rate (eGFR) and serum oxalate/glycolate level) contribute to the risk of developing systemic oxalosis. With the current and promising research into new therapeutic modalities for PH1, these answers would provide knowledge to further improve therapeutic strategies and hopefully help to prevent the current devastating comorbidity and mortality in this patient group.

Objectives:
Primary: Describe the manifestations of systemic oxalosis in a large European cohort of patients with Primary Hyperoxaluria type 1. Secondary: Analysis of two potential clinical thresholds for the occurrence of systemic oxalosis; (I) the degree of renal failure expressed as eGFR, and (II) (the rise in) plasma oxalate/glycolate levels.

Methods:
Retrospective review of all PH1 patients registered in the OxalEurope database, and in whom data of sufficient detail was recorded.

Results (work in progress):
To date, we have included more than 800 PH patients in our database (OxalEurope consortium) which continues to expand. For now, we have performed a sub analysis of 132 PH1 patients.

Of the 132 included patients, 51 (38.6%) were found to have at least one manifestation of systemic oxalosis. Bone disorders represented the most frequent manifestation (18.9% (25/132) at diagnosis and cumulatively 30.3% (40/132) at follow up), followed by cardiac (3.8%, 15.2%), cutaneous- and vascular (3.8%, 15.4%), ophthalmologic (7.6%, 12.9%), neurological (4.5%, 8.3%) amongst other manifestations. We found 30 different combinations of symptoms. The majority of manifestations (94%, 48/51) were found in patients with an eGFR <15 ml/min/1.73m2. PH patients were not routinely screened; e.g. 26.5% had not undergone any ophthalmologic evaluation. We report the first patient with manifestations of oxalosis, an eGFR above 50 ml/min/1.73m2 and plasma oxalate level below 30 µmol/l. Plasma glycolate levels started to increase at higher levels of eGFR compared to plasma oxalate levels.

Conclusion:
This study highlights the heterogeneity of systemic oxalosis. The high number of reported systemic manifestations of oxalosis might be an underestimate due to the large number of non-systematically screened asymptomatic patients. Evidence of systemic oxalosis in a patient with moderate CKD warrants attention. Our results challenge the current assumption that systemic deposition of oxalate starts when the plasma oxalate level is > 30 µmol/l and the eGFR < 40 ml/min/1.73m2. Therefore, we stress the value of annual screening for systemic oxalosis in PH1 patients with CKD2+. Plasma glycolate might be a more accurate predictor of systemic oxalosis than plasma oxalate.

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ACETYLATED CHROMATIN IN APOPTOTIC MICROPARTICLES DRIVES THE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS IN LUPUS NEPHRITIS

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Background:
Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by autoantibodies against nuclear components. The major cause of morbidity and mortality in patient with SLE is lupus nephritis (LN). Patients with SLE suffer from recurrent periods of high disease activity termed flares, which are characterized by increased inflammatory processes in the kidney. The accumulation of cellular waste, especially apoptotic microparticles (MPs), is thought to stimulate immune reactions during these flares. We showed that MPs can induce the formation of neutrophil extracellular traps (NETs), web-like structure of DNA and antimicrobial proteins, expelled during a cell death pathway termed NETosis. In this study, we aimed to analyze the nature of the chromatin contents of apoptotic MPs from patients with LN and to unravel the molecular mechanism underlying MP-induced NETosis.

Methods:
MPs from patients with biopsy-proven active lupus nephritis (LN), patients with LN in remission, SLE patients without LN and healthy controls were characterized by flow cytometry using monoclonal antibodies specific for apoptotic chromatin modifications. Neutrophils were isolated from anti-coagulated blood by Ficoll-density centrifugation and exposed to MPs isolated from plasma of patients with SLE or from apoptotic Human Umbilical Vein Endothelial Cells (HUVECs). HUVECs were treated with the histone deacetylase inhibitor (HDAC) trichostatin A (TSA) before induction of apoptosis to generate MPs containing hyperacetylated chromatin. NETs were quantified by their DNA content and by an in-house developed NET-specific ELISA.

Results:
MPs from patients with active LN contained higher levels of acetylated apoptotic chromatin (H4K8,12,16Ac, H2BK12Ac) compared to MPs from LN patients in remission, patients without nephritis or healthy controls. MPs from patients with SLE as well as from HUVECs could induce NETosis in vitro in a concentration dependent manner. Apoptotic MPs were quickly ingested by neutrophils and induced a fast NETosis program (<60 min), which was independent of the formation of reactive oxygen species and autophagy. MPs containing hyperacetylated chromatin were more potent in inducing NETosis. Moreover, the degree of acetylated chromatin in MPs isolated from LN patients determined their NETosis inducing capacity.

Conclusion:
Patients with active lupus nephritis have higher levels of acetylated chromatin in MPs compared to patients in remission. Furthermore, we showed that the degree of acetylation determines the NETosis-inducing capacity of MPs. Therefore, we suggest that MP-induced NETosis driven by MPs that contain hyperacetylated chromatin amplifies the inflammatory response in patients with lupus nephritis during disease flares.
ANCA-ASSOCIATED VASCULITIS- AND SYSTEMIC LUPUS ERYTHEMATOSUS-INDUCED NEUTROPHIL EXTRACELLULAR TRAPS HAVE INTRINSICALLY DIFFERENT FEATURES

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Background:
Neutrophil extracellular traps (NETs) are immunogenic, extracellular DNA structures that harness important autoantigens to be recognized by the adaptive immune system. NETs are thought to play a pivotal role in the pathogenesis of many systemic autoimmune diseases including ANCA-associated vasculitis (AAV) and systemic lupus erythematosus (SLE). However, it is still unclear how and if NETs can act as a common pathway in the pathophysiology of these clinical divergent autoimmune diseases.

Objectives:
To define the characteristics of NETs in AAV and SLE patients.

Methods:
The present study involved 101 AAV patients, 59 SLE patients and 10 healthy controls. Healthy neutrophils were stimulated with 10% serum of these patients to induce NETs. Quantity of NET induction was measured by a novel, highly-sensitive NET quantification assay using 3D-confocal laser scanning microscopy[1]. Qualitative characteristics of NETs were investigated by immunofluorescence microscopy that detected co-localisation of several established autoantigens on AAV- and SLE-induced NETs, including citrullinated histon-3 (CitH3), neutrophil elastase (NE), high mobility group box-1 (HMGB1), myeloperoxidase (MPO) and proteinase-3 (PR3). Additionally, the morphology, kinetics and mechanism of AAV- and SLE-induced NETosis was visualized by live imaging and electron microscopy.

Results:
Quantifying ex vivo NET induction demonstrated that AAV sera induced significant more NETs (82.22 ± 17.8), compared to SLE sera (11.73 ± 2.4). Also qualitatively, NETs induced by AAV or SLE sera were distinct. In both cases, NETs showed co-localisation of MPO and PR3 with extracellular DNA. However, AAV-induced NETs had significantly higher CitH3 expression than SLE-induced NETs. Interestingly, HMGB1 was exclusively expressed on SLE-induced NETs, which also had a higher expression of NE compared to AAV-induced NETs. Intriguingly, the distinction between AAV and SLE NETs was further corroborated by live imaging demonstrating differences in morphology and chronology of NET induction: in SLE NET-clusters were induced within the 1st hour while in AAV through lytic expulsion non-clustered NETs composed of long, thin DNA-fibres were induced in 2-4 hours.

Conclusions:
The present study demonstrated that NET induction in AAV and SLE resulted in quantitative and qualitative distinct NETs indicating that NET formation in AAV and SLE is likely based on intrinsically different processes. These data increase our understanding of the pathophysiologic relevance of NETs and how they could be considered as a common pathway underpinning different autoimmune diseases.

References
Background. C3 glomerulopathy (C3G) is a rare renal disease in which abnormal complement regulation results in predominant C3 deposits within the glomerulus. Diagnosis is based on characteristic glomerular C3 deposits on immunofluorescence (IF). Based on electron microscopy findings C3G can be subdivided into dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). The disease course and time to reach end stage renal disease is different for adults and children. The aim of this study was to determine and characterize the clinical spectrum of C3G in the pediatric population in the Netherlands.

Methods. Twenty-nine children diagnosed by renal biopsy as C3G, treated in six Dutch Pediatric Nephrology University Medical Centers were retrospectively evaluated. Clinical and laboratory findings at presentation and during follow up were analyzed and therapeutic regimes were noted through chart review. Complement analysis, screening for autoantibodies against factor H as well as DNA analysis for the complement-regulating genes was performed.

Results. Nineteen patients met the histopathological criteria for DDD (65.5%) and 10 for C3GN (34.5%). Mean age at presentation was 7.6 years (median 7.0 years) with a female predominance (62.1%). Most patients presented with proteinuria (93.1%) or (microscopic) hematuria (89.7%), and almost half of the patients presented with nephritic-nephrotic syndrome. In twelve cases (41.4%) an infectious trigger was identified. Nine patients (31.0%) had an impaired eGFR of < 60 ml/min/1.73m² at presentation. Patients with DDD presented more often with proteinuria (p=0.043) and impaired eGFR (p=0.03) than patients with C3GN. Auto-antibodies against complement factor H were found in three patients in the acute phase. Five out of 23 (21.7%) genetically examined patients had a pathogenic variation in one of the complement regulating genes. Various treatment regimens were given in the acute phase. Corticosteroid therapy i.e. methylprednisolone pulse therapy (52%) and oral prednisone (76%) was used in most patients, followed by mycophenolate mofetil (21%). DDD patients compared to C3GN patients significantly needed more additional antihypertensive medication at presentation. Only one patient needed initially renal replacement therapy and remained dependent on dialysis. At last follow up, two other patients had an impaired eGFR (<60 ml/min/1.73m²). During follow up (mean 51 months)12 patients (41.4%) had one relapse and one patient (3.4%) had two relapses. Predictors for having an impaired eGFR at last follow up where an impaired eGFR at presentation (p=0.006) and crescents at first biopsy (p=0.0003).

Conclusions. In this nationwide retrospective cohort of C3G pediatric patients, patients with DDD initially present with more serious renal involvement compared to patients with C3GN. The renal outcome of pediatric C3G patients in general is favorable and better than in adults with C3G as reported in literature.
NEUTROPHIL EXTRACELLULAR TRAPS DRIVE ENDOTHELIAL-TO-MESENCHYMAL TRANSITION AND ASSOCIATE WITH PROTEINURIA IN LUPUS NEPHRITIS

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Background.
The impaired degradation of neutrophil extracellular traps (NETs) has been linked to the development of lupus nephritis (LN) in systemic lupus erythematosus (SLE), but the underlying mechanisms for this association remain obscure. This study aims to unravel how insufficiently degraded NETs may jeopardize glomerular vascular integrity in LN patients.

Methods.
Glomerular and macrovascular endothelial cells were exposed to NETs and subsequent effects on endothelial integrity and function were determined. For in vivo studies, plasma samples and kidneys of proteinuric and non-proteinuric MRL/lpr mice and LN patients were analyzed for NETs and correlated to clinical outcomes.

Results.
Glomerular endothelial cells showed a limited capacity to internalize NETs via the receptor for advanced glycation endproducts (RAGE). An overflow of the phagocytic capacity of endothelial cells for NETs resulted in rapidly altering endothelial cell-cell contacts through elastase-mediated proteolysis of the intercellular junction protein VE-cadherin, thereby facilitating transendothelial albumin passage. NET-associated elastase also promoted the nuclear translocation of junctional β-catenin, thereby inducing endothelial-to-mesenchymal transition (EndMT) in cultured (glomerular) endothelial cells. In vivo, NETs could be identified in glomeruli of diseased MRL/lpr mice and patients with LN, whose glomerular presence correlated with the severity of proteinuria and with markers of EndMT.

Conclusions.
NETs induce EndMT and proteinuria in LN through proteolysis of VE-cadherin and activation of β-catenin signaling. Inhibition of NET-associated elastase is a potential therapeutic target whose local inhibition in the glomerulus may prevent the development of LN in SLE patients.
GENETIC PREDISPOSITION TO INFECTION IN A CASE OF ATYPICAL HEMOLYTIC UREMIC SYNDROME (AHUS)

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Background:
Hemolytic uremic syndrome (HUS) is a major cause of renal failure in childhood. Most cases are caused by infection with Shiga-toxin producing Escherichia coli (STEC). In 5-10% of cases, HUS is not preceded by the STEC infection and is considered atypical (aHUS). These cases are strongly associated with genetic defects leading to dysregulation of the complement system. Often aHUS is triggered by a non-STEC infection, however, genetic predisposition to such infections in aHUS has not yet been studied. Here we present thorough complement analysis of a 2 months old patient in whom aHUS episode coincided with Bordetella pertussis infection (whooping cough), Klebsiella oxytoca sepsis and Moraxella catarrhalis pneumonia.

Methods:
In vitro kinetics of complement activation products (C3bc and TCC) in serum were quantified using ELISA. DNA analysis was performed by Sanger sequencing. Recombinant vitronectin variants were produced in HEK293T cells, purified and used in hemolytic assay with sheep erythrocytes and purified C5b-6, C7, C8 and C9 complement proteins.

Results:
The in vitro complement activation kinetics were compared in patient serum and normal human serum (NHS). In patient serum C3 activation rate (expressed as generation of C3bc) was comparable to the rate in NHS, but the rate of TCC generation was slower. Genetic analysis of TCC components and TCC inhibitors revealed a rare heterozygous variant p.Arg229Cys in vitronectin. Prediction software (SIFT, PolyPhen-2) indicated this change as pathogenic. In vitro experiments using recombinant vitronectin variants have shown that this mutation enhances complement inhibition at TCC level.

Conclusion:
Our work indicates that not only genetic changes leading to uncontrolled complement activation but also these increasing vulnerability to infections contribute to aHUS.
CASE-REPORT: UNUSUAL SEVERE CASE OF HEMOLYTIC UREMIC SYNDROME DUE TO SHIGA TOXIN 2D PRODUCING E.COLI O80:H2

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Background
Hemolytic uremic syndrome (HUS) is a common cause of acute renal failure in children. In over 90% of cases HUS is caused by an infection with Shiga toxin-producing Escherichia coli (STEC). Whereas O157 is still the predominant serotype, non-O157 serotypes are increasingly associated with STEC-HUS. With supportive therapy, STEC-HUS is often self-limiting, with occurrence of chronic sequelae in a small proportion of patients. This is in sharp contrast with atypical HUS (aHUS), a diagnosis per exclusionem, which more often may lead to end stage renal disease and is treated with one of the world’s most expensive drugs, eculizumab. Due to major differences in treatment and prognosis, it is essential to differentiate between STEC-HUS and aHUS. This case shows the pitfalls and challenges to differentiate based on clinical presentation. Furthermore it illustrates the importance and added value of extensive fecal diagnostics.

Case
A previously healthy 16-month old boy presented at the emergency department with convulsions and impaired consciousness. Initial laboratory evaluation showed the triad of HUS. Atypical HUS was considered due to the severe and mainly neurological presentation, absent history of (bloody) diarrhea, and young age of the patient. Consequently, the patient received 600mg of eculizumab on the first day of admission. During the course of admission the patient developed severe multi-organ failure with extensive involvement of brain, kidneys, pancreas and liver. Despite the presentation which was suggestive of atypical HUS later incoming results of additional complement and DNA diagnostics showed no abnormalities. In addition, extensive fecal diagnostics revealed the rare STEC serotype O80:H2. This serotype contained the Stx2d gene and the rarely in human seen eae ξ gene variant, most likely leading to this unusual and severe disease course. Hence, the virulence factors of this O80 strain could explain the highly severe disease presentation.

Conclusion
To differentiate between STEC-HUS and aHUS remains a clinical conundrum. Extensive fecal diagnostics especially in cases with an atypical presentation, are of great importance to differentiate between the two types of HUS. In addition, this case report illustrates the potential very severe disease course of STEC-HUS with the highly virulent 080 serotype and emphasizes the importance of molecular and serotyping assays to estimate the virulence of an STEC strain.
SAFETY AND EFFECTIVENESS OF RESTRICTIVE ECULIZUMAB TREATMENT IN ATYPICAL HEMOLYTIC UREMIC SYNDROME

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Background
Atypical hemolytic uremic syndrome (aHUS) is a rare, but severe form of thrombotic microangiopathy, as a consequence of complement dysregulation. Atypical HUS has a poor outcome with high mortality and over 50% of patients developing end stage renal disease. Since the end of 2012, these outcomes have greatly improved with the introduction of eculizumab. Currently, the duration of treatment is debated. Most guidelines advise lifelong treatment. However, there is no hard evidence to support this advice. Historically, a substantial number of aHUS patients were weaned of plasma therapy, often without disease recurrence. Moreover, the long-term consequences of eculizumab treatment are unknown. In this retrospective study we describe 20 patients who received a restrictive treatment regimen.

Method
All aHUS patients who presented in the Radboudumc, Nijmegen, the Netherlands, between 2012-2016, and who received eculizumab are described. Clinical, diagnostic and follow up data were gathered and reviewed.

Results
Twenty patients (14 adults, 6 children) with aHUS have received eculizumab. Eculizumab was tapered in all and stopped in 17 patients (figure 1). Atypical HUS recurrence occurred in five patients. Due to close monitoring, recurrence was detected early and eculizumab was restarted. No clinical sequela such as proteinuria or progressive kidney dysfunction were detected subsequently. In total, eculizumab has been discontinued in 13 patients without aHUS recurrence, of which 5 are event free for over a year now. With this strategy approximately €11.5 million has been saved.

Conclusion
A restrictive eculizumab regimen in aHUS appears safe and effective, with close monitoring for signs of disease recurrence. Prospective studies are needed to determine optimal duration of treatment with eculizumab and evaluate prediction markers. Recently, a national, prospective study called CUREiHUS has started in the Netherlands.
THE VALUE OF SEROLOGICAL DIAGNOSTICS FOR STEC-HUS UNDER THE LOOP

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Background
Hemolytic uremic syndrome (HUS) is characterized by the triad of thrombocytopenia, hemolytic anemia and acute kidney injury. Discriminating between HUS caused by Shiga toxin-producing Escherichia coli (STEC-HUS) or by a dysregulated complement system (atypical HUS; aHUS) is essential due to major differences in clinical outcomes and treatment possibilities. However, due to the similarity in symptoms, it can be challenging to differentiate clinically between STEC-HUS and aHUS. Atypical HUS is a diagnosis per exclusionem, hence providing proof for the presence of STEC forms the basis for differentiation between aHUS and STEC-HUS.

To diagnose STEC-HUS remains a clinical conundrum. The current golden standard (i.e. fecal diagnostics) used to demonstrate STEC infection appears far from ideal. Serological detection of antibodies against the lipopolysaccharides (LPS) of STEC by enzyme-linked immunosorbent assay (ELISA) has improved STEC-HUS diagnostics significantly. However, this assay revealed multiple limitations, whereas new techniques show promising results. Here we evaluated the use of serotype specific recombinant glycoproteins as antigens in a novel established glyco-iELISA.

Methods
This study retrospectively included all patients (n=61) with clinical STEC-HUS who presented between 1990-2014 in the Radboudumc. Clinical, diagnostic and follow up data were gathered. Serological assays using LPS and glyco-iELISA to detect IgM and IgG against STEC serotype O157 were compared to each other as well as to fecal diagnostics.

Results
Using fecal diagnostics, 54% of the patients were diagnosed with an STEC infection. However, compared to fecal diagnostics, the percentage of patients with evidence of STEC increased to respectively 74% and 77% by LPS-based ELISA and glyco-iELISA. Combining the glyco-iELISA with fecal diagnostics provided evidence for STEC infection in 89% of the patients. More importantly, the stability, reliability, low intra- and inter-assay variability and the absence of cross-reactivity places the glyco-iELISA ahead of the LPS-based ELISA.

Conclusion
Our data demonstrate that the novel glyco-iELISA is a sensitive and accurate serological method to detect STEC-HUS. Combined with fecal diagnostics, the glyco-iELISA improves the STEC detection by 35%.
ECULIZUMAB DOSING REGIMEN IN ATYPICAL HUS: POSSIBILITIES FOR INDIVIDUALIZED TREATMENT

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Background:
Recent studies indicate that eculizumab is often given in excess to aHUS patients. Individualization of treatment is thus highly requested, however, data on pharmacokinetics and pharmacodynamics of eculizumab remain limited.

Methods:
Serum eculizumab and complement activity (CH50) were measured by in-house ELISA-based methods. In total, 209 samples were taken from 11 patients before the eculizumab infusion in the induction (weekly), maintenance (2-weekly) and tapering (every 3, 4 and 5 weeks) phases of therapy. Statistical analysis was performed using linear mixed models.

Results:
The trough eculizumab levels increased with each additional dose during the induction phase (depending on body weight). During maintenance, high eculizumab concentrations of up to 772 µg/mL were observed. The levels decreased with each following dose during tapering (3- and 4-week intervals), however three patients maintained target eculizumab levels over long time periods (30-48 weeks). At intervals of 6-8 weeks target eculizumab levels were no longer attained. Serum samples with eculizumab concentrations ≥50 µg/mL showed full complement blockade.

Conclusion:
Our data provide essential insight for optimization of eculizumab dosing schemes and lessening of therapy burden for the patients and cost of the treatment.
NLRX1 ATTENUATES RENAL FIBROSIS AFTER UNILATERAL URETERAL OBSTRUCTION IN MICE

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Background and objective:
Renal fibrosis is the result of excessive production of collagen in the renal parenchyma. It is the final and irreversible common pathway of all progressive renal diseases, ultimately resulting in life-threatening end-stage renal disease. Our previous results imply that innate immune receptors like Toll-like receptors (TLRs) and NOD-like receptors (NLRs) play different roles in mediating renal fibrosis. Among these receptors NLRX1 is a new NLR family member that is uniquely localized at mitochondria. Although we and others have shown that this receptor protects against acute renal ischemic injury and is a regulator of mitochondrial antiviral immunity, its role in (renal) fibrosis is completely unclear. In this study we therefore examined the role of NLRX1 in fibrosis after unilateral ureteral obstruction (UUO) in mice.

Methods:
C57Bl/6J WT and NLRX1 knock-out (KO) female mice were subjected to complete obstruction of the right ureter. Mice were sacrificed at 1, 3, 7, and 12 days post ureteral obstruction (n = 6-8 mice per group), contralateral kidneys of day 1 were used as control. Renal sections were stained with PASD (tubular injury determination: loss of tubular brush border, flattening of the epithelium and tubular dilatation), and for caspase-3 (apoptosis), α-smooth muscle actin (myofibroblasts), F4/80 (macrophages) and, PicroSirius red (collagen deposition). Active TGF-β1, ureum and creatinine were determined in plasma and kidney homogenates.

Results:
Pathological observation of PASD sections demonstrated increased renal tubular injury at day 3 and 7 in NLRX1 deficient mice compared to WT animals. NLRX1 kidneys moreover displayed an increased number of tubular epithelial cells in apoptosis at day 1. At this time point plasma ureum levels were increased as well in NLRX1 KO mice. F4/80 stained renal sections revealed an increased presence of mature macrophages at day 7 in the NLRX1 KO mice. Interstitial fibrosis was furthermore enhanced in NLRX1 KO kidneys as reflected by increased collagen deposition at day 7 and myofibroblast accumulation at day 3 and 7. In line, renal levels of active pro-fibrotic cytokine TGF-β1 were upregulated in the NLRX1 KO at day 1 and 3 compared to WT animals.

Conclusion:
Our study implies a central role for NLRX1 in protection against the early effects of UUO-induced progressive renal injury and fibrosis. In contrast to previous studied TLR4, TLR2, and NLRP3 we found that NLRX1 attenuates renal fibrosis following UUO by lowering macrophage presence, active TGF-β1 levels, collagen and myofibroblast accumulation. We moreover found that the presence of NLRX1 reduces tubular damage and tubular apoptosis following UUO. The exact mechanism behind this is now under investigation.
THE STRIKING FINDING OF MULTICILIATED PROXIMAL TUBULAR CELLS IN PATIENTS WITH TUBULAR INJURY

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Background:
Cilia are antennae-like structures which are evolutionary highly conserved and have important functions in cell signaling and homeostasis. Two main types of cilia can be distinguished: primary and motile cilia. In kidney epithelial cells, a single primary cilium per cell can be detected, which serves as flow sensor and consists of 9 peripheral microtubular doublets. Motile cilia can be found on multiciliated cells of e.g. lung or ovarian duct and additionally express a central microtubule pair, dynein arms as well as radial spoke proteins (e.g. RSPH4A). These additional structures are required for ciliary motion. The transcriptional program for motile cilia assembly involves activation of FOXJ1 and RFX3. In this study, the unexpected detection of multiciliated cells in kidneys of patients with tubular injury was evaluated.

Methods:
Immunofluorescent staining was performed on patient biopsies with a marker for cilia (Acetylated α-tubulin), marker for specific tubular segments (AQP1, AQP3, AQP4, LTA) and marker to detect motile cilia (RSPH4A). In addition, the expression of transcription factors FOXJ1 and RFX3, driving motile cilia assembly and the formation of multiple cilia per cell was studied. The ciliary ultrastructure was analyzed using transmission electron microscopy.

Results:
Multiciliated cells were initially detected in five patients. All patients were affected by tubular injury with different underlying pathologies. Multiciliated cells showed co-expression of AQP1 and LTA but not AQP3 or AQP4 indicating localization of these cells in the proximal tubules. Furthermore, cilia on multiciliated cells stained positive for RSPH4A and motile cilia structures (9+2) were detected using transmission electron microscopy. Co-expression of FOXJ1 and RFX3 in cells with multiple cilia was observed, indicating activation of motile cilia assembly. Electron microscopic analysis of biopsies from 20 additional patients with severe tubular injury/damage revealed the presence of multiciliated cells in 4 cases (20%).

Conclusions:
In conclusion, multiciliated proximal tubular cells with motile cilia are present in patients with tubular injury. This presence of multiciliated proximal tubule cells was frequently observed in 20% of patients with tubular injury. The mechanism underlying this phenomenon, as well as the possible function of multiciliated cells in the kidney, need further investigation.
PRE-DIALYSIS FLUID STATUS MODIFIES THE RELATION BETWEEN PRE-DIALYSIS SYSTOLIC BLOOD PRESSURE AND OUTCOME IN PREVALENT HEMODIALYSIS PATIENTS. RESULTS OF AN INTERNATIONAL COHORT STUDY ON BEHALF OF THE MONDO INITIATIVE.

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Background: Pre-dialysis fluid overload (FO) is a risk factor for mortality and the most frequent cause of elevated pre-dialysis systolic blood pressure (SBP). However, especially low pre-dialysis SBP is associated with increased mortality in hemodialysis (HD) patients. Whether the combination of pre-dialysis SBP and fluid status (FS) modifies the association with mortality has not yet been investigated. The objective of this study was to investigate the interaction between pre-dialysis FS and pre-dialysis SBP in the association with mortality.

Methods: We included all patients from the MONDO database who had a pre-dialysis multifrequency bioimpedance spectroscopy (MF-BIS) measurement in the year 2011. BP was measured pre- and post-dialysis by the oscillometric method incorporated in the machine. We used all parameters available for the 90-day baseline period before the last MF-BIS of 2011. All-cause mortality was recorded during 1-year follow-up. Associations with outcome were assessed with Cox-models with different levels of adjustment and with a smoothing spline Cox analysis.

Results: We included 8883 patients and found that in patients with pre-dialysis FO (≥+1.1 to +2.5L), low pre-dialysis SBP (<110 mmHg) was associated with an increased risk of death (HR 1.52 (95% CI 1.06-2.17)), as compared to normovolemic (<1.1L to +1.1L)(NV) and normotensive (110-140 mmHg)(NT) patients. An increased risk of death, compared to pre-dialysis NV and NT patients, was also observed in patients with pre-dialysis fluid depletion (FD)(<110mmHg). In NV patients, low pre-dialysis SBP(<110mmHg) associated with a survival benefit (HR 0.46 (95% CI 0.23-0.91)). Post-dialysis FD was associated with a survival benefit. Results were similar but more pronounced when inflammation was present. Higher ultrafiltration rates could not solely explain the higher mortality rates observed in the FO groups.

Conclusion: The relation between SBP and outcome is at least partly dependent on pre-dialysis FS. Low pre-dialysis SBP appears to be disadvantageous in patients with FO or FD, but not in NV patients where low SBP is actually associated with a survival benefit. Post-dialysis FD was found to associate with improved survival. Therefore, we suggest that pre-dialysis SBP levels should ideally be interpreted in the context of FS and not as an isolated marker.

Figure: The association of the combination of pre-dialysis systolic blood pressure (mmHg) and pre-dialysis fluid status (L) with mortality.

Figure legend:
HR: hazard ratio’s of a cox proportional hazards model adjusted for gender, age, dialysis vintage, region (categorical), albumin (g/dL), CRP (ln) (mg/L), LTI (categorical as below <10th percentile vs above), BMI (kg/m2), IDWG (kg), treatment time (categorized >240 and <240 min), dialysate sodium (mEq/L), serum sodium (mEq/L), ultrafiltration rate (mL/kg/h) and the comorbidities congestive heart failure, diabetes mellitus, cardiovascular disease, peripheral vascular disease and cancer. The reference group consists of patients with a pre-dialysis systolic blood pressure (SBP) between 110-140 mmHg and normovolemic (between -1.1L to +1.1L) fluid status pre-dialysis. Patients were stratified into different groups (horizontal and vertical lines) based on pre-dialysis SBP (<110 mmHg, 110-140 mmHg, >140 mmHg) and pre-dialysis fluid status by MF-BIS (<-1.1L, -1.1 to +1.1L, >=+1.1L to +2.5L, >=+2.5L).
A FLUORESCENCE BASED SENSOR FOR ON-LINE ELECTROLYTE MONITORING DURING DIALYSIS
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Background
Patients with end stage renal disease and who are not yet eligible for renal transplant are dependent on chronic dialysis modalities such as peritoneal (PD) or hemodialysis (HD). HD is usually performed 4 hours 3 days/week. In contrast to the natural kidney, conventional HD instrumentation is based on a so-called ‘one-size-fits-all’ approach. In most outpatient centers, the dialysate is prepared with a fixed electrolyte concentration without taking in account the inter-individual differences of essential electrolytes (Na⁺, K⁺ and Ca²⁺). This can lead to acute and chronic cardiovascular complications in dialysis patients. On-line monitoring of these essential electrolytes is an important physiological step towards patient specific dialysate leading to an individualized treatment. Currently, changes in electrolyte concentrations are indirectly measured by conductivity measurements, which are not ion-specific [1].

Method
We propose a fluorescent micro-optofluidic sensor based on the principle of photo-induced electron transfer (PET). The PET principle exploits selective quenching of fluorescence. The intensity of the fluorescence signal is a measure of a specific analyte concentration. To prove the working principle, a micro-optofluidic device is fabricated in polydimethylsiloxane (PDMS) with integrated optical fibers for fluorescence light collection. The PET sensor molecules for sodium ions are covalently grafted in the microchannel. The experimental setup consists of a laser module (λ=450 nm) operating at 4.5 mW, a syringe pump to precisely control the fluid flow and a spectrometer for fluorescence detection.

Results
Firstly the fluorescence response of the dye is evaluated by making a solution of the dye in acetonitrile with a fixed concentration of 20 µm and NaClO₄ is used as a source of sodium ions. Fig 1 shows relationship between the fluorescence emission and sodium concentration. Next, the functionalized chip performance is evaluated for sodium ions (NaCl solution) ranging from 0-50mM. A clear signal and good response time was observed (fig 2).

Conclusions
We have built a proof-of-concept experimental setup and the device for sodium monitoring. The fabricated and developed micro-optofluidic sensor shows promising characteristics in terms of sensitivity and stability. Further work on signal stabilization is under progress.


Fig 1: Fluorescence intensity as a function of sodium concentration. NaClO₄ dissolved in acetonitrile is used as a source of sodium ions. The dye concentration is kept constant at 20 µm and only sodium concentration is changed

Fig 2: Intensity response of the micro-optofluidic functionalized device to different sodium chloride concentrations at pH 7.4 (HEPES buffer).
EVALUATION OF A GENETIC RISK SCORE BASED ON CREATININE ESTIMATED GFR AND ITS ASSOCIATION WITH KIDNEY OUTCOMES

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Background.
Results from cross-sectional genome-wide association studies (GWAS) on creatinine-estimated kidney function (eGFRcrea) can be translated into a Genetic Risk Score (GRS) for CKD. However, it is uncertain whether this GRS corresponds to kidney function per se or merely reflects creatinine metabolism, and whether these cross-sectional associations are also valid longitudinally. Therefore, we examined cross-sectional and longitudinal associations of the GRS with eGFRcrea based outcomes, and compared those with associations of the GRS with cystatin C based outcomes (eGFRcysc). Associations with urinary albumin excretion (UAE) based outcomes were examined to discriminate between impaired kidney function and kidney damage.

Methods.
In the community-based PREVEND Study, we assessed serum creatinine, cystatin-C, and 24h-UAE at baseline and four consecutive examinations. CKD-EPI equations were used to calculate eGFRcrea and eGFRcysc. CKD was defined as eGFR<60mL/min/1.73m2. A GRS was constructed from 52 SNPs identified in a recent meta-analysis of GWAS on eGFRcrea, with weights based on the published SNP effects. Effects of standardized GRS were reported per sd difference. We adjusted for known renal risk factors.

Results.
We included 3,649 subjects (median age 49 [IQR 39-60], median follow-up time 11.0 years [IQR 4.6-11.9], 52% male, N=85 baseline CKD, N=154 incident CKD). At baseline, higher GRS was significantly and independently associated with lower baseline eGFRcrea levels and higher CKD prevalence (Table 1). During follow-up, higher GRS was significantly associated with higher CKD incidence, but no longer after additional adjustment for baseline eGFR. No association with steeper decline of eGFRcrea was found. Similar associations were found with kidney outcomes based on eGFRcysc. We found no robust associations with UAE based outcomes.

Conclusions.
The GRS showed modest but robust associations with baseline eGFRcrea and with prevalent CKD, independent of known risk factors, thus corroborating literature. Associations with incident CKD were likely due to low baseline eGFR, and not due to accelerated eGFR decline. Associations with eGFRcysc based outcomes were largely similar. The GRS was not associated with kidney damage (i.e. UAE). Given these findings, the combined loci for eGFRcrea are likely to truly represent a genetic component to kidney function. However, separate GWAS are needed to identify loci associated with kidney function decline.

Table 1. Independent associations of a Genetic Risk Score based on eGFRcrea with selected kidney outcomes: comparison between creatinine and cystatin C based eGFR.

<table>
<thead>
<tr>
<th>Genetic Risk Score (per sd)</th>
<th>Cross-sectional analyses</th>
<th>Longitudinal analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR estimated by:</td>
<td>Baseline eGFR B ± se</td>
<td>Baseline CKD Odds ratio [95%CI]</td>
</tr>
<tr>
<td>creatinine</td>
<td>-2.04 ± 0.20</td>
<td>1.44 [1.15-1.81]</td>
</tr>
<tr>
<td>cystatin C</td>
<td>-1.58 ± 0.25</td>
<td>1.27 [1.08-1.49]</td>
</tr>
</tbody>
</table>

Estimates from linear, logistic, linear mixed effects, and Cox regression analyses, adjusted for age, sex, BMI, smoking status, diabetes, hypertension, hypercholesterolemia, and history of cardiovascular disease. eGFR, estimated glomerular filtration rate (mL/min/1.73m2); CKD, chronic kidney disease (eGFR<60 mL/min/1.73m2); ∆eGFR, annual change in eGFR (mL/min/1.73m2 per year). *p<0.05, **p<0.01, ***p<0.001.
NPHP1 DELETIONS CAUSE ESRD IN 0.9% OF ADULT-ONSET CASES

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*For the TransplantLines-Genetics consortium.

Background
Nephronophthisis (NPH) is the most prevalent (15%) genetic cause for end-stage renal disease (ESRD) in children. Mutations in the autosomal recessive NPHP1 gene are known to cause 20% of NPH cases, with the homozygous full gene deletion accounting for 80%. The ciliopathy is associated with corticomedullary cysts and reduced kidney size, but as the phenotype is aspecific it can prove difficult to diagnose. Little is known about the prevalence of NPHP1 mutations in adult-onset ESRD. With data generated to perform a genome-wide association study in adult-onset ESRD patients, we aimed to determine the prevalence of homozygous NPHP1 full gene deletions.

Methods
The TransplantLines-Genetics cohort, consisting of 1272 renal transplant recipients was genotyped using the Affymetrix Axiom Tx GWAS Array (Affymetrix, Inc., Santa Clara, California), designed for the iGeneTRAIN consortium. This array contains probes for ~782,000 SNPs, of which ~350,000 were evenly spaced to cover the whole genome. Autosomal copy number variants (deletions and duplications) were determined based on median log2 ratios.

Cases were included in the analysis when they had adult-onset ESRD, defined as start of first renal replacement therapy (RRT) at any age >18 years. Phenotypes were classified according to the 2012 ERA-EDTA Coding system for Primary Renal Disease.

Results
1250 cases met the age criteria, of whom 11 (0.9%) displayed a homozygous deletion of the NPHP1 gene. So far, in seven cases the deletion has independently been validated by multiplex ligation-dependent probe amplification, showing 100% concordance. Validation in the four remaining cases is currently being performed. It is likely that the 0.9% is an underestimation of the overall prevalence of causative deletions, as compound heterozygosity (a full gene deletion on one allele unmasking a pathogenic mutation on the other allele) cannot be assessed with this method.

In the 11 NPHP1 deletion cases median age at start of RRT was 35 years (interquartile range 7), with eight cases aged >30 years and the eldest case being 42 years old. Notably only three out of 11 cases (27%) were phenotypically classified as NPH. The other cases (8/11, 73%) were coded as having chronic kidney disease with unknown etiology (n=5), glomerulonephritis (n=1), sporadic primary reflux nephropathy (n=1) and autosomal dominant polycystic kidney disease (n=1).

Conclusion
NPH is a classical pediatric kidney disease. However, we show in a large unselected cohort that homozygous NPHP1 full gene deletions alone cause 0.9% of all adult-onset ESRD, with the majority of NPHP1 cases being aged >30 years. Considering that other types of mutations in NPHP1 were not analyzed, and the other 18 known NPH genes were not even investigated, NPH is a relatively frequent cause of adult-onset ESRD. Especially as 73% of the cases had received a clinical diagnosis other than NPH, these results warrant wider application of genetic testing in adult-onset ESRD.
FOUR-JOINTED KNOCK-OUT CAUSES A DELAY IN KIDNEY FAILURE IN AN ADPKD MODEL WITH RENAL INJURY

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Background.
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is characterized by the development of bilateral fluid-filled renal cysts which lead to terminal failure of the kidneys. In the majority of cases the disease is caused by a mutation in the PKD1 gene but an extensive signalling network is involved in the progression of this disease. In a previous study we demonstrated that injury-induced tubular epithelial cell proliferation is able to accelerate cyst formation in kidneys of inducible Pkd1-deletion (iksPkd1del) mice (Happé et al, Hum. Mol. Genet. 2009). In particular, our results suggested a role in the injury/repair mechanism for the Planar Cell Polarity (PCP) regulator, Four-jointed (Fjx1), which was aberrantly expressed in Pkd1 conditional deletion (iksPkd1del) mice, when compared with normal mice after injury. Therefore we generated a double mutant lacking both Pkd1 and Fjx1 and we used it to study the effect on disease progression of Fjx1 after injury. Surprisingly the double KO mice lived significantly longer then the single iKspPkd1del mice after injury (median survival: 14 weeks vs 20 weeks; p value<0.05), confirming a role for Fjx1 in injury/repair and disease progression.

Methods.
We use several mouse models, i.e. Fjx1-/- mice, Fjx1-/-; iksPkd1del (Double KO) mice as well as iKspPkd1del (single Pkd1 KO) mice, and we induced toxic renal injury using the nephrotoxic compound 1,2-dichlorovinyl-cysteine (DCVC) after Pkd1-gene inactivation. We then follow tissue- repair and cyst formation in the different models characterizing key pathways involved in this process.

Results.
We have investigated several pathways which are involved in the process of injury repair, both at mRNA and protein level. PCP, assessed using the Golgi position, was comparably aberrant in both single Pkd1 KO and Double KO already early after DCVC suggesting a more complex regulation of PCP and excluding a causal role for this pathway in the longer survival observed. Also proliferation, Hippo pathway, Notch pathway, glucose and fatty acid metabolism, cyst formation and kidney size were comparable in both the genotypes during disease progression. Interestingly, even though cyst formation was not different, we found a significantly lower expression of genes like TGFb, aSMA, Collagen 1 and Vimentin and significantly less areas Sirius Red positive in Double KO mice compared with single Pkd1 KO mice. We also found a reduced number of F4/80+ infiltrates (confirmed both on IHC and qPCR) in Double KO mice. Taken together this data can explain the longer survival observed in the Double KO mice after DCVC injection.

Conclusion.
Our data suggest that Fjx1 disruption protects the cystic kidney against kidney failure, after toxic injury, by delaying fibrosis. Because cyst formation and cyst growth are not affected, our data unveil an interesting (yet unidentified) mechanism affecting fibrogenesis, which might help to explain how fibrosis initiate and progresses in ADPKD.
CD4⁺CD28null T CELLS REQUIRE EXOGENOUS CYTOKINES TO RESPOND TO ALLOGENEIC STIMULATION

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Introduction
Ageing and a pro-inflammatory milieu, such as present in patients with end-stage renal disease, are important drivers of increased T-cell differentiation, which is accompanied by loss of the co-stimulatory molecule CD28. CD4⁺CD28null T cells have a highly cytotoxic, inflammatory profile and respond to IL-15 and IL-21 in particular. These cells have been associated with an increased as well as a decreased risk for rejection after renal transplantation. Therefore, we wanted to investigate the alloreactive potential of CD4⁺CD28null T cells in detail.

Materials and methods
FACS-sorted CD4⁺CD28null and CD4⁺CD28⁺ T cells were stimulated with HLA-mismatched CD3-depleted cells in the absence or presence of exogenous cytokines. The alloreactive potential was evaluated by measuring proliferation, degranulation (CD107a expression), content of cytotoxic molecules and cytokine production.

Results
Compared with CD4⁺CD28⁺ T cells, the CD4⁺CD28null T cells showed an almost absent proliferation, degranulation and cytokine production in response to allogeneic stimulation. Addition of IL-15 (with/without IL-21) to the cell culture increased the frequency of proliferating CD4⁺CD28null T cells significantly up to 30% (p<0.001) without altering CD28 expression. Next to this, the combination of IL-15 and IL-21 also increased CD107a expression within the CD4⁺CD28null T cells (p<0.05). Furthermore, granzyme B and perforin positivity seemed to be higher when IL-15 and IL-21 were added to the allogeneic condition within CD4⁺CD28null T cells compared to the allogeneic condition without these cytokines. Also, allogeneic-expanded CD4⁺CD28null T cells were capable to lyse allogeneic target cells in a specific lysis assay. Finally CD4⁺CD28null T cells after alloantigen stimulation in the presence of IL-15 +/- IL-21 produced more IFN-γ and TNF-α (p<0.05 for IFN-γ and p<0.01 for TNF-α).

Conclusion
CD4⁺CD28null T cells need exogenous cytokines, in particular IL-15, to proliferate and secrete inflammatory cytokines in response to allogeneic stimulation.
INTERSPECIES DIFFERENCES IN PTH-MEDIATED REGULATION OF THE RENAL CALCIUM CHANNEL TRPV5

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*These authors contributed equally to this work

Background
Calcium homeostasis is a delicate balance, maintained by calcium absorption via the intestine, dynamic storage in bones and reabsorption in kidney. The epithelial calcium channel TRPV5 (transient receptor potential vanilloid 5) is expressed in the distal convoluted tubule of the kidney and is the gatekeeper of active calcium reabsorption. TRPV5 is regulated by the calciotropic parathyroid hormone (PTH) on the level of transcription, cell surface trafficking and channel activity. The latter is mediated by protein kinase A (PKA), downstream of PTH-receptor signaling. As previous work was performed on rabbit TRPV5 (rbTRPV5) the aim of the present study was to extend these findings to humans and characterize the expression and function of the human TRPV5 (hTRPV5) channel.

Methods
A phylogenetic tree of TRPV5 orthologues was constructed in RAxML, using sequences imported from EnsEMBL 83. The functional implications of sequence differences between rbTRPV5 and hTRPV5 upon PTH stimulation (10 min) were investigated using a combination of biochemical approaches: i) PKA binding mutants rbTRPV5 R707Q and hTRPV5 Q706R and phosphorylation mutants hTRPV5 S141D (constitutively phosphorylated) and hTRPV5 S141A (non-phosphorylated) were generated; ii) plasma membrane expression was assessed by cell surface biotinylation of transiently transfected human embryonic kidney 293 (HEK-293); iii) functional consequences of these mutations were analyzed by radioactive calcium uptake assays and calcium-sensitive Fura-2-AM imaging; iv) calmodulin binding profiles of the mutants were obtained using a pull-down assay with calmodulin-conjugated beads.

Results
Previously, the PKA-phosphorylation site in rbTRPV5 was traced to a PKA consensus RRxT motif in the carboxyl terminus of the channel. This motif is not conserved in humans (RQxT). Interestingly, while PTH treatment enhanced rbTRPV5 channel function, this was not observed for hTRPV5. Mutation of the human RQxT motif into rabbit RRxT (hTRPV5 Q706R) restored sensitivity to PTH. In rbTRPV5, PKA phosphorylation has previously been shown to impair calmodulin binding to the motif, hampering channel inactivation. However, calmodulin binding was not altered in hTRPV5 Q706R and rbTRPV5 R707Q. Remarkably, the evolutionary change of the RRxT motif coincides with the creation of another putative PKA motif (RGAS to RRA S) in the amino terminus of hTRPV5. Both hTRPV5 S141D and S141A were not stimulated by PKA phosphorylation. Yet, hTRPV5 S141D exhibited reduced channel function, likely due to enhanced calmodulin binding capacity.

Conclusion
The short-term PTH-mediated stimulation of TRPV5, via PKA, is not conserved in humans. Furthermore, an additional phosphorylation site was identified in the amino terminus that inhibits TRPV5 channel activity. These data suggest that PTH regulation of TRPV5 is altered in humans, an observation that is important to future studies and may change the view on the role of PTH in renal calcium handling.
LOWER SERUM CALCIUM IS INDEPENDENTLY ASSOCIATED WITH CKD PROGRESSION

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Background:

Disturbances in serum calcium are common in patients with chronic kidney disease (CKD), but whether they are associated with subsequent renal function decline is less clear. In this study, we address the association between serum calcium at baseline and renal function decline in non-dialysis patients with CKD stages 3a, 3b, 4, and 5 separately.

Methods:

This is an observational study in a healthcare utilization cohort of all adult citizens of Stockholm undertaking creatinine tests during 2006-2011. eGFR was calculated with the CKD-EPI equation, and CKD defined as eGFR <60 ml/min/1.73m², excluding patients undergoing renal replacement therapy. The exposure was serum calcium at inclusion. The outcome was the subsequent change in rate of renal function decline, using linear mixed models stratified by separate CKD stages (3a-5). Dose-response relationships across eGFR levels were investigated via multiplicative interactions.

Results:

15755 individuals were included with eGFR <60 ml/min/1.73m² and concurrent calcium testing at cohort entry. Mean baseline calcium level was 9.6 (SD 0.5) mg/dl and mean eGFR decline was -0.82 (95% confidence interval [CI] -0.90; -0.74) mL/min/1.73m². In advanced CKD stages, a higher serum calcium was associated with a less rapid renal function decline: The crude change in eGFR decline associated with a unit (i.e. mg/dl) increase in calcium at baseline was -0.10 (95% CI -0.36; 0.17, p=0.47), 0.52 (0.20; 0.84, p=0.002), 0.43 (0.09; 0.77, p=0.01) and 0.65 (0.32; 0.98, p=0.001) mL/min/1.73m²/year for patients in CKD stage 3a, 3b, 4, and 5, respectively. Adjustment for baseline confounders did not modify these associations. The lower the baseline eGFR, the stronger the effect of low calcium on subsequent eGFR decline.

Conclusion:

Lower serum calcium was associated with a more rapid CKD progression in stages 3b to 5 in a large population of non-dialysis patients.
QUALITY OF LIFE, PHYSICAL ACTIVITY AND BODY COMPOSITION IN DIALYSIS: COMPARISON OF INTENSIVE HEMODIALYSIS WITH PERITONEAL DIALYSIS AND CONVENTIONAL HEMODIALYSIS

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Background

In dialysis populations, low physical activity (PA) associates with reduced quality of life (QoL) and increased mortality. Physical inactivity relates to abnormalities in body composition (BC), most importantly an increase in fat mass. A higher lean tissue index (LTI; lean tissue mass corrected for height) associates with more steps per day. Recent studies have shown no difference in PA between dialysis patients and stage 5 chronic kidney disease (CKD-5) non-dialysis patients. Intensive hemodialysis (IHD) is associated with improved well-being. Therefore, we hypothesized that IHD is associated with improved QoL, PA and body composition (BC). In this study, we investigated QoL, PA and BC in a unique population of patients on IHD and compared this group to healthy controls and patients on peritoneal dialysis (PD) or conventional hemodialysis (CHD; ≤12 hours hemodialysis per week).

Methods

A total of 16 PD, 36 CHD and 36 IHD patients were included. As a control group, we included 20 healthy individuals. QoL was measured by the mental component summary of the SF-36 survey. PA was evaluated by the amount of steps per day and activity induced energy expenditure (AEE), as determined by the SenseWear pro3 Armband, and the physical component summary of the SF-36 survey. BC was assessed by LTI, measured by the Body Composition Monitor. Multivariate regression analysis was performed to adjust for potential confounding factors, like age, gender, comorbidity (assessed by the Davies Co-morbidity score) and dialysis vintage.

Results

Patients on IHD had a higher AEE compared to patients on CHD (6.76 vs 3.30 kcal/day/kg body weight, p=0.004). The IHD group was younger (mean 52.6 vs. 62.1 years, p<0.004), and had a longer dialysis vintage in the IHD group (mean 112 vs. 36.5 months, p<0.001). In multivariate regression analysis, none of the QoL, PA or BC parameters was associated with dialysis modality, whereas they all were associated with the Davies Co-morbidity score. Age was associated with the amount of steps per day and AEE.

Conclusion

Overall, PA in dialysis patients was lower compared to healthy individuals. The higher PA in terms AEE in this unique IHD population compared with CHD appeared to be explained by differences in age and other confounders. We did not find any differences in QoL or BC between CHD and IHD, in which however differences in age and dialysis vintage between both groups have to be taken into account.
REFERENCE VALUES OF RENAL TUBULAR FUNCTION TESTS ARE DEPENDENT ON AGE AND eGFR

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Background:
Electrolyte disorders can be the consequence of acquired or inherited defects of renal tubular ion transport. These tubular disorders are rare, and knowledge about validated clinical diagnostic tools is sparse. Reference values for tubular function tests arise from small studies in young healthy volunteers. Patients with tubular disorders however frequently are older and/or have a compromised kidney function. In this study we performed tubular function tests in individuals with different ages and eGFR.

Methods:
We performed furosemide tests, thiazide tests, furosemide fludrocortisone tests (FF tests) and DDAVP tests in three groups of individuals: healthy individuals aged 18 to 50 years, healthy individuals of >50 years old and individuals with reduced eGFR (CKD-eGFR stage 2-5 based on the CKD-EPI formula). For each tubular function test, we included 10 individuals per group. Exclusion criteria were pregnancy, severe heart failure, disorders of sodium or potassium balance, tubule-interstitial kidney disease and an urinary alpha1-microglobulin level of ≥ 40 mg/10 mmol creatinine.

Results:
We performed 119 tubular function tests in 53 individuals. The results per test are shown in Table 1.

Table 1: results tubular function tests

<table>
<thead>
<tr>
<th></th>
<th>Young healthy adults</th>
<th>Older healthy adults</th>
<th>CKD subjects</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21 (21-24)</td>
<td>67 (63-68)</td>
<td>68 (57-72)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>28</td>
<td>78</td>
<td>67</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CKD-EPI (ml/min/1.73m2)</td>
<td>120 (109-126)</td>
<td>87 (80-92)</td>
<td>49 (33-61)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Furosemide test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCl at start</td>
<td>1.00 (0.78- 1.76)</td>
<td>1.23 (0.88- 1.56)</td>
<td>1.61 (1.08- 1.96)</td>
<td>0.22</td>
</tr>
<tr>
<td>Maximal ∆FeCl</td>
<td>16.4 (14.9- 19.9)</td>
<td>11.0 (8.3-15.1)</td>
<td>14.0 (11.6- 16.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>Thiazide test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FeCl at start</td>
<td>1.08 (0.89- 1.40)</td>
<td>1.02 (0.75- 1.20)</td>
<td>1.61 (1.30- 2.52)</td>
<td>0.01</td>
</tr>
<tr>
<td>Maximal ∆FeCl</td>
<td>2.9 (2.6-3.9)</td>
<td>2.3 (2.0-2.8)</td>
<td>2.1 (0.9-2.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>FF test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest urine pH</td>
<td>4.8 (4.5-5.1)</td>
<td>4.7 (4.3-4.8)</td>
<td>4.7 (4.4-5.2)</td>
<td>0.66</td>
</tr>
<tr>
<td>DDAVP test</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal urine osmolality (mOsmol)</td>
<td>1002 (869-1074)</td>
<td>820 (799-934)</td>
<td>624 (477-814)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Median values with interquartile ranges.
FeCl = fractional chloride excretion.
∆FeCl = maximal change in FeCl compared to baseline FeCl
*Kruskal Wallis Test

Conclusion:
Reference values for tubular function tests obtained in young healthy adults cannot be simply extrapolated to elderly or subjects with a lower eGFR, except for the FF test. Larger validation studies are needed to determine exact reference values in these patient categories.
PHYSICAL ACTIVITY IN THE FIRST YEAR AFTER LIVING DONOR RENAL TRANSPLANTATION: A LONGITUDINAL ANALYSIS

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Background
Despite the important effects of physical activity (PA) on body composition (BC) and health-related quality of life (HRQOL), only limited information is available in renal transplant recipients. This study aimed to study changes in PA, BC and HRQOL in renal transplant patients with a living kidney donor and healthy kidney donors in the first year after renal transplantation (rTx) or donor-nephrectomy.

Methods
In a prospective longitudinal and cross-sectional analysis of 22 renal transplant patients and 22 healthy kidney donors, PA (number of steps) was measured by a SenseWear pro3. HRQOL was measured by short-form (SF) 36 questionnaires to assess the physical domain of health (physical component summary (PCS) score). Handgrip strength (HGS) was determined, and BC parameters were measured by the Body Composition Monitor©. Measurements were assessed prior to rTx/donation (baseline), 6 and 12 months after surgery.

Results
Data are presented in table 1. In addition, in the recipient group no significant changes in BC parameters were found longitudinally.

Table 1.

<table>
<thead>
<tr>
<th>Longitudinal analyses</th>
<th>Cross-sectional analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Steps/day</strong></td>
<td><strong>Number of Steps/day</strong></td>
</tr>
<tr>
<td>Recipients</td>
<td>Recipients vs. donors</td>
</tr>
<tr>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>6 months post-rTx</td>
<td>6 months post-rTx</td>
</tr>
<tr>
<td>p-value</td>
<td>0.638 (n=14)</td>
</tr>
<tr>
<td>PCS score (%)</td>
<td>45.65 [38.2 – 50.33]</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.001 (n=18)</td>
</tr>
<tr>
<td>12 months post-rTx</td>
<td>51.85 [48.93 – 56.20]</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004 (n=18)</td>
</tr>
<tr>
<td>Handgrip strength (kg)</td>
<td>24.25 [20.00-39.75]</td>
</tr>
<tr>
<td>Baseline</td>
<td>31.25 [24.38-41.00]</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004 (n=18)</td>
</tr>
<tr>
<td>Donor (n=18)</td>
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<td>p-value</td>
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<td>p-value</td>
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Conclusions
PA did not improve 6 months after rTx, however PCS scores significantly increase 12 months after rTx, suggesting improvements in the physical domains of HRQOL. Still, baseline values of healthy donors were not approached. Furthermore, HGS significantly increased after rTx and equalled baseline donor levels. Concomitantly, BC parameters did not change, which may underscore the importance of muscle strength as an indicator of overall health.
PARTIAL EMT TRIGGERS VASOPRESSIN ESCAPE IN SIADH

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Introduction:
In syndrome of inappropriate antidiuretic hormone secretion (SIADH), hyponatremia is limited by onset of vasopressin escape, which is caused by loss of the water channel aquaporin-2 (AQP2) in the renal collecting duct despite high circulating vasopressin. The mechanism causing AQP2 downregulation is not understood partly due to the lack of an unbiased and highly sensitive profiling technique. Recent advances in next-generation sequencing technologies have enabled researchers to investigate transcriptomic changes occurring in single cells and a small number of cells obtained from biopsies or microdissection. Combining RNA-seq and classical microdissection, we investigated transcriptomic changes occurring in the cortical collecting duct (CCD) of rats undergoing vasopressin escape.

Methods:
Male Sprague Dawley rats receiving a sustained dose of dDAVP were given either high water load (50 mL/day) or a lower water load (25 mL/day) matching insensible losses plus a small urinary output. Rats were euthanized at days 1, 2, and 4 (post-water load). CCDs were collected by manual microdissection and transcriptionally profiled using RNA-seq. MpkCCD cells were cultured in serum rich medium for 6 days then serum starved for 2 days. Cell lysates were blotted for AQP2 and cell cycle proteins.

Results:
Time-dependent mRNA abundance changes were mapped to gene sets associated with curated canonical signaling pathways, revealing evidence of perturbation of TGFβ signaling (and epithelial-to-mesenchymal transition [EMT]) on Day 1, simultaneous with the initial fall in AQP2 gene expression. On Day 2, transcriptomic changes mapped to “notch signaling” and “transition from G0 to cell cycle”. When quiescent cultured mpkCCD cells were switched from Go to the proliferative state by serum addition, AQP2 protein abundance was markedly diminished. This means that principal cells need to be in the G0 phase to maintain high AQP2 abundance. Immunofluorescence labeling of microdissected CCDs for V-ATPase and pendrin did not reveal an increase in the proportion of cells undergoing division, and no significant change in the proportions of principal and intercalated cells. Moreover, the abundance of the transcripts of the β- and γ- subunits of ENaC (Scnn1b and Scnn1g) were not altered. Exposure of vasopressin-treated cultured mpkCCD cells to TGFβ resulted in a virtually complete loss of AQP2

Conclusion:
The data demonstrate a role for partial EMT in vasopressin escape with a subsequent shift from the G0 phase into the proliferation with arrest and loss of AQP2 during vasopressin escape.
TELEMETRY-BASED RECORDING OF RENAL OXYGENATION DURING NITRIC OXIDE SYNTHASE INHIBITION: PRELIMINARY OBSERVATIONS

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Background
The kidney needs to strike a delicate balance between oxygen delivery and demand. Renal hypoxia has been advanced as a central factor in the vicious circle of disease progression leading to kidney failure. Nitric oxide (NO) availability plays a crucial role in the homeostasis of renal oxygen delivery. NO synthase (NOS)-inhibition leads to hypertension while decreasing renal blood flow, and thus oxygen delivery. We hypothesized that NOS inhibition would induce renal hypoxia. Here, we report preliminary results in conscious rats during chronic NOS inhibition of telemetrically monitored oxygen pressure (pO\textsubscript{2}) in renal cortex and medulla.

Methods
Oxygen sensitive electrodes were implanted in either the renal cortex (n=4) or medulla (n=3) in healthy rats. After recovery and stabilization, baseline oxygen levels were recorded for one week. Then 40mg/kg/day L-NNA was administered via drinking water to inhibit NOS for 2 weeks. A separate group of rats (n=4), instrumented with blood pressure recording telemeters, went through the same protocol. Terminal glomerular filtration rate (GFR), renal plasma flow (RPF), renal oxygen extraction and natriuresis were assessed under isoflurane anesthesia in all L-NNA rats (n=11) and compared with untreated controls (n=4).

Results
NOS inhibition rapidly induced hypertension (145±6 vs. 108±7 mmHg, P<0.05) and caused progressive proteinuria (74±16 vs. 18±2 mg/day, P<0.05). Cortical oxygenation did not change during two weeks. Medullar oxygenation tended to decrease (-11% compared to baseline), while GFR after 2 weeks was clearly reduced (2152±152 vs. 1484±82 µl/min, P<0.05) and RPF (5405±636 vs. 3167±224 µl/min, P<0.05), leading. Fractional sodium reabsorption/oxygen extraction increased.

Conclusion
These data suggest that oxygen levels in the renal cortex and medulla are well maintained during chronic NOS inhibition, despite a reduced oxygen supply. Possibly, a sustained reduction in kidney perfusion induces a more efficient oxygen utilization, and therefore has neutral effects on renal oxygen balance.
COMBINING STREPTOZOTOCIN AND UNILATERAL NEPHRECTOMY IS AN EFFECTIVE METHOD TO INDUCE EXPERIMENTAL DIABETIC NEPHROPATHY IN THE ‘RESISTANT’ C57BL/6J MOUSE STRAIN

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Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands

Background:
Diabetic nephropathy (DN) is the leading cause of chronic kidney disease. Since current therapies provide only limited protection against progression of DN, animal models are necessary to study pathophysiology and new therapies for this disease. C57Bl/6J is the most widely used mouse strain in experimental disease models. However, this strain is considered as relatively resistant for developing DN. The combination of streptozotocin (STZ), unilateral nephrectomy (UNx) and/or a western diet as a model of DN is currently not described in C57Bl/6J mice. In this study we compared four methods to induce DN in C57Bl/6J mice.

Methods:
Male C57Bl/6J mice were subjected to a control (C) or western (W) diet, streptozotocin (S) (50mg/kg for 5 days) or vehicle treatment, and unilateral nephrectomy (U) or sham surgery. Mice were assigned to 7 groups; C, CS, CSU, W, WS, WSU, WU (n=8-10 per group). Mice were sacrificed 12 weeks after diabetes induction. Glomerular and cortical collagen were determined by image analysis of picrosirius red (PSR) stained kidney sections and glomerular basement membrane (GBM) thickness was determined in the control diet groups using electron microscopy. Urinary albumin and glucose were determined by ELISA. Gene expression levels of transforming growth factor beta 1 (TGF-β1), connecting tissue growth factor (cTGF) and collagen I and IV were determined by qPCR on kidney homogenates.

Results:
Urinary albumin levels were significantly increased in CSU mice compared to C, HS and HU mice (Fig. 1A), while a trend was observed in CS mice. Urinary glucose levels revealed severe glycosuria in CSU mice and moderate glycosuria in CS mice (248±20 and 96±40 mmol/24h resp.) compared to the complete absence of glucose in C urines. WS and WSU showed a mild increase in urinary glucose (10 and 19mmol/24h resp.), suggesting that diabetic mice on a western diet have a higher capability to reabsorb the filtered glucose. PSR staining analysis showed increased deposition of collagen in the cortex and in the glomeruli in CSU mice compared with the C group. Also, CSU mice showed increased mRNA expression of collagen I and IV, TGF-β1 and cTGF (Fig. 1B). In most of these parameters, only a mild trend was observed in WS and WSU mice. Thickening of the GBM was only observed in CSU mice, but not in CS mice (Fig. 1C).

Conclusion:
Our study indicates that the combination of a normal diet, STZ and UNx is the most effective method to induce DN in C57Bl/6J mice over the course of 12 weeks.

Figure 1. Urinary albumin levels in 24h urine (A), cTGF relative mRNA expression in kidney (B) and thickness of the GBM (C). Abbreviations: connective tissue growth factor (cTGF); glomerular basement membrane (GBM). *p<0.05, **p<0.01, ***p<0.001 using One-way ANOVA.
CARDIOVASCULAR EVENTS IN ANCA-ASSOCIATED VASCULITIS: A META-ANALYSIS OF OBSERVATIONAL STUDIES

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Background:
Several chronic inflammatory diseases are associated with cardiovascular disease. The risk for cardiovascular events in ANCA-associated vasculitis is poorly quantified. The aim of the present study is to review the evidence for the increased cardiovascular risk in patients with ANCA-associated vasculitis.

Methods:
A comprehensive systematic review was conducted in accordance with guidelines of preferred reporting items for systematic reviews and meta-analyses (PRISMA). The databases PubMed, Embase.com and the Cochrane Library (Wiley) were searched for original observational studies reporting an estimate of the association between ANCA-associated vasculitis and cardiovascular events, including ischemic heart disease, cerebrovascular accidents and/or peripheral arterial disease. The quality of the included studies was assessed with the Newcastle–Ottawa Scale. Summary estimates were derived with a random-effects model and reported as relative risks.

Results:
1375 studies were identified for a final selection of 7 articles comprising 14098 ANCA-associated vasculitis patients versus general population controls in 6 studies and chronic kidney disease patients in 1 study. Overall, ANCA-associated vasculitis carried a relative risk of 1.65 (95% confidence interval, 1.23–2.22) for all cardiovascular events, 1.60 (1.39–1.84) for ischemic heart disease and 1.20 (0.98–1.48) for cerebrovascular accidents. We did not find studies that addressed the risk for peripheral arterial disease separately. No heterogeneity was seen in the estimates.

Conclusion:
This meta-analysis of observational studies supports an increase in cardiovascular risk in Patients with ANCA-associated vasculitis patients of about 65%, similar to that found in other chronic inflammatory diseases. Hence, there is a clear need for active cardiovascular risk management in patients with ANCA-associated vasculitis.
FERTILITY CARE FOR PATIENTS WITH A CHRONIC KIDNEY DISEASE; AN EVALUATION OF HEALTHCARE PROVIDERS IN THE NEPHROLOGY DEPARTMENT

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Introduction
Fertility disorders (FD) are common in chronic kidney disease (CKD). This study evaluated current fertility care for CKD patients by assessing perspectives of nephrologists and nurses in the nephrology department.

Methods
Two different surveys were distributed for this cross-sectional study amongst all Dutch nephrologists (N=312) and nurses working in nephrology (N=1211).

Results
Response rates were 50.9% (113 nephrologists) and 45.4% (546 nurses). According to 9.4% of nephrologists and 15.6% of nurses, guidelines on fertility care were present in their departments. 61.7% of nephrologists and 23.6% of nurses informed ≥50% of their patients on potential changes in fertility due to a decline in renal function. Fertility subjects discussed by nephrologists included “wish to have children” (91.2%), “risk of pregnancy for patients’ health” (85.8%), and “inheritance of the disease” (81.4%). Barriers retaining nurses from discussing FD were based on “age of the patient” (62.6%), “insufficient training” (55.2%), and “language and ethnicity” (51.6%). 29.1% of nurses felt competent in discussing fertility, 8.3% had sufficient knowledge about fertility, and 75.6% needed to expand their knowledge. Knowledge and competence were associated with providing fertility health care (p<0.01).

Conclusions
In most nephrology departments, guidelines on fertility care for CKD patients are absent. Fertility counseling is routinely provided by most nephrologists, nurses often skip this part of renal healthcare mainly due to lack of competence, insufficient knowledge, and barriers based on cultural diversity. Outcomes identified a need for fertility guidelines in the nephrology department and training and education for nurses on providing fertility care.
Higher Plasma Strontium in Patients with Renal Impairment and T2DM: Implications for Clinical Practice?

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Background
Strontium is a naturally occurring heavy metal, which has biochemical similarities to calcium. In patients with renal function impairment, dysregulation of the calcium-phosphate-PTH-Vitamin D axis, is common. However, the behaviour of strontium in patients with renal function impairment is currently unknown. Therefore we aim to investigate the association between plasma strontium, renal function impairment and the extent of mineral bone disease.

Methods
We performed a cross-sectional analysis in the DIAbetes and LiFestyle Cohort Twente (DIALECT, n=450), performed in the ZGT Hospital, Almelo, The Netherlands. Plasma strontium (pSr²⁺) levels were determined during baseline. pSr²⁺ was log transformed to achieve normal distribution. The difference between pSr²⁺ in patients with and without CKD was determined using the student T test. Linear regression was used to study the association between different determinants and log-transformed pSr²⁺.

Results
In total 400 patients were included in the analyses. pSr²⁺ was higher in patients with CKD than in patients without CKD (308 (325-343) vs 237 (227-247) nmol/l, P<0.001). eGFR was inversely correlated with pSr²⁺, with a higher strontium in patients with a lower eGFR (β=-0.005, P<0.001; see also figure 1). Markers of mineral bone disease correlated significantly with pSr²⁺ as well: plasma phosphate (β=0.198, P=0.048); Serum vitamin D (β=-0.002, P=0.012); LN_PTH (β=0.173, P=0.003); LN_FGF23 (β=-0.123, P<0.001). As expected, the tightly regulated serum calcium levels did not correlate with pSr²⁺ (0.079, P=0.678). In multivariate analyses, eGFR and LN_FGF23 remained the strongest predictors of pSr²⁺.

Conclusion
PSr²⁺ levels are elevated in patients with renal function impairment, and correlate with markers of mineral bone disease. Given the biochemical similarities to calcium, this might have pathophysiological relevance in chronic kidney disease, with respect to vascular calcification and/or bone mineral disease.
Background
Current guidelines do not mention tacrolimus (TAC) as a treatment option and no consensus has been reported on the role of tacrolimus in active lupus nephritis (LN). Recent clinical trials have reported positive effects of tacrolimus-based regimens for treatment of lupus nephritis. In order to translate these trials into clinical practice, we systematically reviewed all clinical studies published thus far that investigated TAC regimens in LN patients and performed a meta-analysis.

Methods
We identified all clinical studies investigating TAC regimens in LN from various databases. Studies were summarized on the basis of treatment target (induction or maintenance), concomitant immunosuppression and quality of the data. A meta-analysis was performed for the efficacy of TAC regimen as induction treatment as well as safety.

Results
239 studies were identified from which 23 were clinical studies performed in LN patients: 6 case series, 9 cohort studies, 2 case-control studies (CCS) and 6 randomized controlled trials (RCTs). Further analysis of the 9 controlled trials showed that 7 studies investigated tacrolimus in combination with steroids and 2 tacrolimus with mycophenolate plus steroids. 5 RCTs investigated TAC regimens as induction treatment and 1 RCTs as maintenance treatment. Strikingly, there was no consensus in any of the studies regarding tacrolimus dosing and target trough levels. Importantly, all the studies were performed in LN patients of Asian ethnicity. In a meta-analysis, TAC regimens achieved a significantly higher total response (RR 1.23, 95% CI 1.12-1.34, p<0.05) and significantly higher complete response (RR 1.48, 95% CI 1.23-1.77, p<0.05). Regarding safety, the occurrence of leukopenia was significantly lower, while the occurrence of increased serum creatinine was higher.

Conclusion
Clinical studies on TAC regimens for LN are limited to patients of Asian ethnicity and hampered by significant heterogeneity. The positive results on clinical efficacy of tacrolimus as induction treatment in LN cannot be extrapolated beyond Asian LN patients. Therefore, further confirmation in multi-ethnic, randomized trials is mandatory. Until then, tacrolimus can be considered in selected LN patients.
LITHIUM INDUCES AEROBIC GLYCOLYSIS IN PRINCIPAL CELLS OF THE COLLECTING DUCT

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Introduction:
Lithium, given to bipolar disorder patients, causes Nephrogenic Diabetes Insipidus (Li-NDI) and an increased intercalated/principal cell ratio of the collecting duct (CD remodeling). Whereas Li-NDI is due to downregulation of principal cell (PC) AQP2, the cause of CD remodeling is unknown, but has been ascribed to metabolic acidosis due to H-ATPase/H-K-exchanger inhibition in α-intercalated cells. However, as we showed that lithium induces PC proliferation, which might coincide with aerobic glycolysis-induced lactic acid formation, we investigated whether the latter process may underlie lithium-induced CD remodeling.

Methods:
In Transwell grown polarized mouse collecting duct (mpkCCD) cells were exposed to LiCl on the apical (10 mM) and basolateral side (1 mM) for 1-2 days. C57BL6/J mice and rats were fed a normal diet with/without 40 mmol LiCl/kg for 10 and 28 days, respectively. 24-hrs water intake and urine output/osmolality was obtained. Kidneys were analyzed for remodeling.

Results:
In mpkCCD cells and mice, lithium induced cell proliferation (increased PCNA levels) coincided with aerobic glycolysis (increased se/excretion of lactate elevated abundance of lactate dehydrogenase, and hexokinase-2, decreased ratio of phospho-pyruvate dehydrogenase (pPDH)/PDH). Lithium-increased urinary lactate was absent in mice lacking the epithelial sodium channel ENaC, the PC entry site for lithium. Moreover, ENaC inhibition via amiloride in rats attenuated lithium-induced CD remodeling.

Conclusion:
We show that ENaC-mediated lithium influx in principal cells induces aerobic glycolysis and that inhibition of principal cell lithium entry attenuates the extent of aerobic glycolysis and CD remodeling. Our data indicate that the lithium-induced aerobic glycolysis of principal cells and the consequent acidification of the micro-environment underlies CD remodeling.
NEPHRIN LOSS PREDICTS LONG-TERM OUTCOME IN PATIENTS WITH MINIMAL CHANGE DISEASE

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Background:
Minimal change disease (MCD) is a common form of nephrotic syndrome, especially in children. In general, MCD patients are responsive to glucocorticoid therapy and show excellent long-term renal survival. However, some patients have a less favourable outcome and even progress to end stage renal disease. These patients are often thought to have progressed to focal segmental glomerulosclerosis (FSGS). We previously found that segmental loss of podocyte markers was present before the development of FSGS in a rat model. In this study we set out to investigate whether loss of podocyte marker nephrin can serve as a biomarker to predict worse prognosis in patients with MCD.

Methods:
86 biopsies of patients diagnosed with MCD by the department of Pathology of the LUMC were collected. Biopsies were stained for periodic acid-Schiff and nephrin. Nephrin loss was scored by two independent researchers blinded for clinical outcome. Of 47 patients, clinical data was available and collected retrospectively. Nephrin loss was correlated with clinical data at time of biopsy and at 1, 4 and 16 weeks follow-up and with long-term follow-up data.

Results:
In 24% of patients, nephrin loss was present. At time of biopsy, patients with nephrin loss in their biopsies had similar clinical characteristics as patients without nephrin loss. However, during follow-up, patients with nephrin loss less frequently achieved remission (64%) compared to patients without nephrin loss (96%, p=0.001) and persistence of proteinuria was slightly more prevalent among patients with nephrin loss (0.097). Also, the 5-year GFR was significantly lower in patients with nephrin loss (61 ml/min/1.73²) compared to patients without nephrin loss (96 ml/min/1.73², p=0.038). The risk of developing renal insufficiency increased with the percentage of glomeruli with nephrin loss (p=0.029, OR=1.03). Finally, patients with nephrin loss more often had FSGS in a second biopsy compared to patients without loss (26.7% in patients with nephrin loss compared to 6.7% in patients without nephrin loss), however this was not statistically significant.

Conclusion:
Our results demonstrate that nephrin loss in biopsies of patients with MCD predicts long-term renal outcome. Also, it shows these patients less often achieve remission, which might indicate more advanced podocyte damage. These data support the use of nephrin as a biomarker for long-term outcome in patients with MCD.
MECHANISMS OF DISTAL TUBULAR IRON LOADING IN EXPERIMENTAL FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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Background
Chronic kidney disease (CKD) affects 5-7% of the worldwide population. The most common manifestation of CKD is focal segmental glomerulosclerosis of which the incidence has increased 3- to 13-fold in the last 20-30 years. Treatment of FSGS is primarily aimed at reducing proteinuria, which is, however, not adequate to prevent disease progression in many patients. There is an unmet need for effective treatment modalities for patients with persistent proteinuria, which could be focused at preventing the tubulointerstitial injury associated with proteinuria. The mechanisms of proteinuria-induced tubulointerstitial injury have not been fully elucidated yet, but may include cellular injury catalyzed by reactive iron. In an experimental model for FSGS, the Thy-1.1 mice, we recently observed increasing distal tubular iron accumulation in line with progressive tubulointerstitial injury. Here, we studied the molecular mechanisms of distal tubular iron loading in progressive FSGS in Thy-1.1 mice as potential targets for intervention.

Methods
Transgenic Thy-1.1 mice were injected with 1 mg anti-Thy-1.1 antibody to induce FSGS or saline as control. Mice were placed in a metabolic cage to collect 24h urine and sacrificed subsequently at the following time points: immediately after injection (D1), day 7 (D7) and day 21 (D21). Localization and expression of proteins involved in iron handling were assessed using immunohistochemistry, Western blot and QPCR.

Results
Injection of a monoclonal anti-Thy-1.1 antibody (mAb) resulted in immediate and persistent proteinuria indicated by increased albuminuria (p<0.01; D7 and D21) compared to control, accompanied by polyuria (p<0.001 D7 and D21). Renal injury was confirmed by standard histology and elevated renal mRNA expression of kidney injury markers IL-6 (p<0.05 D1; p<0.001 D7 and D21) and HO-1 (p<0.05 D1 and p<0.01 D7) in mAb-injected mice, compared to control. Evaluation of renal iron handling in mAb-injected mice demonstrated increased mRNA expression of lipocalin-2 receptor (p<0.001; D7), which is localized in the distal tubules and may facilitate uptake of transferrin-bound iron. Interestingly, mRNA expression of megalin in the proximal tubules was reduced (p<0.05 D7). Although mRNA and protein expression of the iron exporter ferroportin were increased (p<0.05, all timepoints), immunohistochemistry revealed ferroportin localization predominantly in the proximal tubules.

Conclusion
The almost absent expression of ferroportin in the distal tubules in combination with the increased expression of lipocalin-2 receptor may account for distal iron loading during proteinuric FSGS in Thy-1.1 mice. Next, we will use in vivo silencing of the lipocalin-2 receptor to functionally demonstrate its role in distal tubular iron loading in mAb-injected Thy-1.1 mice. Also, we will investigate the effect of body iron status on FSGS disease progression by subjecting the Thy-1.1 mice to iron-rich and iron-deficient diets.
CD44 IS REQUIRED FOR THE PATHOGENESIS OF EXPERIMENTAL CRESCENTIC GLOMERULONEPHRITIS AND COLLAPSING FOCAL SEGMENTAL GLOMERULOSCLEROSIS

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Background:
Activation, migration and proliferation of parietal epithelial cells (PECs) is a key feature of glomerular diseases such as crescentic glomerulonephritis and focal segmental glomerulosclerosis. CD44-positive activated PECs have been identified in proliferative cellular lesions in glomerular disease. However, it remains unknown whether CD44-positive PECs contribute to the pathogenesis of scarring glomerular diseases.

Methods:
Here, we evaluated experimental crescentic glomerulonephritis and the transgenic antiThy1.1 model for collapsing focal segmental glomerulosclerosis in CD44-deficient (cd44/-) and wild type (WT) mice.

Results:
For both models albuminuria was significantly lower in cd44/- mice compared to WT mice. The number of glomerular Ki67-positive proliferating cells was significantly reduced in cd44/- mice compared to WT mice, which was associated with a reduced number of glomerular lesions in crescentic glomerulonephritis. In collapsing FSGS, the extracapillary proliferative cellular lesions were smaller in cd44/- mice, but the number of glomerular lesions was not different compared to WT mice. For crescentic glomerulonephritis the glomerular influx of granulocytes and macrophages was similar. In vitro, human CD24, CD133, CD44-positive PECs displayed haptotactic responses towards collagen I in a CD44 dependent manner.

Conclusions:
In conclusion, CD44-positive proliferating glomerular cells, most likely PECs, are essential in the pathogenesis of scarring glomerular disease.
LONG-TERM LITHIUM TREATMENT CAUSES RENAL INTERSTITIAL FIBROSIS IN MICE


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Introduction:
Lithium is the main treatment of bipolar disorder, but prolonged lithium treatment results in the development of renal interstitial fibrosis in 20% of the patients. Renal fibrosis is a hallmark and common outcome in the development of chronic kidney disease (CKD). To fully understand the development of lithium-induced CKD, the use of animal models is essential. Rats have been instrumental in this and long-term lithium treatment has been shown to induce renal interstitial fibrosis. However, considering the broad knowledge of the renal physiology, the versatility and availability of gene knockout strains, mice are the preferential mammalian model to study lithium-induced CKD, but proper model have never been reported. Therefore, we investigated whether mice could be used as a model to study Li-induced CKD development.

Methods:
10 weeks old male C57Bl/6 mice were fed a normal rodent diet or a diet with 40 mmol/kg food of lithium chloride for 40 weeks. One kidney was used to collect cortex and medulla for RT-qPCR and immunoblot analysis. The other kidney was fixed and stored for immunohistological analysis.

Results:
Chromotrop-Anilinblue (CAB) trichrome staining of the kidneys revealed development of interstitial fibrosis in the cortico-medullary region, dilated tubuli, tubular atrophy and decreased glomerular size. Moreover, lithium treatment increased αSMA staining in renal cortex and outer medulla of mice indicating an increase in myofibroblasts in the interstitial compartments. Although EW3D10 staining showed that the endothelium around collecting ducts was pro-inflammatory, no increase was observed for inflammatory markers in RT-qPCR analysis.

Conclusion:
We show that 40 weeks lithium treatment results in the development of renal interstitial fibrosis and that mice can be used as a model to study Li-induced CKD development. In line with data in rats, no increase was observed for inflammatory markers.
ACTIVATION OF PROTEASE-ACTIVATED RECEPTOR 1 LEADS TO EPITHELIAL-TO-MESENCHYMAL TRANSITION IN PROXIMAL TUBULAR EPITHELIAL CELLS

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Background:
Diabetic nephropathy is the leading cause of chronic kidney disease worldwide. Protease-activated receptor-1 (PAR-1) has been shown to potentiate diabetic nephropathy by driving mesangial expansion in a streptozotocin-induced diabetes model. Importantly however, PAR-1 deficiency not only reduced glomerular damage but also reduced tubular atrophy in diabetic mice. Although the latter could be a mere reflection of the primary glomerular damage in this model, PAR-1 has been shown to induce epithelial-to-mesenchymal transition (EMT) in the setting of gastric cancer and pulmonary fibrosis. Therefore we hypothesized that PAR-1 directly drives tubular injury by stimulating EMT, and consequently we assessed the effect of PAR-1 activation on EMT of proximal tubular epithelial cells (PTECs).

Methods:
To test our hypothesis, murine immortalized PTECs were stimulated with thrombin or PAR-1 specific agonist peptide (TFLLRN-NH₂; TRAP-6). Protein and mRNA expression of epithelial and mesenchymal markers was assessed using immunofluorescence, western blot and qPCR analysis.
To determine the role of PAR-1 on primary tubular damage in vivo, wild type and PAR-1⁻/⁻ mice were subjected to unilateral ureter obstruction. Eight mice in each group were sacrificed 1, 3, 7, and 10 days after the obstruction. Kidneys were harvested and processed for (immuno)histochemical analysis.

Results:
We show that PAR-1 activation in PTECs leads to increased mRNA expression levels of the mesenchymal markers fibronectin and collagen I and decreased expression levels of epithelial markers ZO-1 and AQP1. Additionally, western blot analysis and immunofluorescent staining revealed that PAR-1 stimulation leads to increased protein expression of α-SMA and vimentin, suggesting that PAR-1 activation induces EMT in PTECs. Accordingly with PAR-1-induced EMT, ongoing experiments show that tubular damage is reduced in PAR-1⁻/⁻ mice as compared to wild type mice upon unilateral ureter obstruction.

Conclusion:
Overall, these data suggest that PAR-1 drives tubular injury by inducing EMT in proximal tubular epithelial cells.
A HETEROZYGOUS \textit{DE NOVO} MUTATION IN SLC41A1 CAUSES HYPOMAGNESEMIA AND RENAL MAGNESIUM WASTING

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Background
Over the last decades, the identification of hereditary hypomagnesemia-causing genes has increased our understanding of the mechanisms underlying renal magnesium (Mg\textsuperscript{2+}) reabsorption. In the kidney, urinary Mg\textsuperscript{2+} excretion is determined in the distal convoluted tubule, as no Mg\textsuperscript{2+} is reabsorbed beyond this segment. In the distal convoluted tubule, the epithelial Mg\textsuperscript{2+} channel transient receptor potential melastatin type 6 (TRPM6) mediates active Mg\textsuperscript{2+} reabsorption from the pro-urine into the cell. However, to date the molecular identity of the proteins facilitating Mg\textsuperscript{2+} extrusion from the cell towards the blood remains elusive.

Methods
In this study, a three-year-old girl was identified with tetanic convulsions caused by severe hypomagnesemia and hypocalcemia. A Mg\textsuperscript{2+}-loading test showed urinary Mg\textsuperscript{2+} wasting, demonstrating a renal origin of the disease. The aim of this study was to identify the gene mutation that is causative for hypomagnesemia in this patient. Moreover, we investigated the molecular mechanism of disease by cellular Mg\textsuperscript{2+} transport experiments and by gene expression knockdown experiments in the zebrafish model.

Results
Whole exome sequencing identified a heterozygous \textit{de novo} p.Ile98Phe mutation in the SLC41A1 gene. \textsuperscript{25}Mg\textsuperscript{2+} transport assays in human embryonic kidney 293 cells overexpressing SLC41A1 demonstrated that this transporter mediates cellular Mg\textsuperscript{2+} extrusion. To examine the pathogenicity of the mutation, a slc41a1 zebrafish knockdown model was generated using two separate morpholino approaches. Slc41a1 knockdown resulted in a 20% reduction of the total magnesium content. Overexpression of SLC41A1 in slc41a1\textsuperscript{-}knockdown zebrafish restored the total magnesium content. Conversely, total magnesium levels were not normalized in slc41a1\textsuperscript{-}knockdown zebrafish overexpressing the patient's SLC41A1 mutant. Co-expression of wild-type and mutant SLC41A1 in zebrafish disturbed the Mg\textsuperscript{2+} balance, demonstrating that mutant SLC41A1 has a dominant negative effect over wild-type SLC41A1.

Conclusions
In conclusion, a heterozygous \textit{de novo} mutation in SLC41A1 causes hypomagnesemia and renal Mg\textsuperscript{2+} wasting. These findings demonstrate the essential role of SLC41A1 in renal Mg\textsuperscript{2+} handling and maintenance of body Mg\textsuperscript{2+} balance. Based on our experiments, we propose that SLC41A1 may facilitate basolateral Mg\textsuperscript{2+} extrusion in the distal convoluted tubule.
DEFICIENT ENDOTHELIAL HEPARAN SULFATE PREVENTS RENAL INFLAMMATION AND FIBROSIS IN MURINE DIABETIC NEPHROPATHY

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Background
Recent findings suggest a role for inflammation in the development of diabetic nephropathy. Endothelial heparan sulfate (HS) is known for its cytokine/chemokine binding capacities and presentation to high affinity receptors on leukocytes. For the sulfation and function of HS the enzyme N-deacetylase N-sulfotransferase-1 (NDST-1) is essential. In this study we aim to assess the role of endothelial HS in the development of renal inflammation and fibrosis in a mouse diabetic nephropathy model.

Methods
To induce diabetes, age matched C57Bl/6J–WT and Tie2 Cre+ NDST-1f/f mice were intraperitoneally injected with streptozotocin (50 mg/kg). Control mice received citrate buffer (n=8-10/group). At baseline, two and eight weeks follow up urine and plasma were collected and plasma glucose, urinary creatinine and albuminuria were measured. Two months after diabetes induction the animals were sacrificed and kidneys were immunohistochemically stained for macrophages, MCP-1, collagen III, αSMA and WT1. Expression of collagen I, fibronectin and desmin was measured using qRT-PCR.

Results
Diabetes induction was evidenced by significant increased values of blood glucose and albuminuria, without differences between NDST-1WT and NDST-1−/− animals. Compared to NDST-1WT, NDST-1−/− animals showed decreased interstitial macrophages (p<0.05), despite increased MCP-1 expression. NDST-1−/− animals also showed a reduced interstitial fibrosis evidenced by reduced density of αSMA-positive myofibroblasts (p<0.01) and less collagen III deposition (p<0.001). The reduction in fibrosis was confirmed by a reduced mRNA expression of collagen I (p<0.001) and fibronectin (p<0.001). Furthermore, glomerulosclerosis was reduced in the NDST-1−/− animals (p<0.001). Podocyte damage marker desmin was upregulated in diabetic NDST-1WT animals and recovered in the diabetic NDST-1−/− mice (p<0.001) without affecting podocyte cell numbers.

Conclusion
Our results show the role of endothelial HS in the development of renal inflammation and subsequent fibrosis in diabetic nephropathy in mice. These results suggest that HS can be a possible target for therapy in diabetic nephropathy.
COMPLEMENT ACTIVATION BIOMARKER C4D IS ASSOCIATED WITH DIABETIC NEPHROPATHY

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Background:
Complement activation plays a role in various renal diseases. Experimental models suggest that complement activation is involved in the pathogenesis of diabetic nephropathy (DN). The aim of this study was to determine the prevalence and significance of complement deposits in a large cohort of renal tissue from diabetic patients with and without DN.

Methods:
We investigated the presence of glomerular C4d, C1q, MBL, and C5b-9 depositions in 159 kidneys from autopsied patients with type 1 or type 2 diabetes mellitus. Diabetic patients were divided into 2 groups: patients with histologically proven DN and patients without DN; confirmed by light- and electron microscopy. Kidneys were re-evaluated histologically. Complement deposition patterns were scored blinded to the clinical and histological data. We included an additional control cohort of 41 kidneys from autopsied non-diabetic patients without renal disease.

Results:
DN was present in 63% of autopsied diabetic patients. C1q and C4d were present in glomeruli of 37% and 38% of all patients with diabetes, respectively. In all diabetic patients, C5b-9 was present in arterioles and arterial branches. MBL was infrequently observed (6% of all diabetic patients). Patients with type 1 diabetes had a significantly higher prevalence of C4d in glomeruli (p=0.012), C4d in arterioles (p=0.007), and C5b-9 in glomeruli (p=0.013) than patients with type 2 diabetes. Compared to patients without DN, patients with DN had significantly higher prevalence of C4d in glomeruli (p=0.019), both in glomerular capillary walls (p=0.019) and in mesangial cells (p=0.041), and in arterioles (p=0.022). The prevalence of glomerular C4d and C5b-9 was correlated with higher classes of DN (p<0.001). In the total cohort of diabetic patients, presence of glomerular C4d was associated with more glomerular hyalinosis (p=0.020), IFTA (p<0.001), arteriosclerosis (p=0.017), and arteriolar hyalinosis (p=0.017). Non-diabetic controls without renal disease had significantly less C4d and C5b-9 in glomeruli, less C1q, C4d, and C5b-9 in arterioles, and less C1q in arterial branches compared to diabetic patients with or without DN (p<0.05).

Conclusions:
Evidence for complement activation is present in more than 35% of kidneys from diabetic patients. Glomerular C4d is associated with DN, and is correlated with higher classes of DN.
DZP: Dialysecafe zonder personeel!
Voor en door patiënten

Gerard Coumou, Bernie van Daatselaar

Inleiding
Bij ons centrum hebben wij de overtuiging dat de patiënt de regie in eigen handen kan nemen. Wij moeten dan wel helder communiceren over de sociale, technische en medische mogelijkheden. ‘Zelfmanagement’ is daarom speerpunt bij ons. Het staat voor: de patiënt optimaal ondersteunen bij het maken van zorgkeuzes, dit zodat de patiënt zijn aandoening zo goed mogelijk kan inpassen in zijn leven.

We willen de patiënt zélf meer aan zet, meer aan het woord, maar vooral zelf de beslissingen laten nemen over zijn manier van leven; dus ook over zijn manier van dialyseren. In de Stuurgroep Zelfmanagement kwamen we er al snel achter dat zelfmanagement bij dialyse patiënten betekent dat hij zijn LEVEN vooropzet en daarna pas naar zijn ziekte kijkt. Om niet óver patiënten te praten maar mét, vroegen we een patiënt om mee te draaien in onze Stuurgroep. Deze patiënt opende ons pas echt de ogen: we ontdekte dat ‘de patiënt’ verder was in zijn zelfstandigheid dan wij dachten, verder ook, dan dat wijzelf zijn.

Methode
Een van de dingen die we doen om zelfmanagement te bevorderen is het houden van een DZP: Dialysecafe Zonder Personeel, zonder zorgverleners; voor één door patiënten. Hierin is dus géén zorgverlener actief. Een patiënt heeft de leiding. Een maatschappelijk werker faciliteert op dit moment nog, deze heeft geen inhoudelijke rol. Dialysecafe: éénmaal per kwartaal, gedurende 2,5 uur. Er hebben zes DZP’s plaatsgevonden. Per café komen er ongeveer 15 voornamelijk thuispatiënten. In februari komt er, op verzoek van de patiënten, een partnercafé. Wanneer de patiënten dit nodig vinden wordt er een deskundige uitgenodigd. De deskundige doet mee in de groep; hij is volwaardig deelnemer. Wanneer er voldoende draagkracht onder de patiënten is om de cafés draaiende te houden gaat het DZP daadwerkelijk zonder personeel verder.

Resultaten
De patiënt is en kan er eerlijk zijn. Hij hoeft geen sociaal of medisch wenselijke antwoorden te geven; men is immers onder elkaar. Het gaat om het ervaren van leven als dialyse patiënt; daar heeft een zorgverlener geen verstand van. De deskundigheid komt van de patiënten zélf. Samen weten ze nagenoeg overal antwoord op te geven; vaak praktischer dan de deskundige. Onderwerpen worden door de patiënten voorgesteld. Sommige patiënten komen er informatie halen, andere brengen hierdoor ontstaat er een informele sfeer. Het accent is door de patiënt verlegd van: de béste zorg, naar de best pássende zorg bij hem.

Samenvatting/discussie
Patiënten willen en kunnen hun eigen leven - op hun manier - inrichten. Zorgverleners moeten niet meer alleen uitgaan van standaarden, maar vooral luisteren en vragen aan de patiënt wat hij wil; dat weet hij als geen ander. Wij zorgverleners met onze regels en protocollen staan daar vaak onnodig bij in de weg.

Conclusie
Patiënten zijn deskundig in het managen van hun ziekte; de patiënt is leidend. Lotgenotencontact biedt openheid en eerlijkheid, dit geeft een synergie die met een zorgverlener niet kan worden bereikt.

**Waarom dit onderzoek?**

Gezien de prevalentie van ondervoeding onder dialysepatiënten is het belangrijk de voedingstoestand goed te monitoren. Wij gebruiken voor het bepalen van de voedingstoestand bij dialysepatiënten de Subjective Global Assessment (SGA). Wij het gevoel dat de uitkomst van de SGA geen goed beeld geeft van de voedingstoestand van onze dialyse patiënten. Wij hadden het vermoeden dat de patiënten in een slechtere voedingstoestand verkeren dan uit de SGA kwam. Hierdoor zijn wij gaan kijken naar andere, aanvullende, mogelijkheden om de voedingstoestand beter in kaart te brengen.

Kijkend naar een omschrijving van de voedingstoestand, bestaat dit uit 3 onderdelen, namelijk [4]:

- Intake, behoefte en verlies,
- Functionality,
- lichaamssamenstelling.

Daarom hebben wij ervoor gekozen om naast de SGA, ook handknijpkracht en lichaamssamenstelling te gaan meten. Doordat wij dan alle onderdelen van de voedingstoestand beoordelen, kunnen wij een beter onderbouwde uitspraak doen omtrent de voedingstoestand.

**Methoden**

De SGA wordt volgens protocol bij alle dialyse patiënten afgenomen. Wij hebben besloten de handknijpkracht bij alle dialyse patiënten te gaan meten. Het meten van lichaamssamenstelling was voor ons nieuw. De Body Composition Monitor (BCM) van Fresenius is n.a.v. een eerder onderzoek aangeschaft. Tijdens dat onderzoek zijn ook de gegevens van vet massa, vet massa index, vet vrije massa en vet vrije massa index verzameld. Deze gegevens zijn afgezet tegen de referentiewaarden die Fresenius Medical Care geeft bij het gebruik van de BCM [5,6].

Voor dit onderzoek nemen wij alleen de patiënten mee waarbij zowel een SGA, handknijpkracht en lichaamssamenstelling is gemeten. Wij hebben van 18 hemodialysepatiënten zowel SGA, handknijpkracht als lichaamssamenstelling.

Om meer ervaring te krijgen met de uitkomsten van de VVMI vanuit de BCM, hebben wij ervoor gekozen deze ook af te zetten tegen de waarden uit het Zakboek diëtetiek [7] en de afkapwaarden in de criteria ondervoeding van ESPEN (VVMI: mannen < 17 kg/m² en vrouwen < 15 kg/m²).

**Werking Body Composition Monitor**

De body Composition Monitor werkt volgens het principe van bio impedantie spectroscopie (BIS) waarbij gebruik gemaakt van fysiologische modellering en mengsel vergelijkingen (Cole-Cole plot en Hanai formules) om eerst de elektrische weerstand van ECW en ICW te bepalen en vervolgens de berekening van de volumes van deze compartimenten te maken. Dit is ook nodig voor de identificatie van overhydratie. De BCM is op meerdere manieren gevalideerd [6]De determinantenant zijn gevalideerd t.o.v. de gouden standaarden die gelden. Zo is ECW gevalideerd t.o.v. bromide verdunning, ICW t.o.v. totaal lichaamsKalium, TBW t.o.v. deuterium verdunning, VVM t.o.v. Dual Energy X-ray Absorptiometry (DEXA), VM – 4 compartimenten model t.o.v. DEXA, Luchtverplaatsing (plethysmografie) en onder water weging. Body Cell Mass t.o.v. MRI en totaal lichaamsKalium. Referentiewaarden zijn beschikbaar voor overhydratie (OH), vetvrije massa index (LTI) en vetmassa index (FTI). De referentiewaarden zijn gebaseerd op een populatie van 2071 gezonde proefpersonen tussen de 2 en 95 jaar met een BMI tussen 18 - 35 kg / m2. De referentiewaarden zijn specifiek voor leeftijd en geslacht [5,6].

**Resultaten.**

Kijkend naar de SGA score van deze 18 patiënten, bevinden zich geen patiënten in de categorie “ernstige ondervoeding” (score 1 en 2), 13 patiënten (72.2%) in “matig tot lichte ondervoeding” (score 3,4 en 5) (allen score 4 of 5) en 5 patiënten (27.8%) in “normaal gevoed” (score 6 en 7).

Van deze 18 patiënten knepen 10 patiënten (55.6%) < P10 (Referentiewaarden van Dodds).

Kijkend naar de vet vrije massa index, zitten 6 patiënten (33.3%) < P10 (referentiewaarden van Fresenius Medical Care). 4 van deze 6 patiënten knepen ook < P10.

Er waren ook 6 patiënten die < P10 knepen, maar wel een VVMI hadden > P10.
5 van de 6 patiënten met een VVMI < P10 hadden een SGA score 5 en 1 patiënt een SGA score 4. Als wij de waarden van VVMI afzetten tegen de ESPEN criteria, dan zien wij dat 15 patiënten (83.3%) een te lage VVMI hebben. Als wij de waarden van VMMI afzetten tegen de waarden uit het Zakboek diëtetiek, dan hebben ook 15 patiënten (83.3%) een te lage VVMI. Daarnaast zien wij dat 12 patiënten (66.6%) > p90 zitten wat betreft vet massa. (referentiewaarden van Fresenius Medical Care).

**Consequenties voor de praktijk**
Gezien de uitkomsten van de SGA, het feit dat er geen enkele patiënt een score 1,2 of 3 heeft willen wij intern de procedure rondom SGA weer bekijken en zien of dit aangescherpt kan worden. Door dit onderzoek zijn wij van mening dat er bij dialyse patiënten naar meer parameters gekeken moet worden voor het bepalen van de voedingstoestand dan de SGA. Het meten van functionaliteit en lichaamssamenstelling is een waardevolle aanvulling. Voor ons betekend dit onderzoek dat wij het meten van lichaamssamenstelling bij dialyse patiënten volledig willen implementeren. Geen consequentie, maar wat ons wel op valt, is dat er een groot verschil zit tussen de referentie waarden van Fresenius Medical Care voor VVMI en die uit het zakboek diëtetiek en de ESPEN criteria. Verder leidt dit onderzoek tot nieuwe vragen en maakt de mogelijkheid van het meten van lichaamssamenstelling meer onderzoek hieraan mogelijk.

**Literatuur:**
- DNN position paper CNF Nutritional Assessment
- Carrero, J.J. (2011). Causes of CKD; undernutrition and anorexia; 2nd course on Nutrition and Metabolism, Lyon France
- Chamney, P.W. (2007) A whole-body model to distinguish excess fluid from the hydration of major body tissues
HET VERBETEREN VAN DE VOEDINGSTOESTAND DOOR DE INZET VAN EEN WEI-EIWITSHAKE TIJDENS DE DIALYSE BIJ CENTRUMHEMODIALYSEPATIENTEN MET EEN PROTEIN CATABOLIC RATE <1

Amidouche-van Laar D.H.I., Norbart H.A., Overmeer-Haverkamp S., Twigt A.C. & Wentink-Koopman A.M.*

* Alle auteurs hebben een gelijke bijdrage geleverd

Inleiding
Ondervoeding is een veelvoorkomend probleem bij dialysepatiënten en wordt geassocieerd met een verhoogde morbiditeit en mortaliteit. Binnen onze organisatie wordt op 2 van de 3 locaties al enkele jaren gescreend op ondervoeding dmv de Subjective Global Assessment (SGA). In 2014 bleek de prevalentie ondervoeding bij de centrumhemodialysepatiënten (CHD patiënten) op ca 40% te liggen. Ivm het streven om het percentage ondervoede patiënten terug te dringen heeft de afdeling diëtetiek onderzocht of het verhogen van de eiwitinname dmv een wei-eiwitshake tijdens de dialysebehandeling ten goede zou komen aan de voedingstoestand.

Methoden
Op al onze locaties kwamen tussen april 2015 en april 2016 CHD patiënten met een protein catabolic rate (PCR) <1.0 in aanmerking voor de whey-perfection shake van Body&Fit (WP shake). Van hen is het geslacht, de afkomst, de dialyseduur en de eventuele aanwezigheid van Diabetes Mellitus (DM) in kaart gebracht. De PCR is bij aanvang van de shake, na 3, 6, 9 en 12 maanden beoordeeld, evenals, indien van toepassing, de SGA score aan het begin en einde van de onderzoeksperiode. Een controlegroep is niet meegenomen in dit onderzoek gezien het kleine aantal. Ook is geen gewichtscorrectie toegepast voor de PCR bij mensen met een hoog gewicht en is geen rekening gehouden met de invloed van katabolie/anabolie op de PCR.

Resultaten
In de onderzoeksperiode zijn 34 patiënten (n=34) gestart met de WP shake; 18 mannen (53%) en 16 vrouwen (47%), 26 patiënten zijn van het kaukasisch ras (76%), 7 van het negroïde ras (21%) en 1 van het mongoloïde ras (3%). De gemiddelde leeftijd van de onderzoeksgroep is 65.3 jaar (min 26, max 88). De dialyseduur is gemiddeld 3.6 jaar (min 6 maanden, max 13 jaar) en 26% heeft DM.

Paired Samples Correlations

<table>
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<th>N Correlation</th>
<th>Sig.</th>
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<td>27</td>
<td>.399</td>
<td>.039</td>
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<td>.007</td>
<td>.979</td>
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</table>

Tabel 1: Statistische analyse van de eiwitinname en voedingstoestand.

Conclusie
De afdeling diëtetiek heeft onderzoek gedaan naar de invloed van het verhogen van de eiwitinname tijdens de dialysebehandeling dmv het inzetten van een WP shake in relatie tot de voedingstoestand bij haar CHD patiënten. Uit een voorlopige analyse lijkt er een significant positief effect van het inzetten van de WP shake op de PCR in de eerste 9 maanden. Echter, er is vooral nog geen effect te zien op de voedingstoestand. Momenteel wordt bekeken of de gegevens uit het verleden betreft de voedingstoestand (voor de start van het onderzoek) gebruikt kunnen worden als referentie.
Inleiding
Door toenemende kosten van de dialysezorg geven kostenverzekeraars aan dat gestreefd moet worden naar zelfstandige vormen van dialyse. Thuisdialyse is een goed voorbeeld, maar deze behandeling wordt om verschillende redenen door slechts een klein aantal patiënten gekozen. Patiënten gaven aan behoefte te hebben aan een zelfstandige vorm van dialyse in het centrum. Diapriva beschikt over 15 eigen slaapkamers voor nachtelijke hemodialyse, die overdag uitermate geschikt zijn voor ZelfZorgdialyse (ZZ). Ook maatschappelijk gezien is dit een belangrijke ontwikkeling, omdat in de toekomst meer zorg met minder mensen en minder geld uitgevoerd moet worden. Voor dit project is het belangrijk dat er een zelfredzaamheidsinstrument wordt ontwikkeld, wat op iedere nierfalenpolikliniek kan worden gebruikt. Dit instrument maakt direct duidelijk of een patiënt geschikt is voor een zelfstandige behandeling en of hoeveel ondersteuning nodig is van een zorgprofessional.

Methoden
Het uitgangspunt is om met behulp van een opleidingsplan volledig zelfstandige dialyse mogelijk te maken, met hulp achter de hand als het echt nodig is. De patiënt leert om de dialysehandeling zelfstandig uit te voeren, met als evt. uitzondering het aanprikken van de shunt of het aan sluiten van de lijn. De opleiding wordt afgesloten met een examen, dat iedere 6 maanden wordt herhaald.

Na de opleiding bepaalt de patiënt zelf wanneer hij/zij komt voor de dialyse. Ook de behoefte aan een artsenvisite komt vanuit de patiënt, maar vindt ten minste maandelijkse plaats. De opleiding wordt afgesloten met een examen, dat iedere 6 maanden wordt herhaald.

Vanuit de afdeling zijn de voordelen een personeelsbesparing na de opleiding (1 op 5/6 patiënten); tijdens het opleiden is meer personeel nodig vanwege één op één opleiding.

Resultaten
Begin 2016 is de ZZ gestart met twee patiënten en uitgebreid naar 6. Patiënten zijn erg tevreden: men is “minder patiënt” en heeft meer grip op de behandeling. De wetenschap dat er altijd iemand is bij vragen of problemen wordt als heel positief beoordeeld. De extra tijd en de reistijd wordt niet als probleem gezien.

Conclusie
Een goede tussenvorm tussen passieve centrumdialyse en thuishemodialyse, met verbetering van de eigen regie over de behandeling en een bijdrage aan de maatschappelijke problemen die op ons afkomen. Screening op zelfredzaamheid in het nierfalentraject is de volgende stap in de toekomst.
Inleiding
Niertransplantatie is een ingrijpende behandeling met als doel de kwaliteit van leven aanmerkelijk te verbeteren. Het is echter ook een behandeling die, zeker als de patiënt tevoren met dialyse behandeld werd, de wereld op zijn kop kan zetten. Van een vertrouwde en veilige omgeving op de dialyse afdeling wordt in een nieuwe situatie een grote zelfstandigheid verwacht: de patiënt is in staat signalen van ziek-zijn op te pakken en hierop adequaat te reageren. Psychiatrische stoornissen, gebruik van alcohol en drugs en compliantie ten aanzien van de behandeling zijn items die gewoonlijk worden meegenomen in de beoordeling voor transplantatie. Echter, de sociale achtergrond, mentale en cognitieve status en coping gedrag worden lang niet altijd betrokken in de beoordeling. Social support en wijze van communicatie zijn van belang om te beoordelen of en bij wie de patiënt ondersteuning kan vragen. De vraag die steeds weer opduikt is: hoe kunnen wij de patiënt niet alleen medisch optimaal voorbereiden op een transplantatie, maar ook op psychosociaal en maatschappelijk gebied beter begeleiden? Een beschikbare methode hiervoor is de SIPAT: Stanford Integrated Psychosocial Assessment for Transplant, een handvat voor het psychosociaal screenen van transplantatiekandidaten.

Methoden
Op de afdeling nierziekten van het Radboudumc is een vragenlijst ontwikkeld, deels gebaseerd op SIPAT. Het doel is de informatie die veelal wel bekend is bij de eigen nefroloog, medisch maatschappelijk werker of verpleegkundige na te vragen, verder te beschrijven en voor te leggen aan het transplantatieteam. Deze vragenlijst kan door de verschillende professionals ingevuld worden. Het is mogelijk om in de vragenlijst aan te geven welke items extra aandacht verdienen bij opname en bezoek aan de polikliniek; voor items die mogelijk een belemmering voor transplantatie zijn kan een plan van aanpak gemaakt worden. Het gebruik van gekleurde symbolen maken de lijst zeer overzichtelijk.

Resultaten
Na pilots in enkele ziekenhuizen is de vragenlijst in november 2016 geïntroduceerd bij alle collega’s in de gehele transplantatieregio van het Radboudumc. Inmiddels is deze voor ongeveer 100 patiënten ingevuld. Het invullen van de lijst kost 5 tot 15 minuten. Om alle informatie te verzamelen kan een extra gesprek nodig zijn. Alle betrokken zorgverleners zijn verrast door de grote hoeveelheid informatie die de vragenlijst oplevert. Patiënten zijn positief, zij ervaren dit als goede zorg. In enkele gecompliceerde casussen heeft dat er al toe bijgedragen dat het transplantatieproces en de periode erna probleemloos verlopen zijn. In één geval is besloten dat patiënt voorlopig niet in aanmerking komt voor transplantatie.

Conclusie
De ingevulde vragenlijst levert zinvolle, aanvullende informatie op die gebruikt kan worden bij de voorbereiding op niertransplantatie, de opname rondom de operatie en naderhand bij bezoek aan de polikliniek en draagt zo bij aan adequate transplantatiezorg.
PARTNERGROEP

Mirjam Splinter maatschappelijk werker Catharina ziekenhuis
Bep Princen maatschappelijk werker Bernhoven

Inleiding
De confrontatie met een chronische nierziekte heeft vaak grote gevolgen voor het dagelijks leven. Mensen die zich voorbereiden op de naderende nierfunctie vervangende behandeling of mensen die deze behandeling ondergaan merken de grote invloed die de ziekte heeft op zowel de lichamelijke conditie als het dagelijks leven.

Partners maken het hele ziekteproces bewust mee en ook hun leven veranderd waarschijnlijk. Taken moeten wellicht anders verdeeld worden, de rollen kunnen veranderen en misschien dat niet alle activiteiten die zij graag doen nog mogelijk zijn. De partner maakt zich wellicht zorgen en kan zich machteloos voelen.

In het ziekenhuis staat de patiënt vaak centraal. Ook de omgeving geeft vaak de meeste aandacht aan degene die de behandeling ondergaat en wordt er weinig/minder geïnformeerd naar de partner.

Methode
Maatschappelijk werkers Mirjam Splinter van het Catharina ziekenhuis en Bep Princen van Bernhoven zetten middels een partnergroep juist de partner centraal. Door de bijeenkomsten kan de partner zijn verhaal vertellen. Daarnaast kan het luisteren naar ervaringen van groepsgenoten zorgen voor herkenning, erkenning en ondersteuning. De partnergroep bestaat uit 5 bijeenkomsten waarin steeds een ander thema centraal staat; kennismaking, omgaan met veranderingen, creatieve expressie, verzorgen of verdwijnen?, intimiteit, sociale steun en de toekomst.

Resultaten
In Bernhoven en in het Catharina ziekenhuis hebben Bep en Mirjam samen een groep gedraaid. Gemiddeld hebben de partners de partnergroep met een 8,5 beoordeeld. In de evaluatie hebben ze aangegeven geleerd te hebben van anderen, de verhalen van anderen te herkennen, het gevoel te hebben er niet alleen voor te staan en beter te kunnen relativeren. De partners wilde graag een terugkombijeenkomst over 1 jaar.