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Plasma Cadmium is Associated with Increased Risk of Long-Term Kidney Graft Failure

**CONCLUSION:**
Plasma cadmium is independently associated with increased risk of long-term kidney graft failure

- **Baseline phenotyping** including plasma cadmium (Cd) measurement
- **Median follow-up** ~5 years
- **Median Cd** 58 ng/L (IQR, 43–75)
- **78 Graft failure events**
- **13, 26, 39 events over increasing tertiles of Cd**

672 outpatient kidney transplant recipients
With a functioning graft ≥1 year

Median Cd 58 ng/L (IQR, 43–75)

78 Graft failure events

13, 26, 39 events over increasing tertiles of Cd

Risk of graft failure

- **Graft failure-free survival (%)**
  - Tertile 1
  - Tertile 2
  - Tertile 3
  - P<0.001

- **Multivariable-adjusted HR**
  - P<0.001

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Plasma cadmium is associated with increased risk of long-term kidney graft failure.

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Abbreviations: eGFR, estimated Glomerular Filtration Rate; HLA, human leukocyte antigens; KTR, kidney transplant recipients.
ABSTRACT

The kidney is one of the most sensitive organs to cadmium-induced toxicity, particularly in conditions of long-term oxidative stress. We hypothesized that, in kidney transplant recipients, nephrotoxic exposure to cadmium represents an overlooked hazard for optimal graft function. To test this, we performed a prospective cohort study and included 672 outpatient kidney transplant recipients with a functioning graft of beyond one year. The median plasma cadmium was 58 ng/L. During a median 4.9 years of follow-up, 78 kidney transplant recipients developed graft failure with a significantly different distribution across tertiles of plasma cadmium (13, 26, and 39 events, respectively). Plasma cadmium was associated with an increased risk of graft failure (hazard ratio 1.96, 95% confidence interval 1.56–2.47 per log₂ ng/L). Similarly, a dose-response relationship was observed over increasing tertiles of plasma cadmium, after adjustments for potential confounders (donor, recipient, transplant and lifestyle characteristics), robust in both competing risk and sensitivity analyses. These findings were also consistent for kidney function decline (graft failure or doubling of serum creatinine). Thus, plasma cadmium is independently associated with an increased risk of long-term kidney graft failure and decline in kidney function. Further studies are needed to investigate whether exposure to cadmium represents an otherwise overlooked modifiable risk factor for adverse long-term graft outcomes in different populations.

Key words: Cadmium, oxidative stress, kidney transplant recipients, tubular damage, long-term graft failure, kidney function decline.
INTRODUCTION

Kidney transplantation is the gold-standard treatment for most patients with end-stage kidney disease (ESKD). Notwithstanding that advances in transplant research have largely improved 1-year graft survival rates beyond 90%, improvement of long-term graft survival continues to lag behind. Diagnosis and prevention of long-term kidney graft failure is subsidized by systematic identification of both immune and non-immune mechanisms that –over a background of donor and recipient risk factors– enclose potential hazards for adverse graft end-points.

There is increasing international awareness that heavy metals are meaningful chronic kidney disease (CKD) risk factors. Cadmium is a toxic heavy metal, of which primary sources of exposure in the general population are food and tobacco. Once absorbed, it is retained in the system in a long-lasting manner, with the kidney being the primary organ in which cadmium-accumulates and causes toxicity. Reason is that after being bound to metallothionein and temporarily stored in the liver, the cadmium-metallothionein-complex is released into the circulation, filtered by the glomerulus and subsequently reabsorbed by the proximal tubule epithelial cells, wherein cadmium accumulates with a half-life of up to 45 years. Cadmium-induced oxidative stress poses a major hazard for kidney integrity. Its exposure has been associated with glomerular and proximal tubular damage, proteinuria and organ dysfunction. Both occupational and environmental cadmium exposure have been shown to be associated with greater urinary excretion of kidney damage biomarkers, and with increased risk of ESKD and renal replacement treatment.

Better detection techniques allowing for quantification of smaller amounts of heavy metals have made it possible to find harmful effects on health below levels formerly considered as thresholds of toxicity, thereby increasing recognition of adverse consequences of chronic environmental–non-occupational– exposure to heavy metals. Cadmium, in
particular, has been associated with increased risk of CKD even at low levels of exposure.\textsuperscript{14,23} Moreover, in settings of long-term, ongoing oxidative stress, cadmium-induced nephrotoxicity has been associated with impaired kidney function, even at concentrations that are otherwise considered non-toxic.\textsuperscript{24–26} Kidney transplant recipients (KTR) are chronically exposed to oxidative stress due to maintenance immunosuppressive therapy, decreased kidney clearance, and other, often co-occurring pro-oxidant conditions, such as aging, hypertension, and diabetes.\textsuperscript{27} We, therefore, hypothesized that cadmium exposure represents an overlooked hazard for preserved graft functioning. To date, however, there is a paucity of studies devoted to investigating whether cadmium may independently contribute to increased risk of adverse long-term kidney graft end-points.

In the Netherlands, environmental cadmium exposure rates are relatively low and other sources than food do not significantly increase cadmium exposure,\textsuperscript{28} which makes the TransplantLines Food and Nutrition Biobank and Cohort Study\textsuperscript{29} ideal for epidemiologic studies evaluating whether cadmium –at even relatively low levels– associates with increased risk of adverse long-term kidney graft end-points. With a strong body of evidence suggesting that the hazardous exposure to cadmium may be susceptible to clinical monitoring and modifiable by non-toxic therapeutic interventions, assessment and characterization of cadmium-associated risk may provide rationale for development of novel risk-management strategies post-kidney transplantation.\textsuperscript{30} Although the majority of circulating cadmium is in red blood cells, the proximal tubule –which of the kidney is the most sensitive part to the toxic effects of cadmium– may not only be exposed to plasma containing cadmium via diffusion from red blood cells on its serosal side, but also on its luminal side where it is exposed to plasma ultrafiltrate, which is known to contain the cadmium-metallothionein-complex.\textsuperscript{31} Because plasma is an intermediate in both potential pathways of exposure of the kidney, we set out to investigate the association of plasma cadmium concentrations with
adverse kidney graft outcomes in this large cohort of KTR. We additionally aimed to identify subgroups of KTR at particularly high risk according to potential pathophysiology-based effect-modifiers. In secondary analyses, we also investigated the association of plasma cadmium concentration with long-term kidney function decline and patient survival endpoints.

RESULTS

Baseline Characteristics

We included 672 KTR (53±13 years-old, 58% male). Mean eGFR was 43±20 mL/min/1.73 m². Median cadmium concentration was 58 (IQR, 43–75) ng/L. Using cut-offs of 500 and 1500 ng/L for hazardous and toxic concentrations, respectively, a single study subject was observed in each of such categories. Detailed description of baseline characteristics by tertiles of the study population according to plasma cadmium distribution is shown in Table 1.

Cadmium and Risk of Late Graft Failure

During a median follow-up of 4.9 (IQR, 3.4–5.5) years, 78 KTR developed graft failure (12%), with a significantly different distribution across tertiles of plasma cadmium (13, 26, and 39 events, respectively; \( P < 0.001 \); Figure 1A). In crude analyses, cadmium concentration was associated with risk of graft failure (HR 1.89, 95% CI 1.47–2.43 per \( \log_2 \) ng/L; \( P < 0.001 \)). We consistently found that patients in either the middle or the highest tertile of cadmium concentration were at higher risk of graft failure (HR 2.19, 95% CI 1.13–4.27; and, HR 3.38, 95% CI 1.80–6.33; respectively) compared to patients in the lowest tertile (reference). In multivariable-adjusted analyses, these findings remained materially unchanged (Table 2; Figure 2).

Effect-Modification and Stratified Analyses
Effect-modification of the association between plasma cadmium and risk of graft failure are shown in Supplemental Table 1. Aspartate aminotransferase and alanine aminotransferase were found significant effect-modifiers ($P_{interaction}$ 0.003 and 0.005, respectively). In subsequent stratified analyses (cut-off point 25 U/L), we found that the association of plasma cadmium with risk of graft failure was significant across both patients' strata, however, KTR with levels of liver enzymes higher than 25 U/L were at particularly increased risk of graft failure (Figure 3).

**Description of Extreme Outliers**

Description of clinical characteristics of extreme outliers is provided in Supplemental Results.

**Sensitivity Analyses**

We identified 32 outliers (plasma cadmium >123 ng/L). In sensitivity analyses with exclusion of all and extreme outliers from the third tertile, plasma cadmium remained significantly associated with risk of graft failure (HR 3.17, 95% CI 1.66–6.05; and 3.29 (1.74–6.20), respectively). This finding remained materially unchanged in further multivariable-adjusted analyses. Table 2 provides e-values for the observed coefficient estimate and lower limit of the confidence interval in death-censored and competing risk analyses of graft failure, per doubling of plasma cadmium and for patients in the third tertile after exclusion of extreme outliers.

**Cadmium and Risk of Kidney Function Decline, Graft Loss and All-Cause Mortality**

During a median follow-up of 4.9 (IQR, 3.4–5.5) years, 95, 137, and 190 patients developed kidney function decline, died, or were recorded for the composite end-point graft loss respectively. Supplemental Table 2 summarizes the number of events of all outcomes under study overall the study population and by tertiles of plasma cadmium distribution. A Kaplan–Meier curve for the secondary end-point kidney function decline, according to tertiles of plasma cadmium distribution (22, 29, and 44 events, respectively; $P$=0.001) is shown in
**Figure 1B.** Plasma cadmium was independently associated with kidney function decline in both continuous and categorical analyses, as well as after exclusion of outliers (Table 3). Plasma cadmium was also independently associated with graft loss (Supplemental Tables 3). The association with all-cause mortality was mainly driven by graft failure (Supplemental Tables 4 and 5).

**Serial Plasma Cadmium Levels in a Sample Population of the TransplantLines Cohort and Biobank Study**

In Supplemental Figure 1 we show box plots with medians (IQR) of plasma cadmium concentration of 46 KTR (mean age 52±14 years-old, eGFR 43±28 mL/min/1.73 m²) from the TransplantLines Prospective Cohort and Biobank Study. Median (IQR) plasma cadmium concentrations were 78 (71–93), 70 (60–100), 76 (67–98), 79 (63–89) ng/L, at 3-months, 6-months, 1-year, and 2-years post-transplantation, respectively. Median (IQR) intra-individual coefficient of variation post-transplantation was 2.9% (1.9–4.5), and we did not find signs of a significant change in plasma cadmium levels post-transplantation ($P=0.89$). In **Supplemental Figure 2** we show that (A) plasma cadmium at 3-months post-transplantation was significantly different than plasma cadmium at admission for transplantation (median (interquartile range), 78 (71–93) and 100 (75–126) ng/L, respectively; $P<0.001$), and that (B) plasma cadmium at transplantation was significantly associated (Std. $\beta=0.71$, $P<0.001$) with plasma cadmium at 3-months post-transplantation ($R^2=0.51$).

**Blood versus Plasma Cadmium Levels in Participants of the TransplantLines Cohort and Biobank Study**

In Supplemental Figure 3 we show the association of whole blood cadmium with plasma cadmium concentration (Std. $\beta=0.52$, $P=0.001$) in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study. In **Supplemental Figure 4** we show the association of (A) plasma (Std. $\beta=-0.19$, $P=0.046$) and (B) whole blood (Std. $\beta=0.07$, $P=0.47$) cadmium
concentrations with eGFR. Plasma but not blood cadmium was significantly associated with estimated glomerular filtration rate. In further analyses with adjustment for hematocrit, the association between plasma cadmium and eGFR became stronger (Std. $\beta=-0.24$, $P=0.01$), and the association between whole blood cadmium and eGFR changed towards a non-significant inverse association (Std. $\beta=-0.02$, $P=0.81$).

**DISCUSSION**

In a large cohort of outpatient KTR, this study shows that plasma cadmium is independently and consistently associated with risk of long-term kidney graft failure and function decline. In line with previous literature in the field, we observed a dose-dependent association between cadmium concentration and risk of adverse long-term kidney function end-points.\(^7\) These findings are in agreement with previous evidence indicating that the kidney is the most sensitive target organ of cadmium-induced body burden,\(^7,10-17\) and with current international awareness of heavy metals as meaningful risk factors in CKD patients.\(^3,4\) Particularly in the outpatient-kidney transplant setting, this is the first clinical study describing a prospective association of cadmium with adverse long-term end-points. The current study also provides clinical data to suggest that the hazardous association between plasma cadmium and long-term graft failure is particularly substantial in patients with relatively higher liver enzymes levels. Our results point towards cadmium exposure as a potentially modifiable –yet rather overlooked– risk factor for long-term graft failure in KTR, and may raise the question whether plasma cadmium monitoring and non-toxic therapeutic interventions to decrease bodily cadmium concentrations could represent novel risk management strategies to decrease the burden of long-term kidney graft failure.

To our knowledge, the current is the first study to investigate the association of plasma cadmium with clinical end-points. Most of previous studies on mammals have measured cadmium in urine or whole blood samples.\(^15\) Our findings on that on that plasma cadmium,
but not whole blood cadmium was significantly and inversely associated with eGFR and that plasma cadmium was strongly associated with graft failure may provide rationale and further support for our hypothesis that plasma rather than whole blood cadmium is suitable for the study of cadmium-associated nephrotoxicity and adverse long-term outcomes.

Food and tobacco are the primary sources of cadmium exposure in the general population. After ingestion or inhalation, cadmium is temporarily stored in the liver bound to metallothionein. Pathophysiologically in agreement with the effect-modification of liver enzymes on cadmium-associated risk of graft failure hereby reported, cadmium-metallothionein is thereafter – upon hepatocytes turnover – released into the circulation, filtered at the glomerulus, and reabsorbed at the proximal tubule as a result of its preferential uptake by receptor-mediated endocytosis. With a kidney half-life of up to 45 years, a build-up of cadmium in the proximal tubule will ensue. Herein, cadmium is degraded in endosomes and lysosomes, releasing free Cd$^{2+}$ into the cytosol, where it generates reactive oxygen species (e.g., superoxide anion, hydrogen peroxide, and hydroxyl radicals) and activates redox sensitive transcription factors (e.g., NF-kB, AP-1 and Nrf2), which play a major role in cadmium-associated kidney pathophysiology through activation of cell death pathways involving p53, thus linking long-term cadmium exposure with proximal tubular cell apoptosis (HK-2 cells) and impaired reabsorption of low molecular weight proteins. In line, it has been found that cadmium exposure is associated with increased urinary excretion of N-acetyl-$\beta$-D-glucosaminidase (NAG), retinol binding protein, and $\alpha_1$- and $\beta_2$-microglobulin. It is thought that as tubular injury progresses, more generalized tubular dysfunction occurs. Prozialeck et al. recently showed that kidney injury molecule-1 (KIM-1) outperforms classic biomarkers of cadmium-induced nephrotoxicity. Further studies, and particularly human studies, have shown that urinary KIM-1 displays a better dose-response association with long-term low-dose cadmium exposure. Although in the current study we show that plasma
cadmium strongly correlates with urinary excretion of two novel tubular damage biomarkers, \textit{i.e.} epidermal growth factor and liver-type fatty acid-binding protein,\textsuperscript{41} future investigations in KTR are warranted to investigate the association of plasma cadmium with urinary excretion of other low-molecular weight proteins and KIM-1. Finally, although potential cadmium-associated glomerular injury has received relatively little attention, it should be underscored that there is a meaningful body of evidence linking cadmium exposure with glomerular damage and decreased glomerular filtration rate.\textsuperscript{7,14,19,22,23,42,43}

Because cadmium-induced hypertension has been previously reported, it could be hypothesized that at least part of the cadmium-associated risk of graft failure is attributable to an intermediary role of augmented blood pressure.\textsuperscript{44–47} Although across tertiles of plasma cadmium distribution systolic blood pressure was not different, we did observe a direct relation with use of antihypertensive medication. It should be noted, however, that in the present study the association between cadmium and graft failure was independent of systolic blood pressure, which supports that cadmium is linked to kidney tissue injury and dysfunction through proposed direct mechanisms at the kidney proximal tubule.

It should be realized that the current study is etiological in nature, which needs to be separated from prediction research.\textsuperscript{48} Whereas the latter is a distinct field of epidemiologic research aimed at predicting the risk of an outcome according to a model of statistically significant predictors, which not necessarily represent causal associations, etiological studies aim to understand a certain pathway of a disease in an attempt to prevent its onset or progression.\textsuperscript{48} This differentiation is relevant because in both scientific and clinical practice, the two kinds of analyses are often confused, reportedly resulting in poor-quality publications with limited interpretability and applicability. We remark on that, whereas its observational design does not allow causality assumptions, the current study is etiological in nature, and that taking together our findings and those of previous studies showing a plausible biological link
between cadmium exposure and kidney damage, it is possible to support an etiological role of cadmium in pathways of disease that contribute to increased risk of graft failure in KTR.

Previous cohort studies performed in the general population have shown that cadmium is adversely associated with survival. We therefore additionally aimed to provide data on patients' survival and the composite end-point graft loss to account for both graft and patients' survival. When studying the broader end-point graft loss (defined as graft failure or death), increased cadmium-associated risk was consistent in analyses of patients in the highest tertile of plasma cadmium distribution, as well as in analyses of continuous increment of plasma cadmium. On the other hand, we observed that an apparent association of cadmium with all-cause mortality was mainly driven by graft failure, as shown in graft failure-censored analyses. These findings remark on the epidemiological relevance of cadmium exposure, as accounted by the clinically relevant end-point graft loss, whereas they emphasize that cadmium-associated hazard acts mainly through its nephrotoxic effects to increase the burden of adverse end-points in the long-term setting post-kidney transplantation.

Remarkably, our study was conducted in the northern part of The Netherlands, an area with known low environmental exposure rates to cadmium, both in soil and air. The lifelong Dutch dietary intake of cadmium is below the European Food Safety Authority tolerable weekly cadmium intake of 2500 ng/kg body weight. The largest Western-European cohort study on cadmium, the Cadmibel study conducted in Belgium, reported whole blood cadmium concentrations –within the normal range– to be associated with kidney tubular dysfunction. Mining and metal industry countries, e.g. China – which is the world's leading country on cadmium production since 2014 –, have markedly increased patients' cadmium exposure. Due to the dose-dependent effect suggested by the results of the current and previous studies, consequences of cadmium-associated kidney tissue injury may likely be more hazardous in
such populations, yet we emphasize that heavy metals exposure-associated CKD risk has been reported across all geographic regions.

Taken together, these findings underscore that cadmium monitoring, reduction of environmental exposure, and non-toxic therapeutic interventions to decrease bodily cadmium concentrations, may be novel risk management strategies to decrease the current burden of long-term kidney graft failure. Because the kidney is thought to be the organ most critically vulnerable to cadmium accumulation, monitoring its specific organ built-up—by means, e.g., of an in vivo X-ray fluorescence technique that using plane polarized X-rays allows a non-invasive assessment of kidney cortex cadmium—may be a particularly useful mean to assess the effects of accumulated cadmium on long-term kidney function end-points. Chelation therapy, used in heavy metal poisoning and iron overload syndrome, could henceforth offer an otherwise underestimated therapeutic approach. Lin et al. have repeatedly shown that the excretion of lead, a heavy metal with comparable nephrotoxicity to cadmium, can be increased by using Ca-EDTA (calcium ethylenediaminetetraacetic acid) chelation, which has been shown to slow progression rates of ESKD. Such results are promising for a potential cadmium-chelation therapeutic approach, particularly in KTR as being a population of high vulnerability to oxidative stress challenge and at high risk of kidney function impairment. Whether a novel cadmium-chelation pharmacological strategy may improve long-term graft survival rates warrants further studies.

We performed a prospective study in a large cohort of KTR, whom were sequentially recruited during outpatient visits at our university hospital, and then closely monitored by regular check-up in the outpatient clinic during a substantial follow-up period; which granted comprehensive and updated end-points evaluation, without loss to follow-up. Additional strengths of the current study are that our findings on the association of plasma cadmium with increased risk of graft failure were observed in a dose-response fashion in line with the
literature, were robust in competing risk analyses as well as in sensitivity analyses with exclusion of outliers, and consistent over the secondary end-point in which graft failure is combined with kidney function decline (graft failure or doubling of serum creatinine). With baseline data being extensively collected, we were able to perform analyses with adjustment for several potential confounders. Whereas we acknowledge that we were not able to adjust our main analyses for SES in the whole study population, we provide the results of sensitivity analyses in a sample population of consecutively enrolled 198 KTR, to ponder towards the notion that the association of cadmium with risk of graft failure is independent of SES in Dutch KTR, which may also be in line with previous literature showing that SES does not influence the risk of CKD nor the risk of adverse long-term outcomes post-kidney transplantation in the egalitarian Dutch population.\textsuperscript{63,64} Next, although exposure was assessed using a single measure, we studied serial plasma cadmium levels in a sample population of the TransplantLines Cohort and Biobank Study,\textsuperscript{34} in which we found low intra-individual variability, indicative of relatively stable plasma cadmium levels over time post-transplantation. This finding additionally underscores that even at low levels, the nephrotoxic exposure to cadmium may represent an overlooked hazard for preserved graft functioning. We also acknowledge that our predominantly Caucasian study population was derived from a single center from the northern part of The Netherlands, which, as described before, calls for prudence to extrapolate these results to a different population regarding potential environmental contamination and exposure to cadmium.

Our results, however, show for the first time that plasma cadmium is independently associated with long-term risk kidney graft failure, which was robust to several sensitivity analyses and consistent over additional graft function end-points, thus holding the plea for future studies to confirm our results and externally validate our findings among different populations of KTR. We also call out for future studies to confirm our findings by comparing
whole blood cadmium versus plasma cadmium concentrations for the study of cadmium-associated nephrotoxicity and adverse kidney outcomes. We did not have data on urinary cadmium excretion, which might be a better marker of total body cadmium accumulation and therefore even stronger associated with eGFR and graft failure. Future studies will have to compare the prospective associations of plasma cadmium, whole blood cadmium and urinary cadmium with adverse kidney outcomes to sort this out. Next, we observed that cadmium associated with risk of graft failure in a dose-response fashion, which has been consistently shown in previous literature and underscored to evidence causal cadmium-risk associations.12,65,66 While the prospective design of this study provides signals to formulate hypotheses regarding a causal link between cadmium and adverse kidney graft outcomes, we acknowledge that its observational nature prevents us from distinguishing whether plasma cadmium increases with decreasing eGFR or whether increased plasma cadmium levels cause a reduction in eGFR, and it does not allow for hard conclusions on causality. Neither could the potential presence of reversed causation, nor the possibility of residual confounding be entirely excluded. Despite the substantial number of potential confounders for which we adjusted, observational findings on the association between cadmium and risk of graft failure are, by definition, prone to confounding, which is in line with the moderate to low e-values hereby reported.67 Finally, because we found that plasma cadmium concentrations at admission for transplantation were significantly higher than at 3-months post-transplantation, and were also highly correlated with plasma cadmium at 3-months post-transplantation (in the sample population of KTR from the TransplantLines Prospective Cohort and Biobank Study), we hypothesize that cadmium exposure prior to transplantation may represent an otherwise overlooked contributing factor for increased risk of ESKD in the first place. Our findings warrant future studies to investigate a potential increased risk of ESKD associated with long-term cadmium exposure, even at relatively low levels as those of the KTR in this study, and to
independently replicate our findings in different populations with regards to SES and environmental determinants of cadmium exposure.

In conclusion, the current study shows that in a Dutch cohort of outpatient KTR, higher plasma cadmium concentrations were independently associated with increased risk of long-term graft failure and kidney function decline. Cadmium exposure may be a potentially modifiable –yet rather overlooked– risk factor for adverse long-term kidney graft end-points. Our findings on a particularly strong association between plasma cadmium and risk of kidney graft failure among patients with relatively higher liver enzymes levels, may contribute with pathophysiological support to our findings, and be clinically relevant to aid on generating individualized follow-up strategies of outpatient KTR. Further studies are needed to confirm our results and to validate these findings in different populations with regards to exposure. Whether clinical monitoring of bodily cadmium concentrations, reduction of environmental exposure, and non-toxic therapeutic interventions to decrease system cadmium in outpatient KTR may represent novel risk management strategies to decrease the burden of long-term kidney graft failure remains to be investigated in future studies.

METHODS

Study Population

Between November 2008 and March 2011, all adult KTR with a functioning allograft ≥1-year, visiting the outpatient clinic of the University Medical Center Groningen (The Netherlands) were invited to participate in the TransplantLines Food and Nutrition Biobank and Cohort Study, as described previously. A total of 707 of 817 (87%) eligible KTR signed informed consent. Pancreas transplant patients (n=1) and patients missing plasma cadmium measurements (n=34) were excluded from the current analyses, resulting in 672 KTR, of whom data is hereby presented (a flowchart is shown in Supplemental Figure 5). Additional information can be found in Supplemental Methods (Online Supplemental Material). The
The study protocol has been approved by the institutional review board (METc 2008/186) and was conducted in accordance with the Declaration of Helsinki and Declaration of Istanbul.

Data Collection and Definitions

Medical and transplantation history as well as medication use were extracted from electronic patient records, including clinical history of past acute rejection. According to a strict protocol, all patients were asked to collect a 24 hours urine collection sample during the day before to their visit at the outpatient clinic. Blood was drawn in the morning after completion of the 24 hours urine collection. The measurement of clinical and laboratory parameters has been described in Supplemental Methods and in detail elsewhere. Blood and plasma cadmium concentrations were determined with use of an inductively coupled plasma mass spectrometer (ICP-MS, Varian 820-MS; Varian, Palo Alto, USA) with a validated method for the measurement of heavy metals in plasma as detailed in Supplemental Methods. Information on alcohol consumption and smoking behavior was obtained by using a questionnaire. Diabetes was defined as the usage of antidiabetics or a fasting blood glucose ≥7.0 mmol/L. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. In the first n=198 consecutively enrolled KTR, socioeconomic status was investigated using a self-report questionnaire at inclusion, categorizing education as described elsewhere according to the International Standard Classification of Education: bachelor, master or doctorate graduate (level 1), postsecondary or non-tertiary or short-cycle tertiary education (level 2), upper secondary education (level 3), lower secondary education (level 4), and primary or below primary education (level 5). To investigate financial status, participants were asked to choose among four possible categories: Short, enough, good, or excellent monthly budget.
As described elsewhere, dietary intake was assessed using a 177 food items validated semi-quantitative food frequency questionnaire (FFQ) developed and updated at Wageningen University. Further information on the FFQ can be found in Supplemental Methods.

Clinical End-points

The primary end-point of this study was graft failure, defined as the requirement of dialysis or re-transplantation. Secondary end-points were kidney function decline (defined as doubling of serum creatinine or graft failure), graft loss (defined as graft failure or death) and all-cause mortality. These endpoints were chosen to adhere to current recommendations and state of the art in the field. For the analyses of graft failure, kidney function decline, and graft loss, patients who died with a functioning graft were censored at time of death. The study of all-cause mortality was performed with and without censoring at graft failure. The surveillance system of the outpatient program at our university hospital ensures updated information on patient status and events of graft failure as assessed by a nephrologist. Within this system, patients visit the outpatient clinic with declining frequency, in accordance with the guidelines of the American Society of Transplantation. End-points were recorded until September, 2015. General practitioners or referring nephrologists were contacted in case the status of a patient was unknown. No patients were lost to follow-up.

Serial Measurements in Participants the Ongoing TransplantLines Cohort and Biobank Study

Additionally, to investigate plasma cadmium levels over time, we requested follow-up plasma samples (at admission for transplantation, and at 3-months, 6-months, 1-year, and 2-years post-kidney transplantation) from 46 KTR consecutively enrolled between February 2016 and May 2017 in the ongoing TransplantLines Prospective Cohort and Biobank Study. Cadmium plasma concentrations were determined using inductively coupled plasma mass spectrometry, as described in detail in Supplemental Methods.
Blood versus Plasma Cadmium in Participants of the Ongoing TransplantLines Cohort and Biobank Study

We also measured whole blood and plasma cadmium levels in 116 outpatient KTR at a median of 5.2 (IQR, 1.6–11.1) years post-transplantation—which is comparable with transplant vintage of our prospective cohort study population—, to compare whole blood versus plasma cadmium concentrations and to investigate the cross-sectional between cadmium concentration in each of these samples and eGFR.

Statistical Analyses

Data analyses were performed by using SPSS 23.0 for Windows (IBM, Chicago, Illinois, USA), GraphPad Prism 7.02 software (GraphPad Software Inc., San Diego, CA, USA), and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics of study subjects were described by subgroup of patients according to tertiles of plasma cadmium distribution. Normally distributed variables are described as mean (SD), and skewed variables as median (IQR). Categorical variables are expressed as n (number) with percentage (%). Differences were studied with the chi-squared test for categorical variables and by means of linear regression analyses for continuous variables. Variables with skewed distribution were natural log transformed, i.e., transplant vintage, cold ischemia time, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, low density lipoprotein cholesterol, triglycerides, and blood glucose. A two-sided P value <0.05 was considered significant.

Analyses for testing difference and calculating intra-individual coefficient of variation (CV) for follow-up plasma cadmium levels in KTR of the TransplantLines Cohort and Biobank Study can be found in Supplemental Methods.

Prospective analyses

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In prospective analyses of the primary end-point graft failure, a Kaplan-Meier curve and a log-rank test were performed to study whether the distribution of events was significantly different by subgroups of KTR according to tertiles of plasma cadmium concentration. The association of plasma cadmium concentration with risk of graft failure was further examined incorporating time to event by means of Cox proportional-hazards regression analyses (all assumptions were met as described in Supplemental Methods), in which plasma cadmium was log₂-transformed to estimate regression coefficients per doubling of plasma cadmium concentration. For these analyses, risk of death with a functioning graft was accounted by censoring at time of death and by performing competing risk analyses according to Fine and Gray. To illustrate the association of plasma cadmium (log₂-transformed) with risk of graft failure, data were fitted using median plasma cadmium concentration (58 ng/L) as reference value. To study the effect of potential confounders, several Cox regression models were fitted to the data. We performed adjustment for age and sex in model 1; and, eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens (HLA) mismatches, and donor type in model 2. Subsequently, we additively adjusted for body mass index, systolic blood pressure, glucose, and history of diabetes in model 3; and, lifestyle-related risk factors (i.e., smoking status and alcohol consumption) in model 4; induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab) in model 5; and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood) in model 6. Potential effect-modification by donor age, donor sex, donor type, recipient age, recipient sex, cold ischemia time, history of delayed graft function, eGFR, history of diabetes, systolic blood pressure, use of antihypertensive medication, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were tested.
by fitting models containing both main effects and their cross-product terms. The Bonferroni-adjusted significance threshold ($P_{\text{interaction}} < 0.006$; calculated as described in Supplemental Methods) was considered to indicate the performance of stratified prospective analyses. For these analyses, cut-off points of originally continuous variables were determined to concede clinically meaningful patients' strata.

**Sensitivity Analyses**

We identified plasma cadmium outliers by using Turkey's fences (as described in Supplemental Methods), and analyzed Cox regression models analogous to the overall prospective analyses. Estimates are shown for patients pertaining to tertile 3 of plasma cadmium distribution in relation to patients pertaining to tertile 1 (reference group). Using the HR and CI calculated per doubling of plasma cadmium and for patients in tertile 3 of plasma cadmium distribution after exclusion of extreme outliers, we performed further sensitivity analyses as recommended for observational studies by means of providing e-values for both the observed association estimate and the limit of the CI closest to the null. We also performed sensitivity analyses in which we studied whether the association of cadmium with risk of late graft failure is independent of adjustment for SES.

**Secondary Analyses**

In secondary analyses, we studied the association of plasma cadmium with the secondary end-points kidney function decline, graft loss, and all-cause mortality, by means of Cox regression models analogous to the study of the primary end-point graft failure.

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DISCLOSURE
The authors hereby declare that no conflict of interest exists. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.
FIGURES LEGENDS

Figure 1. Kaplan–Meier curve for (A) death-censored graft failure ($n_{\text{events}}=78$) and (B) kidney function decline ($n_{\text{events}}=95$), according to tertiles of plasma cadmium distribution. Tertile 1: $\leq 48$ ng/L; tertile 2: 48–68 ng/L; tertile 3: $\geq 69$ ng/L. $P$ values were calculated by Log-rank test. Graft failure was defined as return to dialysis or re-transplantation. Kidney function decline was defined as doubling of serum creatinine or graft failure.

Figure 2. Association of plasma cadmium concentration with risk of death-censored graft failure in the (A) overall study population; (B) with exclusion of all outliers; and (C) with exclusion of extreme outliers. Data were fitted by Cox proportional-hazards regression using median plasma cadmium concentration (58 ng/L) as reference value. The black line represents the hazard ratio and the grey area represents the 95% confidence interval.

Figure 3. Stratified analyses of the association of plasma cadmium with risk of graft failure. $P_{\text{interaction}}$ was calculated by fitting models which contain both main effects and their cross-product term. Bonferroni-adjusted significance threshold $P_{\text{interaction}} < 0.01$ was considered to indicate the performance of stratified analyses shown hereby. Cut-off points of originally continuous variables were determined to concede clinically meaningful patients' strata. Within each subgroup, hazard ratios (95% CI) were calculated per log$_2$ (ng/L) change in plasma cadmium, and adjusted for age and sex. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.
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**Supplemental Figure 6.** Flowchart depicting the phases of inclusion of the study population

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### TABLES

#### Table 1. Baseline characteristics of 672 kidney transplant recipients

| Baseline characteristics | Tertile 1 (≤48 ng/L) | Tertile 2 (48-68 ng/L) | Tertile 3 (≥69 ng/L) | \( P_{\text{trend}} \) |
|--------------------------|----------------------|------------------------|----------------------|-----------------|
| **Demographics and anthropometrics** | | | | |
| Age, years | 48 (14) | 54 (12) | 56 (11) | <0.001 |
| Sex (male), n (%) | 142 (63) | 132 (60) | 113 (50) | 0.01 |
| Body mass index, kg/m\(^2\) | 26.5 (4.6) | 27.0 (4.9) | 26.6 (4.7) | 0.78 |
| Waist circumference, cm | 98 (14) | 99 (15) | 99 (15) | 0.71 |
| **Smoking status** | | | | 0.005 |
| Never, n (%) | 101 (45) | 94 (42) | 72 (32) | |
| Former, n (%) | 90 (40) | 88 (40) | 107 (47) | |
| Current, n (%) | 21 (9) | 27 (12) | 32 (14) | |
| **Alcohol use** | | | | 0.32 |
| 0 g/d, n (%) | 18 (8) | 27 (12) | 30 (13) | |
| 0–10 g/d, n (%) | 123 (55) | 127 (57) | 119 (53) | |
| 0–30 g/d, n (%) | 43 (19) | 44 (20) | 44 (20) | |
| >30 g/d, n (%) | 15 (7) | 5 (2) | 10 (4) | |
| Systolic blood pressure, mmHg | 134 (17) | 136 (16) | 137 (19) | 0.18 |
| Diastolic blood pressure, mmHg | 83 (11) | 83 (11) | 82 (11) | 0.60 |
| Use of antihypertensive medication, n (%) | 187 (84) | 197 (89) | 208 (92) | 0.02 |
### Dietary intake

|                          | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
|--------------------------|--------------|--------------|--------------|---------|
| Total energy intake, kCal/d | 2259 (633)   | 2088 (634)   | 2152 (587)   | 0.45    |
| Cereals, g/d             | 187 (147–231)| 176 (146–211)| 178 (138–212)| 0.21    |
| Potatoes, g/d, median (IQR) | 111 (70–146) | 118 (73–166)| 122 (76–173)| 0.29    |
| Vegetables, g/d, median (IQR) | 80 (56–116) | 80 (48–124)| 75 (53–107)| 0.54    |
| Fruits, g/d, median (IQR) | 100 (48–189) | 110 (53–197)| 104 (39–186)| 0.22    |
| Legumes, g/d, median (IQR) | 29 (14–48)   | 30 (18–45)   | 31 (17–43)   | 0.88    |
| Nuts, g/d, median (IQR)  | 5.6 (1.1–10.6)| 5.1 (1.9–10.4)| 4.5 (1.4–8.9)| 0.41    |
| Meat, g/d                | 94 (73–112)  | 95 (77–118)  | 98 (75–117)  | 0.03    |
| Dairy products, g/d      | 389 (239–482)| 374 (245–510)| 361 (264–514)| 0.54    |
| Fish and seafood, g/d, median (IQR) | 13 (7–21) | 16 (6–23) | 13 (6–24) | 0.85 |

### Kidney function and transplant history

|                          | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
|--------------------------|--------------|--------------|--------------|---------|
| eGFR, mL/min/1.73 m²     | 60 (19)      | 52 (18)      | 45 (19)      | <0.001  |
| Proteinuria, n (%)       | 43 (19)      | 50 (23)      | 57 (25)      | 0.31    |
| Urinary protein excretion, g/24 h, median (IQR) | 0.15 (0.02–0.28) | 0.19 (0.02–0.35) | 0.21 (0.02–0.45) | 0.01 |
| Dialysis vintage, months, median (IQR) | 20 (5–43) | 25 (10–48) | 30 (11–55) | 0.001 |
| Transplant vintage, years, median (IQR) | 7 (3–13) | 5 (1–12) | 5 (1–10) | 0.003 |
| Acute rejection, n (%)   | 53 (24)      | 64 (29)      | 60 (27)      | 0.46    |
| Cold ischemia time, hrs, median (IQR) | 13 (2–21) | 16 (3–21) | 15 (3–21) | 0.09 |
| Warm ischemia time, minutes | 42 (15) | 44 (16) | 44 (15) | 0.36 |
| HLA mismatches           | 2.1 (1.5)    | 2.1 (1.6)    | 2.4 (1.6)    | 0.69    |
| Donor type, deceased, n (%) | 133 (59) | 150 (68) | 158 (70) | 0.05 |

### Primary kidney disease, n (%)

|                          | Median (IQR) | Median (IQR) | Median (IQR) | p-value |
|--------------------------|--------------|--------------|--------------|---------|
| Glomerulosclerosis       | 70 (31)      | 61 (28)      | 60 (27)      | 0.40    |
| Condition                                      | Group 1 | Group 2 | Group 3 | p-value |
|-----------------------------------------------|---------|---------|---------|---------|
| Glomerulonephritis                            | 19 (9)  | 19 (9)  | 13 (6)  |         |
| Tubulointerstitial nephritis                   | 32 (14) | 20 (9)  | 24 (11) |         |
| Polycystic kidney disease                      | 40 (18) | 47 (21) | 54 (24) |         |
| Kidney hypo/dysplasia                          | 10 (5)  | 12 (5)  | 7 (3)   |         |
| Renovascular disease                           | 8 (4)   | 15 (7)  | 16 (7)  |         |
| Diabetes                                       | 7 (3)   | 10 (5)  | 15 (7)  |         |
| Other/miscellaneous                             | 38 (17) | 38 (17) | 37 (16) |         |
| **Immunosuppressive therapy**                  |         |         |         |         |
| Use of calcineurin inhibitor, n (%)            | 110 (49)| 136 (61)| 139 (62)| 0.01    |
| Use of proliferation inhibitor, n (%)          | 196 (88)| 180 (81)| 184 (81)| 0.12    |
| Corticosteroids dose <10 mg/24 h, n (%)        | 95 (42) | 97 (44) | 84 (37) | 0.35    |
| **Liver function parameters**                  |         |         |         |         |
| ASAT, U/L, median (IQR)                        | 21 (18–26) | 22 (19–27) | 22 (18–27) | 0.09 |
| ALAT, U/L, median (IQR)                        | 19 (14–26) | 19 (14–26) | 18 (14–26) | 0.93 |
| Alkaline phosphatase, U/L, median (IQR)        | 66 (51–81) | 67 (55–85) | 68 (54–91) | 0.06 |
| GGT, U/L, median (IQR)                         | 25 (19–34) | 28 (19–46) | 28 (18–45) | 0.02 |
| **Fasting lipids**                             |         |         |         |         |
| Total cholesterol, mmol/L                      | 4.9 (1.0) | 5.1 (1.1) | 5.3 (1.2) | 0.01 |
| HDL cholesterol, mmol/L                        | 1.4 (0.4) | 1.4 (0.5) | 1.4 (0.5) | 0.39 |
| LDL cholesterol, mmol/L                        | 2.8 (2.3–3.4) | 2.8 (2.3–3.6) | 3.0 (2.4–3.6) | 0.05 |
| Triglycerides, mmol/L, median (IQR)            | 1.7 (1.2–2.2) | 1.6 (1.2–2.1) | 1.8 (1.3–2.7) | 0.08 |
| **Diabetes and glucose homeostasis**           |         |         |         |         |
| Diabetes, n (%)                                | 41 (18) | 58 (26) | 64 (28) | 0.03    |
| Glucose, mmol/L, median (IQR)                  | 5.2 (4.7–5.8) | 5.2 (4.8–6.1) | 5.3 (4.8–6.2) | 0.09 |
HbA\(_1\)C, \%  
5.9 (0.7) 6.0 (1.0) 6.1 (0.8) 0.04

**Markers of tubular toxicity**

| Marker                  | Tertile 1 (≤48 ng/L) | Tertile 2 (48–68 ng/L) | Tertile 3 (≥69 ng/L) | p-value |
|-------------------------|----------------------|------------------------|----------------------|---------|
| uEGF, ng/mL median (IQR)| 5.45 (2.99–8.13)     | 3.99 (2.16–7.21)       | 3.57 (1.47–7.26)     | <0.001  |
| uLFABP, ng/mL median (IQR)| 0.65 (0.27–2.11)     | 0.91 (0.43–3.13)       | 1.21 (0.50–5.90)     | <0.001  |

Values presented as mean (SD) unless stated otherwise. Differences among tertiles of the plasma cadmium distribution (Tertile 1: ≤48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: ≥69 ng/L) were studied by means of analysis of variance or the linear regression test for continuous variables and by means of the chi-squared test for categorical variables. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein cholesterol; HLA, human leukocyte antigens; LDL, low-density lipoprotein cholesterol; uEGF, urinary epidermal growth factor; uLFABP, urinary liver-type fatty acid binding protein.
Table 2. Association of cadmium with risk of graft failure

| Models          | Cadmium per log₂ (ng/L) | Tertiles of cadmium | Tertile 1 (n=224) | Tertile 2 (n=222) | Tertile 3 (n=226) | ¹Tertile 3 (n=194) | ²Tertile 3 (n=215) |
|----------------|-------------------------|---------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                |                         | Ref.               | HR (95% CI)       | HR (95% CI)       | HR (95% CI)       | HR (95% CI)       | E-values          |
| Death-censored analyses |             |                     |                   |                   |                   |                   |                   |
| Crude          | 1.89 (1.47–2.43)        | <0.001              | 2.48, 1.94        | 2.19 (1.13–4.27)  | 3.38 (1.80–6.33)  | 3.17 (1.66–6.05)  | 3.29 (1.74–6.20)  | 3.93, 2.29        |
| Model 1        | 1.96 (1.56–2.47)        | <0.001              | 2.56, 2.06        | 2.67 (1.36–5.26)  | 4.31 (2.25–8.22)  | 4.23 (2.16–8.27)  | 4.26 (2.21–8.21)  | 4.78, 2.85        |
| Model 2        | 1.88 (1.31–2.69)        | <0.001              | 2.46, 1.70        | 2.49 (1.14–5.43)  | 3.11 (1.41–6.86)  | 2.50 (1.14–5.48)  | 3.09 (1.37–6.93)  | 3.72, 1.79        |
| Model 3        | 1.87 (1.30–2.69)        | <0.001              | 2.45, 1.69        | 2.48 (1.14–5.41)  | 3.08 (1.40–6.82)  | 2.47 (1.12–5.42)  | 3.07 (1.36–6.90)  | 3.73, 1.78        |
| Model 4        | 1.93 (1.36–2.75)        | <0.001              | 2.52, 1.78        | 2.57 (1.16–5.70)  | 3.36 (1.50–7.54)  | 3.45 (1.48–5.05)  | 3.34 (1.46–7.64)  | 3.98, 1.92        |
| Model 5        | 1.87 (1.33–2.62)        | <0.001              | 2.45, 1.73        | 2.50 (1.14–5.47)  | 3.03 (1.37–6.69)  | 3.22 (1.40–7.39)  | 2.96 (1.31–6.66)  | 3.62, 1.70        |
| Model 6        | 1.81 (1.28–2.56)        | <0.001              | 2.38, 1.66        | 2.31 (1.02–5.22)  | 2.82 (1.25–6.40)  | 2.89 (1.24–5.00)  | 2.76 (1.20–6.35)  | 3.42, 1.53        |
| Competing risk analyses |         |                     |                   |                   |                   |                   |                   |                   |
| Crude          | 1.90 (1.49–2.42)        | <0.001              | 2.49, 1.96        | 2.04 (1.05–3.96)  | 3.09 (1.65–5.78)  | 2.83 (1.48–5.41)  | 2.99 (1.59–5.63)  | 3.65, 2.10        |
| Model 1        | 1.97 (1.66–2.35)        | <0.001              | 2.57, 2.19        | 2.57 (1.33–4.97)  | 4.13 (2.22–7.69)  | 4.01 (2.11–7.63)  | 4.07 (2.17–7.64)  | 4.62, 2.80        |
| Model 2        | 1.81 (1.43–2.30)        | <0.001              | 2.38, 1.88        | 2.17 (1.02–4.62)  | 2.80 (1.32–5.94)  | 2.69 (1.22–5.93)  | 2.69 (1.24–5.80)  | 3.35, 1.59        |
| Model 3        | 1.81 (1.41–2.30)        | <0.001              | 2.38, 1.85        | 2.28 (0.99–5.26)  | 3.00 (1.35–6.67)  | 2.98 (1.30–6.83)  | 2.95 (1.30–6.67)  | 3.61, 1.69        |
| Model 4        | 1.76 (1.41–2.20)        | <0.001              | 2.32, 1.85        | 2.12 (0.99–4.55)  | 2.74 (1.26–5.55)  | 2.70 (1.21–6.04)  | 2.68 (1.22–5.86)  | 3.34, 1.56        |
| Model 5        | 1.79 (1.42–2.26)        | <0.001              | 2.35, 1.87        | 2.15 (0.98–4.71)  | 2.65 (1.22–5.77)  | 2.54 (1.16–5.57)  | 2.54 (1.18–5.45)  | 3.20, 1.49        |
| Model 6        | 1.99 (1.38–2.85)        | <0.001              | 2.59, 1.81        | 1.96 (0.90–4.26)  | 2.66 (1.24–5.68)  | 2.89 (1.24–6.75)  | 2.59 (1.17–5.71)  | 3.25, 1.47        |
Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with risk of graft failure \((n_{\text{events}}=78)\), accounting for death (with a functioning graft) by censoring at time of death or by performing competing risk analyses according to Fine and Gray.\(^ {33}\) Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (Tertile 1: \(\leq 48\) ng/L; Tertile 2: 48–68 ng/L; Tertile 3: \(\geq 69\) ng/L) in the overall population, and without \(^ {\dagger}\) all \((n=32)\) and \(^ {\ddagger}\) extreme \((n=11)\) outliers. \(^ {*}\)E-values are calculated for the association estimate (HR) and the limit of the confidence interval closest to the null per doubling of plasma cadmium and for patients in the third tertile of plasma cadmium distribution after exclusion of extreme outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); smoking and alcohol use (model 4); induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab; model 5); and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood; model 6).
| Models     | Tertile 1 \((n=224)\) | Tertile 2 \((n=222)\) | Tertile 3 \((n=226)\) | †Tertile 3 \((n=194)\) | ‡Tertile 3 \((n=215)\) |
|------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|
|            | HR (95% CI)             | HR (95% CI)            | HR (95% CI)            | HR (95% CI)              | HR (95% CI)            |
|            | Ref.                    | P value                |                        |                          |                        |
| Crude      | 1.00                    | 1.00                   | 1.00                   | 1.00                     | 1.00                   |
| Model 1    | 1.78 (1.41–2.26)        | <0.001                 | 1.00                   | 1.76 (1.00–3.10)         | 2.84 (1.67–4.83)       |
| Model 2    | 1.61 (1.17–2.22)        | 0.003                  | 1.00                   | 1.56 (0.82–2.98)         | 2.18 (1.14–4.17)       |
| Model 3    | 1.60 (1.16–2.20)        | 0.004                  | 1.00                   | 1.55 (0.81–2.95)         | 2.15 (1.12–4.11)       |
| Model 4    | 1.74 (1.27–2.38)        | 0.001                  | 1.00                   | 1.93 (0.97–3.84)         | 2.75 (1.37–5.53)       |
| Model 5    | 1.61 (1.17–2.20)        | 0.003                  | 1.00                   | 1.57 (0.82–3.01)         | 2.14 (1.11–4.12)       |
| Model 6    | 1.59 (1.15–2.21)        | 0.006                  | 1.00                   | 1.54 (0.76–3.11)         | 2.11 (1.05–4.23)       |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with kidney function decline \((n_{\text{events}}=95)\). Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (tertile 1: \(\leq 48\) ng/L; tertile 2: \(48–68\) ng/L; tertile 3: \(\geq 69\) ng/L) in the overall population, and without \(^{†}\) all \((n=32)\) and \(^{‡}\) extreme \((n=11)\) outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); smoking and alcohol use (model 4); induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab; model 5); and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood; model 6).
Plasma Cadmium is Associated with Increased Risk of
Long-Term Kidney Graft Failure:
A Prospective Cohort Study

Main figures
| Effect-modifiers | Subgroup cut-off point | Events (n) | Hazard ratio (95% CI) per log₂ ng/L | Pinteraction |
|------------------|------------------------|------------|----------------------------------|--------------|
| ASAT             | ≤ 25 U/L               | 55         | 1.81 (1.38-2.37)                 | 0.003        |
|                  | > 25 U/L               | 23         | 2.61 (1.52-4.51)                 |              |
| ALAT             | ≤ 25 U/L               | 60         | 1.74 (1.34-2.28)                 | 0.005        |
|                  | > 25 U/L               | 18         | 3.89 (1.92-7.88)                 |              |

HR (95% CI) per log₂ ng/L
Plasma Cadmium is Associated with Increased Risk of Long-Term Kidney Graft Failure

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Plasma cadmium is associated with increased risk of long-term kidney graft failure.

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Abbreviations: eGFR, estimated Glomerular Filtration Rate; HLA, human leukocyte antigens; KTR, kidney transplant recipients.
ABSTRACT

The kidney is one of the most sensitive organs to cadmium-induced toxicity, particularly in conditions of long-term oxidative stress. We hypothesized that, in kidney transplant recipients, nephrotoxic exposure to cadmium represents an overlooked hazard for optimal graft function. To test this, we performed a prospective cohort study and included 672 outpatient kidney transplant recipients with a functioning graft of beyond one year. The median plasma cadmium was 58 ng/L. During a median 4.9 years of follow-up, 78 kidney transplant recipients developed graft failure with a significantly different distribution across tertiles of plasma cadmium (13, 26, and 39 events, respectively). Plasma cadmium was associated with an increased risk of graft failure (hazard ratio 1.96, 95% confidence interval 1.56–2.47 per log₂ ng/L). Similarly, a dose-response relationship was observed over increasing tertiles of plasma cadmium, after adjustments for potential confounders (donor, recipient, transplant and lifestyle characteristics), robust in both competing risk and sensitivity analyses. These findings were also consistent for kidney function decline (graft failure or doubling of serum creatinine). Thus, plasma cadmium is independently associated with an increased risk of long-term kidney graft failure and decline in kidney function. Further studies are needed to investigate whether exposure to cadmium represents an otherwise overlooked modifiable risk factor for adverse long-term graft outcomes in different populations.

Key words: Cadmium, oxidative stress, kidney transplant recipients, tubular damage, long-term graft failure, kidney function decline.
INTRODUCTION

Kidney transplantation is the gold-standard treatment for most patients with end-stage kidney disease (ESKD). Notwithstanding that advances in transplant research have largely improved 1-year graft survival rates beyond 90%, improvement of long-term graft survival continues to lag behind.\(^1\) Diagnosis and prevention of long-term kidney graft failure is subsidized by systematic identification of both immune and non-immune mechanisms that –over a background of donor and recipient risk factors– enclose potential hazards for adverse graft endpoints.\(^2\)

There is increasing international awareness that heavy metals are meaningful chronic kidney disease (CKD) risk factors.\(^3,4\) Cadmium is a toxic heavy metal, of which primary sources of exposure in the general population are food and tobacco.\(^5\) Once absorbed, it is retained in the system in a long-lasting manner, with the kidney being the primary organ in which cadmium-accumulates and causes toxicity. Reason is that after being bound to metallothionein and temporarily stored in the liver, the cadmium-metallothionein-complex is released into the circulation, filtered by the glomerulus and subsequently reabsorbed by the proximal tubule epithelial cells, wherein cadmium accumulates with a half-life of up to 45 years.\(^6–9\) Cadmium-induced oxidative stress poses a major hazard for kidney integrity. Its exposure has been associated with glomerular and proximal tubular damage, proteinuria and organ dysfunction.\(^7–17\) Both occupational and environmental cadmium exposure have been shown to be associated with greater urinary excretion of kidney damage biomarkers, and with increased risk of ESKD and renal replacement treatment.\(^7,14,18–23\)

Better detection techniques allowing for quantification of smaller amounts of heavy metals have made it possible to find harmful effects on health below levels formerly considered as thresholds of toxicity, thereby increasing recognition of adverse consequences of chronic environmental –non-occupational– exposure to heavy metals. Cadmium, in particular, has been
associated with increased risk of CKD even at low levels of exposure.\textsuperscript{14,23} Moreover, in settings of long-term, ongoing oxidative stress, cadmium-induced nephrotoxicity has been associated with impaired kidney function, even at concentrations that are otherwise considered non-toxic.\textsuperscript{24–26} Kidney transplant recipients (KTR) are chronically exposed to oxidative stress due to maintenance immunosuppressive therapy, decreased kidney clearance, and other, often co-occurring pro-oxidant conditions, such as aging, hypertension, and diabetes.\textsuperscript{27} We, therefore, hypothesized that cadmium exposure represents an overlooked hazard for preserved graft functioning. To date, however, there is a paucity of studies devoted to investigating whether cadmium may independently contribute to increased risk of adverse long-term kidney graft end-points.

In the Netherlands, environmental cadmium exposure rates are relatively low and other sources than food do not significantly increase cadmium exposure,\textsuperscript{28} which makes the TransplantLines Food and Nutrition Biobank and Cohort Study\textsuperscript{29} ideal for epidemiologic studies evaluating whether cadmium—at even relatively low levels—associates with increased risk of adverse long-term kidney graft end-points. With a strong body of evidence suggesting that the hazardous exposure to cadmium may be susceptible to clinical monitoring and modifiable by non-toxic therapeutic interventions, assessment and characterization of cadmium-associated risk may provide rationale for development of novel risk-management strategies post-kidney transplantation.\textsuperscript{30} Although the majority of circulating cadmium is in red blood cells, the proximal tubule—which of the kidney is the most sensitive part to the toxic effects of cadmium—may not only be exposed to plasma containing cadmium via diffusion from red blood cells on its serosal side, but also on its luminal side where it is exposed to plasma ultrafiltrate, which is known to contain the cadmium-metallothionein-complex.\textsuperscript{31} Because plasma is an intermediate in both potential pathways of exposure of the kidney, we set out to investigate the association of plasma cadmium concentrations with adverse kidney graft...
outcomes in this large cohort of KTR. We additionally aimed to identify subgroups of KTR at
particularly high risk according to potential pathophysiology-based effect-modifiers. In
secondary analyses, we also investigated the association of plasma cadmium concentration with
long-term kidney function decline and patient survival end-points.

RESULTS

Baseline Characteristics

We included 672 KTR (53±13 years-old, 58% male). Mean eGFR was 43±20 mL/min/1.73 m².
Median cadmium concentration was 58 (IQR, 43–75) ng/L. Using cut-offs of 500 and 1500
ng/L for hazardous and toxic concentrations, respectively, a single study subject was observed
in each of such categories. Detailed description of baseline characteristics by tertiles of the
study population according to plasma cadmium distribution is shown in Table 1.

Cadmium and Risk of Late Graft Failure

During a median follow-up of 4.9 (IQR, 3.4–5.5) years, 78 KTR developed graft failure (12%),
with a significantly different distribution across tertiles of plasma cadmium (13, 26, and 39
events, respectively; P<0.001; Figure 1A). In crude analyses, cadmium concentration was
associated with risk of graft failure (HR 1.89, 95% CI 1.47–2.43 per log₂ ng/L; P<0.001). We
consistently found that patients in either the middle or the highest tertile of cadmium
concentration were at higher risk of graft failure (HR 2.19, 95% CI 1.13–4.27; and, HR 3.38,
95% CI 1.80–6.33; respectively) compared to patients in the lowest tertile (reference). In
multivariable-adjusted analyses, these findings remained materially unchanged (Table 2;
Figure 2).

Effect-Modification and Stratified Analyses

Effect-modification of the association between plasma cadmium and risk of graft failure are
shown in Supplemental Table 1. Aspartate aminotransferase and alanine aminotransferase
were found significant effect-modifiers ($P_{\text{interaction}}$ 0.003 and 0.005, respectively). In subsequent stratified analyses (cut-off point 25 U/L), we found that the association of plasma cadmium with risk of graft failure was significant across both patients' strata, however, KTR with levels of liver enzymes higher than 25 U/L were at particularly increased risk of graft failure (Figure 3).

**Description of Extreme Outliers**

Description of clinical characteristics of extreme outliers is provided in *Supplemental Results*.

**Sensitivity Analyses**

We identified 32 outliers (plasma cadmium >123 ng/L). In sensitivity analyses with exclusion of all and extreme outliers from the third tertile, plasma cadmium remained significantly associated with risk of graft failure (HR 3.17, 95% CI 1.66–6.05; and 3.29 (1.74–6.20), respectively). This finding remained materially unchanged in further multivariable-adjusted analyses. *Table 2* provides e-values for the observed coefficient estimate and lower limit of the confidence interval in death-censored and competing risk analyses of graft failure, per doubling of plasma cadmium and for patients in the third tertile after exclusion of extreme outliers.

**Cadmium and Risk of Kidney Function Decline, Graft Loss and All-Cause Mortality**

During a median follow-up of 4.9 (IQR, 3.4–5.5) years, 95, 137, and 190 patients developed kidney function decline, died, or were recorded for the composite end-point graft loss respectively. *Supplemental Table 2* summarizes the number of events of all outcomes under study overall the study population and by tertiles of plasma cadmium distribution. A Kaplan–Meier curve for the secondary end-point kidney function decline, according to tertiles of plasma cadmium distribution (22, 29, and 44 events, respectively; $P=0.001$) is shown in Figure 1B. Plasma cadmium was independently associated with kidney function decline in both continuous and categorical analyses, as well as after exclusion of outliers (*Table 3*). Plasma cadmium was also independently associated with graft loss (*Supplemental Tables 3*).
association with all-cause mortality was mainly driven by graft failure (Supplemental Tables 4 and 5).

Serial Plasma Cadmium Levels in a Sample Population of the TransplantLines Cohort and Biobank Study

In Supplemental Figure 1 we show box plots with medians (IQR) of plasma cadmium concentration of 46 KTR (mean age 52±14 years-old, eGFR 43±28 mL/min/1.73 m²) from the TransplantLines Prospective Cohort and Biobank Study. Median (IQR) plasma cadmium concentrations were 78 (71─93), 70 (60─100), 76 (67─98), 79 (63─89) ng/L, at 3-months, 6-months, 1-year, and 2-years post-transplantation, respectively. Median (IQR) intra-individual coefficient of variation post-transplantation was 2.9% (1.9─4.5), and we did not find signs of a significant change in plasma cadmium levels post-transplantation (P=0.89). In Supplemental Figure 2 we show that (A) plasma cadmium at 3-months post-transplantation was significantly different than plasma cadmium at admission for transplantation (median (interquartile range), 78 (71─93) and 100 (75─126) ng/L, respectively; P<0.001), and that (B) plasma cadmium at transplantation was significantly associated (Std. β=0.71, P<0.001) with plasma cadmium at 3-months post-transplantation (R²=0.51).

Blood versus Plasma Cadmium Levels in Participants of the TransplantLines Cohort and Biobank Study

In Supplemental Figure 3 we show the association of whole blood cadmium with plasma cadmium concentration (Std. β=0.52, P=0.001) in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study. In Supplemental Figure 4 we show the association of (A) plasma (Std. β=─0.19, P=0.046) and (B) whole blood (Std. β=0.07, P=0.47) cadmium concentrations with eGFR. Plasma but not blood cadmium was significantly associated with estimated glomerular filtration rate. In further analyses with adjustment for hematocrit, the association between plasma cadmium and eGFR became stronger (Std. β=─0.24, P=0.01), and the
association between whole blood cadmium and eGFR changed towards a non-significant inverse association (Std. β=−0.02, P=0.81).

DISCUSSION

In a large cohort of outpatient KTR, this study shows that plasma cadmium is independently and consistently associated with risk of long-term kidney graft failure and function decline. In line with previous literature in the field, we observed a dose-dependent association between cadmium concentration and risk of adverse long-term kidney function end-points. These findings are in agreement with previous evidence indicating that the kidney is the most sensitive target organ of cadmium-induced body burden, and with current international awareness of heavy metals as meaningful risk factors in CKD patients. Particularly in the outpatient-kidney transplant setting, this is the first clinical study describing a prospective association of cadmium with adverse long-term end-points. The current study also provides clinical data to suggest that the hazardous association between plasma cadmium and long-term graft failure is particularly substantial in patients with relatively higher liver enzymes levels. Our results point towards cadmium exposure as a potentially modifiable—yet rather overlooked—risk factor for long-term graft failure in KTR, and may raise the question whether plasma cadmium monitoring and non-toxic therapeutic interventions to decrease bodily cadmium concentrations could represent novel risk management strategies to decrease the burden of long-term kidney graft failure.

To our knowledge, the current is the first study to investigate the association of plasma cadmium with clinical end-points. Most of previous studies on mammals have measured cadmium in urine or whole blood samples. Our findings on that on that plasma cadmium, but not whole blood cadmium was significantly and inversely associated with eGFR and that plasma cadmium was strongly associated with graft failure may provide rationale and further
support for our hypothesis that plasma rather than whole blood cadmium is suitable for the study of cadmium-associated nephrotoxicity and adverse long-term outcomes.

Food and tobacco are the primary sources of cadmium exposure in the general population. After ingestion or inhalation, cadmium is temporarily stored in the liver bound to metallothionein. Pathophysiologically in agreement with the effect-modification of liver enzymes on cadmium-associated risk of graft failure hereby reported, cadmium-metallothionein is thereafter – upon hepatocytes turnover – released into the circulation, filtered at the glomerulus, and reabsorbed at the proximal tubule as a result of its preferential uptake by receptor-mediated endocytosis. With a kidney half-life of up to 45 years, a build-up of cadmium in the proximal tubule will ensue. Herein, cadmium is degraded in endosomes and lysosomes, releasing free Cd$^{2+}$ into the cytosol, where it generates reactive oxygen species (e.g., superoxide anion, hydrogen peroxide, and hydroxyl radicals) and activates redox sensitive transcription factors (e.g., NF-κB, AP-1 and Nrf2), which play a major role in cadmium-associated kidney pathophysiology through activation of cell death pathways involving p53, thus linking long-term cadmium exposure with proximal tubular cell apoptosis (HK-2 cells) and impaired reabsorption of low molecular weight proteins. In line, it has been found that cadmium exposure is associated with increased urinary excretion of N-acetyl-$\beta$-D-glucosaminidase (NAG), retinol binding protein, and $\alpha_1$- and $\beta_2$-microglobulin. It is thought that as tubular injury progresses, more generalized tubular dysfunction occurs. Prozialeck et al. recently showed that kidney injury molecule-1 (KIM-1) outperforms classic biomarkers of cadmium-induced nephrotoxicity. Further studies, and particularly human studies, have shown that urinary KIM-1 displays a better dose-response association with long-term low-dose cadmium exposure. Although in the current study we show that plasma cadmium strongly correlates with urinary excretion of two novel tubular damage biomarkers, i.e. epidermal growth factor and liver-type fatty acid-binding protein, future investigations in KTR are...
warranted to investigate the association of plasma cadmium with urinary excretion of other low-molecular weight proteins and KIM-1. Finally, although potential cadmium-associated glomerular injury has received relatively little attention, it should be underscored that there is a meaningful body of evidence linking cadmium exposure with glomerular damage and decreased glomerular filtration rate.\textsuperscript{7,14,19,22,23,42,43}

Because cadmium-induced hypertension has been previously reported, it could be hypothesized that at least part of the cadmium-associated risk of graft failure is attributable to an intermediary role of augmented blood pressure.\textsuperscript{44–47} Although across tertiles of plasma cadmium distribution systolic blood pressure was not different, we did observe a direct relation with use of antihypertensive medication. It should be noted, however, that in the present study the association between cadmium and graft failure was independent of systolic blood pressure, which supports that cadmium is linked to kidney tissue injury and dysfunction through proposed direct mechanisms at the kidney proximal tubule.

It should be realized that the current study is etiological in nature, which needs to be separated from prediction research.\textsuperscript{48} Whereas the latter is a distinct field of epidemiologic research aimed at predicting the risk of an outcome according to a model of statistically significant predictors, which not necessarily represent causal associations, etiological studies aim to understand a certain pathway of a disease in an attempt to prevent its onset or progression.\textsuperscript{48} This differentiation is relevant because in both scientific and clinical practice, the two kinds of analyses are often confused, reportedly resulting in poor-quality publications with limited interpretability and applicability. We remark on that, whereas its observational design does not allow causality assumptions, the current study is etiological in nature, and that taking together our findings and those of previous studies showing a plausible biological link between cadmium exposure and kidney damage, it is possible to support an etiological role of cadmium in pathways of disease that contribute to increased risk of graft failure in KTR.
Previous cohort studies performed in the general population have shown that cadmium is adversely associated with survival.\textsuperscript{49} We therefore additionally aimed to provide data on patients' survival and the composite end-point graft loss to account for both graft and patients' survival. When studying the broader end-point graft loss (defined as graft failure or death), increased cadmium-associated risk was consistent in analyses of patients in the highest tertile of plasma cadmium distribution, as well as in analyses of continuous increment of plasma cadmium. On the other hand, we observed that an apparent association of cadmium with all-cause mortality was mainly driven by graft failure, as shown in graft failure-censored analyses. These findings remark on the epidemiological relevance of cadmium exposure, as accounted by the clinically relevant end-point graft loss, whereas they emphasize that cadmium-associated hazard acts mainly through its nephrotoxic effects to increase the burden of adverse end-points in the long-term setting post-kidney transplantation.

Remarkably, our study was conducted in the northern part of The Netherlands, an area with known low environmental exposure rates to cadmium, both in soil and air.\textsuperscript{50} The life-long Dutch dietary intake of cadmium is below the European Food Safety Authority tolerable weekly cadmium intake of 2500 ng/kg body weight.\textsuperscript{28} The largest Western-European cohort study on cadmium, the Cadmibel study conducted in Belgium, reported whole blood cadmium concentrations –within the normal range– to be associated with kidney tubular dysfunction.\textsuperscript{18} Mining and metal industry countries, e.g. China –which is the world's leading country on cadmium production since 2014–, have markedly increased patients' cadmium exposure.\textsuperscript{51–53} Due to the dose-dependent effect suggested by the results of the current and previous studies, consequences of cadmium-associated kidney tissue injury may likely be more hazardous in such populations.\textsuperscript{7,18,28,50,51,53} Yet we emphasize that heavy metals exposure-associated CKD risk has been reported across all geographic regions.\textsuperscript{54}
Taken together, these findings underscore that cadmium monitoring, reduction of environmental exposure, and non-toxic therapeutic interventions to decrease bodily cadmium concentrations, may be novel risk management strategies to decrease the current burden of long-term kidney graft failure. Because the kidney is thought to be the organ most critically vulnerable to cadmium accumulation, monitoring its specific organ built-up—by means, e.g., of an in vivo X-ray fluorescence technique that using plane polarized X-rays allows a non-invasive assessment of kidney cortex cadmium—may be a particularly useful mean to assess the effects of accumulated cadmium on long-term kidney function end-points. Chelation therapy, used in heavy metal poisoning and iron overload syndrome, could henceforth offer an otherwise underestimated therapeutic approach. Lin et al. have repeatedly shown that the excretion of lead, a heavy metal with comparable nephrotoxicity to cadmium, can be increased by using Ca-EDTA (calcium ethylenediaminetetraacetic acid) chelation, which has been shown to slow progression rates of ESKD. Such results are promising for a potential cadmium-chelation therapeutic approach, particularly in KTR as being a population of high vulnerability to oxidative stress challenge and at high risk of kidney function impairment. Whether a novel cadmium-chelation pharmacological strategy may improve long-term graft survival rates warrants further studies.

We performed a prospective study in a large cohort of KTR, whom were sequentially recruited during outpatient visits at our university hospital, and then closely monitored by regular check-up in the outpatient clinic during a substantial follow-up period; which granted comprehensive and updated end-points evaluation, without loss to follow-up. Additional strengths of the current study are that our findings on the association of plasma cadmium with increased risk of graft failure were observed in a dose-response fashion in line with the literature, were robust in competing risk analyses as well as in sensitivity analyses with exclusion of outliers, and consistent over the secondary end-point in which graft failure is
combined with kidney function decline (graft failure or doubling of serum creatinine). With baseline data being extensively collected, we were able to perform analyses with adjustment for several potential confounders. Whereas we acknowledge that we were not able to adjust our main analyses for SES in the whole study population, we provide the results of sensitivity analyses in a sample population of consecutively enrolled 198 KTR, to ponder towards the notion that the association of cadmium with risk of graft failure is independent of SES in Dutch KTR, which may also be in line with previous literature showing that SES does not influence the risk of CKD nor the risk of adverse long-term outcomes post-kidney transplantation in the egalitarian Dutch population. Next, although exposure was assessed using a single measure, we studied serial plasma cadmium levels in a sample population of the TransplantLines Cohort and Biobank Study, in which we found low intra-individual variability, indicative of relatively stable plasma cadmium levels over time post-transplantation. This finding additionally underscores that even at low levels, the nephrotoxic exposure to cadmium may represent an overlooked hazard for preserved graft functioning. We also acknowledge that our predominantly Caucasian study population was derived from a single center from the northern part of The Netherlands, which, as described before, calls for prudence to extrapolate these results to a different population regarding potential environmental contamination and exposure to cadmium.

Our results, however, show for the first time that plasma cadmium is independently associated with long-term risk kidney graft failure, which was robust to several sensitivity analyses and consistent over additional graft function end-points, thus holding the plea for future studies to confirm our results and externally validate our findings among different populations of KTR. We also call out for future studies to confirm our findings by comparing whole blood cadmium versus plasma cadmium concentrations for the study of cadmium-associated nephrotoxicity and adverse kidney outcomes. We did not have data on urinary
cadmium excretion, which might be a better marker of total body cadmium accumulation and therefore even stronger associated with eGFR and graft failure. Future studies will have to compare the prospective associations of plasma cadmium, whole blood cadmium and urinary cadmium with adverse kidney outcomes to sort this out. Next, we observed that cadmium associated with risk of graft failure in a dose-response fashion, which has been consistently shown in previous literature and underscored to evidence causal cadmium-risk associations.\textsuperscript{12,65,66} While the prospective design of this study provides signals to formulate hypotheses regarding a causal link between cadmium and adverse kidney graft outcomes, we acknowledge that its observational nature prevents us from distinguishing whether plasma cadmium increases with decreasing eGFR or whether increased plasma cadmium levels cause a reduction in eGFR, and it does not allow for hard conclusions on causality. Neither could the potential presence of reversed causation, nor the possibility of residual confounding be entirely excluded. Despite the substantial number of potential confounders for which we adjusted, observational findings on the association between cadmium and risk of graft failure are, by definition, prone to confounding, which is in line with the moderate to low e-values hereby reported.\textsuperscript{67} Finally, because we found that plasma cadmium concentrations at admission for transplantation were significantly higher than at 3-months post-transplantation, and were also highly correlated with plasma cadmium at 3-months post-transplantation (in the sample population of KTR from the TransplantLines Prospective Cohort and Biobank Study), we hypothesize that cadmium exposure prior to transplantation may represent an otherwise overlooked contributing factor for increased risk of ESKD in the first place. Our findings warrant future studies to investigate a potential increased risk of ESKD associated with long-term cadmium exposure, even at relatively low levels as those of the KTR in this study, and to independently replicate our findings in different populations with regards to SES and environmental determinants of cadmium exposure.
In conclusion, the current study shows that in a Dutch cohort of outpatient KTR, higher plasma cadmium concentrations were independently associated with increased risk of long-term graft failure and kidney function decline. Cadmium exposure may be a potentially modifiable –yet rather overlooked– risk factor for adverse long-term kidney graft end-points. Our findings on a particularly strong association between plasma cadmium and risk of kidney graft failure among patients with relatively higher liver enzymes levels, may contribute with pathophysiological support to our findings, and be clinically relevant to aid on generating individualized follow-up strategies of outpatient KTR. Further studies are needed to confirm our results and to validate these findings in different populations with regards to exposure. Whether clinical monitoring of bodily cadmium concentrations, reduction of environmental exposure, and non-toxic therapeutic interventions to decrease system cadmium in outpatient KTR may represent novel risk management strategies to decrease the burden of long-term kidney graft failure remains to be investigated in future studies.

METHODS

Study Population

Between November 2008 and March 2011, all adult KTR with a functioning allograft ≥1-year, visiting the outpatient clinic of the University Medical Center Groningen (The Netherlands) were invited to participate in the TransplantLines Food and Nutrition Biobank and Cohort Study, as described previously.29 A total of 707 of 817 (87%) eligible KTR signed informed consent. Pancreas transplant patients (n=1) and patients missing plasma cadmium measurements (n=34) were excluded from the current analyses, resulting in 672 KTR, of whom data is hereby presented (a flowchart is shown in Supplemental Figure 5). Additional information can be found in Supplemental Methods (Online Supplemental Material). The study protocol has been approved by the institutional review board (METc 2008/186) and was conducted in accordance with the Declaration of Helsinki and Declaration of Istanbul.
Data Collection and Definitions

Medical and transplantation history as well as medication use were extracted from electronic patient records, including clinical history of past acute rejection. According to a strict protocol, all patients were asked to collect a 24 hours urine collection sample during the day before to their visit at the outpatient clinic. Blood was drawn in the morning after completion of the 24 hours urine collection. The measurement of clinical and laboratory parameters has been described in Supplemental Methods and in detail elsewhere. Blood and plasma cadmium concentrations were determined with use of an inductively coupled plasma mass spectrometer (ICP-MS, Varian 820-MS; Varian, Palo Alto, USA) with a validated method for the measurement of heavy metals in plasma as detailed in Supplemental Methods. Information on alcohol consumption and smoking behavior was obtained by using a questionnaire. Diabetes was defined as the usage of antidiabetics or a fasting blood glucose ≥7.0 mmol/L. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation. In the first n=198 consecutively enrolled KTR, socioeconomic status was investigated using a self-report questionnaire at inclusion, categorizing education as described elsewhere according to the International Standard Classification of Education: bachelor, master or doctorate graduate (level 1), postsecondary or non-tertiary or short-cycle tertiary education (level 2), upper secondary education (level 3), lower secondary education (level 4), and primary or below primary education (level 5). To investigate financial status, participants were asked to choose among four possible categories: Short, enough, good, or excellent monthly budget.

As described elsewhere, dietary intake was assessed using a 177 food items validated semi-quantitative food frequency questionnaire (FFQ) developed and updated at Wageningen University. Further information on the FFQ can be found in Supplemental Methods.

Clinical End-points
The primary end-point of this study was graft failure, defined as the requirement of dialysis or re-transplantation. Secondary end-points were kidney function decline (defined as doubling of serum creatinine or graft failure), graft loss (defined as graft failure or death) and all-cause mortality. These endpoints were chosen to adhere to current recommendations and state of the art in the field.\textsuperscript{73–76} For the analyses of graft failure, kidney function decline, and graft loss, patients who died with a functioning graft were censored at time of death. The study of all-cause mortality was performed with and without censoring at graft failure. The surveillance system of the outpatient program at our university hospital ensures updated information on patient status and events of graft failure as assessed by a nephrologist. Within this system, patients visit the outpatient clinic with declining frequency, in accordance with the guidelines of the American Society of Transplantation.\textsuperscript{77} End-points were recorded until September, 2015. General practitioners or referring nephrologists were contacted in case the status of a patient was unknown. No patients were lost to follow-up.

Serial Measurements in Participants the Ongoing TransplantLines Cohort and Biobank Study

Additionally, to investigate plasma cadmium levels over time, we requested follow-up plasma samples (at admission for transplantation, and at 3-months, 6-months, 1-year, and 2-years post-kidney transplantation) from 46 KTR consecutively enrolled between February 2016 and May 2017 in the ongoing TransplantLines Prospective Cohort and Biobank Study.\textsuperscript{34} Cadmium plasma concentrations were determined using inductively coupled plasma mass spectrometry, as described in detail in Supplemental Methods.

Blood versus Plasma Cadmium in Participants of the Ongoing TransplantLines Cohort and Biobank Study

We also measured whole blood and plasma cadmium levels in 116 outpatient KTR at a median of 5.2 (IQR, 1.6–11.1) years post-transplantation—which is comparable with transplant vintage
of our prospective cohort study population—, to compare whole blood versus plasma cadmium concentrations and to investigate the cross-sectional between cadmium concentration in each of these samples and eGFR.

**Statistical Analyses**

Data analyses were performed by using SPSS 23.0 for Windows (IBM, Chicago, Illinois, USA), GraphPad Prism 7.02 software (GraphPad Software Inc., San Diego, CA, USA), and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics of study subjects were described by subgroup of patients according to tertiles of plasma cadmium distribution. Normally distributed variables are described as mean (SD), and skewed variables as median (IQR). Categorical variables are expressed as n (number) with percentage (%). Differences were studied with the chi-squared test for categorical variables and by means of linear regression analyses for continuous variables. Variables with skewed distribution were natural log transformed, *i.e.*, transplant vintage, cold ischemia time, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, low density lipoprotein cholesterol, triglycerides, and blood glucose. A two-sided *P* value <0.05 was considered significant.

Analyses for testing difference and calculating intra-individual coefficient of variation (CV) for follow-up plasma cadmium levels in KTR of the TransplantLines Cohort and Biobank Study can be found in *Supplemental Methods*.

**Prospective analyses**

In prospective analyses of the primary end-point graft failure, a Kaplan-Meier curve and a log-rank test were performed to study whether the distribution of events was significantly different by subgroups of KTR according to tertiles of plasma cadmium concentration. The association of plasma cadmium concentration with risk of graft failure was further examined incorporating time to event by means of Cox proportional-hazards regression analyses (all assumptions were
met as described in Supplemental Methods), in which plasma cadmium was log₂-transformed to estimate regression coefficients per doubling of plasma cadmium concentration. For these analyses, risk of death with a functioning graft was accounted by censoring at time of death and by performing competing risk analyses according to Fine and Gray. To illustrate the association of plasma cadmium (log₂-transformed) with risk of graft failure, data were fitted using median plasma cadmium concentration (58 ng/L) as reference value. To study the effect of potential confounders, several Cox regression models were fitted to the data. We performed adjustment for age and sex in model 1; and, eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens (HLA) mismatches, and donor type in model 2. Subsequently, we additively adjusted for body mass index, systolic blood pressure, glucose, and history of diabetes in model 3; and, lifestyle-related risk factors (i.e., smoking status and alcohol consumption) in model 4; induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab) in model 5; and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood) in model 6.

Potential effect-modification by donor age, donor sex, donor type, recipient age, recipient sex, cold ischemia time, history of delayed graft function, eGFR, history of diabetes, systolic blood pressure, use of antihypertensive medication, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were tested by fitting models containing both main effects and their cross-product terms. The Bonferroni-adjusted significance threshold ($P_{\text{interaction}} < 0.006$; calculated as described in Supplemental Methods) was considered to indicate the performance of stratified prospective analyses. For these analyses, cut-off points of originally continuous variables were determined to concede clinically meaningful patients' strata.

**Sensitivity Analyses**
We identified plasma cadmium outliers by using Turkey’s fences (as described in Supplemental Methods), and analyzed Cox regression models analogous to the overall prospective analyses. Estimates are shown for patients pertaining to tertile 3 of plasma cadmium distribution in relation to patients pertaining to tertile 1 (reference group). Using the HR and CI calculated per doubling of plasma cadmium and for patients in tertile 3 of plasma cadmium distribution after exclusion of extreme outliers, we performed further sensitivity analyses as recommended for observational studies by means of providing e-values for both the observed association estimate and the limit of the CI closest to the null. We also performed sensitivity analyses in which we studied whether the association of cadmium with risk of late graft failure is independent of adjustment for SES.

Secondary Analyses

In secondary analyses, we studied the association of plasma cadmium with the secondary end-points kidney function decline, graft loss, and all-cause mortality, by means of Cox regression models analogous to the study of the primary end-point graft failure.

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DISCLOSURE

The authors hereby declare that no conflict of interest exists. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.
FIGURES LEGENDS

**Figure 1.** Kaplan–Meier curve for (A) death-censored graft failure ($n_{\text{events}}=78$) and (B) kidney function decline ($n_{\text{events}}=95$), according to tertiles of plasma cadmium distribution. Tertile 1: $\leq 48$ ng/L; tertile 2: 48–68 ng/L; tertile 3: $\geq 69$ ng/L. $P$ values were calculated by Log-rank test. Graft failure was defined as return to dialysis or re-transplantation. Kidney function decline was defined as doubling of serum creatinine or graft failure.

**Figure 2.** Association of plasma cadmium concentration with risk of death-censored graft failure in the (A) overall study population; (B) with exclusion of all outliers; and (C) with exclusion of extreme outliers. Data were fitted by Cox proportional-hazards regression using median plasma cadmium concentration (58 ng/L) as reference value. The black line represents the hazard ratio and the grey area represents the 95% confidence interval.

**Figure 3.** Stratified analyses of the association of plasma cadmium with risk of graft failure. $P_{\text{interaction}}$ was calculated by fitting models which contain both main effects and their cross-product term. Bonferroni-adjusted significance threshold $P_{\text{interaction}}<0.01$ was considered to indicate the performance of stratified analyses shown hereby. Cut-off points of originally continuous variables were determined to concede clinically meaningful patients' strata. Within each subgroup, hazard ratios (95% CI) were calculated per log$_2$ (ng/L) change in plasma cadmium, and adjusted for age and sex. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.
# Supplemental Material

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# TABLES

**Table 1. Baseline characteristics of 672 kidney transplant recipients**

| Baseline characteristics | Tertile 1 (≤48 ng/L) | Tertile 2 (48-68 ng/L) | Tertile 3 (≥69 ng/L) | $P_{\text{trend}}$ |
|--------------------------|----------------------|------------------------|----------------------|------------------|
| **Demographics and anthropometrics** | | | | |
| Age, years | 48 (14) | 54 (12) | 56 (11) | <0.001 |
| Sex (male), n (%) | 142 (63) | 132 (60) | 113 (50) | 0.01 |
| Body mass index, kg/m² | 26.5 (4.6) | 27.0 (4.9) | 26.6 (4.7) | 0.78 |
| Waist circumference, cm | 98 (14) | 99 (15) | 99 (15) | 0.71 |
| **Smoking status** | | | | 0.005 |
| Never, n (%) | 101 (45) | 94 (42) | 72 (32) |
| Former, n (%) | 90 (40) | 88 (40) | 107 (47) |
| Current, n (%) | 21 (9) | 27 (12) | 32 (14) |
| **Alcohol use** | | | | 0.32 |
| 0 g/d, n (%) | 18 (8) | 27 (12) | 30 (13) |
| 0–10 g/d, n (%) | 123 (55) | 127 (57) | 119 (53) |
| 0–30 g/d, n (%) | 43 (19) | 44 (20) | 44 (20) |
| >30 g/d, n (%) | 15 (7) | 5 (2) | 10 (4) |
| Systolic blood pressure, mmHg | 134 (17) | 136 (16) | 137 (19) | 0.18 |
| Diastolic blood pressure, mmHg | 83 (11) | 83 (11) | 82 (11) | 0.60 |
| Use of antihypertensive medication, n (%) | 187 (84) | 197 (89) | 208 (92) | 0.02 |
### Dietary intake

|                          | Median (IQR)   |
|--------------------------|----------------|
| Total energy intake, kCal/d | 2259 (633)     |
| Cereals, g/d             | 187 (147–231)  |
| Potatoes, g/d, median (IQR) | 111 (70–146)  |
| Vegetables, g/d, median (IQR) | 80 (56–116)   |
| Fruits, g/d, median (IQR) | 100 (48–189)  |
| Legumes, g/d, median (IQR) | 29 (14–48)    |
| Nuts, g/d, median (IQR)   | 5.6 (1.1–10.6) |
| Meat, g/d                | 94 (73–112)    |
| Dairy products, g/d       | 389 (239–482)  |
| Fish and seafood, g/d, median (IQR) | 13 (7–21)   |

### Kidney function and transplant history

|                          | Median (IQR)   |
|--------------------------|----------------|
| eGFR, mL/min/1.73 m²      | 60 (19)        |
| Proteinuria, n (%)        | 43 (19)        |
| Urinary protein excretion, g/24 h, median (IQR) | 0.15 (0.02–0.28) |
| Dialysis vintage, months, median (IQR) | 20 (5–43)     |
| Transplant vintage, years, median (IQR) | 7 (3–13)      |
| Acute rejection, n (%)    | 53 (24)        |
| Cold ischemia time, hrs, median (IQR) | 13 (2–21)     |
| Warm ischemia time, minutes | 42 (15)       |
| HLA mismatches            | 2.1 (1.5)      |
| Donor type, deceased, n (%) | 133 (59)      |

### Primary kidney disease, n (%)

- Glomerulosclerosis: 70 (31)
  - Median (IQR): 60 (27)
  - Kidney International 12(2)
  - The International Society of Nephrology (http://www.isn-online.org/site/cms)
| Disease                                      | Count 1 | Count 2 | Count 3 |
|----------------------------------------------|---------|---------|---------|
| Glomerulonephritis                           | 19 (9)  | 19 (9)  | 13 (6)  |
| Tubulointerstitial nephritis                 | 32 (14) | 20 (9)  | 24 (11) |
| Polycystic kidney disease                    | 40 (18) | 47 (21) | 54 (24) |
| Kidney hypo/dysplasia                        | 10 (5)  | 12 (5)  | 7 (3)   |
| Renovascular disease                         | 8 (4)   | 15 (7)  | 16 (7)  |
| Diabetes                                     | 7 (3)   | 10 (5)  | 15 (7)  |
| Other/miscellaneous                           | 38 (17) | 38 (17) | 37 (16) |
| **Immunosuppressive therapy**                |         |         |         |
| Use of calcineurin inhibitor, n (%)          | 110 (49)| 136 (61)| 139 (62)| 0.01  |
| Use of proliferation inhibitor, n (%)        | 196 (88)| 180 (81)| 184 (81)| 0.12  |
| Corticosteroids dose <10 mg/24 h, n (%)      | 95 (42) | 97 (44) | 84 (37) | 0.35  |
| **Liver function parameters**                |         |         |         |
| ASAT, U/L, median (IQR)                      | 21 (18‒26) | 22 (19‒27) | 22 (18‒27) | 0.09  |
| ALAT, U/L, median (IQR)                      | 19 (14‒26) | 19 (14‒26) | 18 (14‒26) | 0.93  |
| Alkaline phosphatase, U/L, median (IQR)      | 66 (51‒81) | 67 (55‒85) | 68 (54‒91) | 0.06  |
| GGT, U/L, median (IQR)                       | 25 (19‒34) | 28 (19‒46) | 28 (18‒45) | 0.02  |
| **Fasting lipids**                           |         |         |         |
| Total cholesterol, mmol/L                    | 4.9 (1.0) | 5.1 (1.1) | 5.3 (1.2) | 0.01  |
| HDL cholesterol, mmol/L                      | 1.4 (0.4) | 1.4 (0.5) | 1.4 (0.5) | 0.39  |
| LDL cholesterol, mmol/L                      | 2.8 (2.3‒3.4) | 2.8 (2.3‒3.6) | 3.0 (2.4‒3.6) | 0.05  |
| Triglycerides, mmol/L, median (IQR)          | 1.7 (1.2‒2.2) | 1.6 (1.2‒2.1) | 1.8 (1.3‒2.7) | 0.08  |
| **Diabetes and glucose homeostasis**         |         |         |         |
| Diabetes, n (%)                              | 41 (18) | 58 (26) | 64 (28) | 0.03  |
| Glucose, mmol/L, median (IQR)                | 5.2 (4.7‒5.8) | 5.2 (4.8‒6.1) | 5.3 (4.8‒6.2) | 0.09  |
### Markers of tubular toxicity

| Marker          | Tertile 1 (≤48 ng/L) | Tertile 2 (48–68 ng/L) | Tertile 3 (≥69 ng/L) | p-value |
|-----------------|-----------------------|------------------------|----------------------|---------|
| **HbA1C, %**    | 5.9 (0.7)             | 6.0 (1.0)              | 6.1 (0.8)            | 0.04    |
| **uEGF, ng/mL, median (IQR)** | 5.45 (2.99–8.13) | 3.99 (2.16–7.21) | 3.57 (1.47–7.26) | <0.001 |
| **uLFABP, ng/mL, median (IQR)** | 0.65 (0.27–2.11) | 0.91 (0.43–3.13) | 1.21 (0.50–5.90) | <0.001 |

Values presented as mean (SD) unless stated otherwise. Differences among tertiles of the plasma cadmium distribution (Tertile 1: ≤48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: ≥69 ng/L) were studied by means of analysis of variance or the linear regression test for continuous variables and by means of the chi-squared test for categorical variables. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein cholesterol; HLA, human leukocyte antigens; LDL, low-density lipoprotein cholesterol; uEGF, urinary epidermal growth factor; uLFABP, urinary liver-type fatty acid binding protein.
Table 2. Association of cadmium with risk of graft failure

| Models | Cadmium per log2 (ng/L) (n=672) | Tertiles of cadmium (n=224) | Tertile 2 (n=222) | Tertile 3 (n=226) | Tertile 3 (n=194) | Tertile 3 (n=215) |
|--------|---------------------------------|-----------------------------|-------------------|-------------------|-------------------|-------------------|
|        | HR (95% CI) | P value | E-values* | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | E-values* |
| Death-censored analyses | | | | | | | | |
| Crude  | 1.89 (1.47–2.43) | <0.001 | 2.48, 1.94 | 1.00 | 2.19 (1.13–4.27) | 3.38 (1.80–6.33) | 3.17 (1.66–6.05) | 3.29 (1.74–6.20) | 3.93, 2.29 |
| Model 1 | 1.96 (1.56–2.47) | <0.001 | 2.56, 2.06 | 1.00 | 2.67 (1.36–5.26) | 4.31 (2.25–8.22) | 4.23 (2.16–8.27) | 4.26 (2.21–8.21) | 4.78, 2.85 |
| Model 2 | 1.88 (1.31–2.69) | 0.001 | 2.46, 1.70 | 1.00 | 2.49 (1.14–5.43) | 3.11 (1.41–6.86) | 2.50 (1.14–5.48) | 3.09 (1.37–6.93) | 3.72, 1.79 |
| Model 3 | 1.87 (1.30–2.69) | 0.001 | 2.45, 1.69 | 1.00 | 2.48 (1.14–5.43) | 3.08 (1.40–6.82) | 2.47 (1.12–5.42) | 3.07 (1.36–6.90) | 3.73, 1.78 |
| Model 4 | 1.93 (1.36–2.75) | <0.001 | 2.52, 1.78 | 1.00 | 2.57 (1.16–5.70) | 3.36 (1.50–7.54) | 3.45 (1.48–7.05) | 3.34 (1.46–6.74) | 3.98, 1.92 |
| Model 5 | 1.87 (1.33–2.62) | <0.001 | 2.45, 1.73 | 1.00 | 2.50 (1.14–5.47) | 3.03 (1.37–6.82) | 2.98 (1.30–6.83) | 2.96 (1.31–6.66) | 3.62, 1.70 |
| Model 6 | 1.81 (1.28–2.56) | 0.001 | 2.38, 1.66 | 1.00 | 2.31 (1.02–5.22) | 2.82 (1.25–6.40) | 2.89 (1.24–5.00) | 2.76 (1.20–6.35) | 3.42, 1.53 |
| Competing risk analyses | | | | | | | | |
| Crude  | 1.90 (1.49–2.42) | <0.001 | 2.49, 1.96 | 1.00 | 2.04 (1.05–3.96) | 3.09 (1.65–5.78) | 2.83 (1.48–5.41) | 2.99 (1.59–5.63) | 3.65, 2.10 |
| Model 1 | 1.97 (1.66–2.35) | <0.001 | 2.57, 2.19 | 1.00 | 2.57 (1.33–4.97) | 4.13 (2.22–7.69) | 4.01 (2.11–7.63) | 4.07 (2.17–7.64) | 4.62, 2.80 |
| Model 2 | 1.81 (1.43–2.30) | <0.001 | 2.38, 1.88 | 1.00 | 2.17 (1.02–4.62) | 2.80 (1.32–5.94) | 2.69 (1.22–5.93) | 2.69 (1.24–5.80) | 3.35, 1.59 |
| Model 3 | 1.81 (1.41–2.30) | <0.001 | 2.38, 1.85 | 1.00 | 2.28 (0.99–5.26) | 3.00 (1.35–6.67) | 2.98 (1.30–6.83) | 2.95 (1.30–6.67) | 3.61, 1.69 |
| Model 4 | 1.76 (1.41–2.20) | <0.001 | 2.32, 1.85 | 1.00 | 2.12 (0.99–4.55) | 2.74 (1.26–5.55) | 2.70 (1.21–6.04) | 2.68 (1.22–5.86) | 3.34, 1.56 |
| Model 5 | 1.79 (1.42–2.26) | <0.001 | 2.35, 1.87 | 1.00 | 2.15 (0.98–4.71) | 2.65 (1.22–5.77) | 2.54 (1.16–5.57) | 2.54 (1.18–5.45) | 3.20, 1.49 |
| Model 6 | 1.99 (1.38–2.85) | <0.001 | 2.59, 1.81 | 1.00 | 1.96 (0.90–4.26) | 2.66 (1.24–5.68) | 2.89 (1.24–6.75) | 2.59 (1.17–5.71) | 3.25, 1.47 |
Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with risk of graft failure \( (n_{\text{events}}=78) \), accounting for death (with a functioning graft) by censoring at time of death or by performing competing risk analyses according to Fine and Gray.\(^{33}\) Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (Tertile 1: \( \leq 48 \) ng/L; Tertile 2: 48–68 ng/L; Tertile 3: \( \geq 69 \) ng/L) in the overall population, and without †all \( (n=32) \) and ‡extreme \( (n=11) \) outliers. *E-values are calculated for the association estimate (HR) and the limit of the confidence interval closest to the null per doubling of plasma cadmium and for patients in the third tertile of plasma cadmium distribution after exclusion of extreme outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); smoking and alcohol use (model 4); induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab; model 5); and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood; model 6).
Table 3. Association of cadmium with kidney function decline

| Models          | Crude          | Model 1    | Model 2    | Model 3    | Model 4    | Model 5    | Model 6    |
|-----------------|----------------|------------|------------|------------|------------|------------|------------|
|                 | HR (95% CI)    | HR (95% CI)| HR (95% CI)| HR (95% CI)| HR (95% CI)| HR (95% CI)| HR (95% CI)|
|                 | *(n=672)*      | *(n=224)*  | *(n=222)*  | *(n=226)*  | *(n=194)*  | *(n=215)*  |
| Crude           | 1.66 (1.29–2.15) | 1.00 | 1.44 (0.83–2.51) | 2.24 (1.34–3.74) | 2.16 (1.27–3.67) | 2.20 (1.31–3.70) |
| Model 1         | 1.78 (1.41–2.26) | 1.00 | 1.76 (1.00–3.10) | 2.84 (1.67–4.83) | 2.84 (1.64–4.94) | 2.82 (1.64–4.84) |
| Model 2         | 1.61 (1.17–2.22) | 1.00 | 1.56 (0.82–2.98) | 2.18 (1.14–4.17) | 2.39 (1.21–4.72) | 2.16 (1.11–4.19) |
| Model 3         | 1.60 (1.16–2.20) | 1.00 | 1.55 (0.81–2.95) | 2.15 (1.12–4.11) | 2.35 (1.19–4.65) | 2.13 (1.09–4.13) |
| Model 4         | 1.74 (1.27–2.38) | 1.00 | 1.93 (0.97–3.84) | 2.75 (1.37–5.53) | 2.94 (1.42–6.10) | 2.72 (1.33–5.54) |
| Model 5         | 1.61 (1.17–2.20) | 1.00 | 1.57 (0.82–3.01) | 2.14 (1.11–4.12) | 2.36 (1.19–4.70) | 2.11 (1.08–4.13) |
| Model 6         | 1.59 (1.15–2.21) | 1.00 | 1.54 (0.76–3.11) | 2.11 (1.05–4.23) | 2.26 (1.10–4.63) | 2.04 (1.01–4.13) |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with kidney function decline (n\text{events}=95). Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (tertile 1: ≤48 ng/L; tertile 2: 48–68 ng/L; tertile 3: ≥69 ng/L) in the overall population, and without †all (n=32) and ‡extreme (n=11) outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); smoking and alcohol use (model 4); induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab; model 5); and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood; model 6).
Plasma Cadmium is Associated with Increased Risk of Long-Term Kidney Graft Failure: A Prospective Cohort Study

Main figures
A. Death-Censored Graft Failure

B. Death-Censored Graft Failure

C. Death-Censored Graft Failure
| Effect-modifiers | Subgroup cut-off point | Events | Hazard ratio (95% CI) per log₂ ng/L | P interaction |
|------------------|------------------------|--------|------------------------------------|---------------|
|                  | ≤ 25 U/L               | 55     | 1.81 (1.38-2.37)                   | 0.003         |
|                  | > 25 U/L               | 23     | 2.61 (1.52-4.51)                   |               |
| ASAT             |                        |        |                                    |               |
|                  | ≤ 25 U/L               | 60     | 1.74 (1.34-2.28)                   | 0.005         |
|                  | > 25 U/L               | 18     | 3.89 (1.92-7.88)                   |               |
| ALAT             |                        |        |                                    |               |

HR (95% CI) per log₂ ng/L
CONCLUSION:
Plasma cadmium is independently associated with increased risk of long-term kidney graft failure.
Plasma Cadmium is Associated with Increased Risk of Long-Term Kidney Graft Failure: A Prospective Cohort Study

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Running Title: Cadmium & Risk of Kidney Graft Failure

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SUPPLEMENTAL MATERIAL

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References
SUPPLEMENTAL METHODS

Study population
Excluded patients were comparable to the study population in terms of age, sex, BMI, waist circumference, smoking status, alcohol use, blood pressure, use of antihypertensive therapy, dietary intake, primary kidney disease, transplant history, eGFR and urinary protein excretion.

Cadmium analysis
Cadmium plasma concentrations were determined with use of an inductively coupled plasma mass spectrometer (ICP-MS, Varian 820-MS; Varian, Palo Alto, USA) with a modified method for the measurement of low concentrations of heavy metals in plasma using a standard addition method. Standards were made by addition to blanc plasma known amounts of cadmium to obtain added concentrations of: 50; 100; 200; 300; 400 and 500 ng/L. Control samples were made by spiking blanc plasma with known amounts of cadmium to obtain added concentrations of respectively: 75 (low); 225 (medium) and 450 ng/L (high). Sample preparation consisted of diluting 100 µL sample with 1.0 mL dilution reagent. The dilution reagent contained 0.005% Triton X100, 0.005% EDTA and 0.1 mg/L Yttrium as internal standard. Characteristics of this method are summarized in Supplemental Table 6.

Other laboratory measurements
Urinary epidermal growth factor was measured by ELISA (R&D Systems, Minneapolis, MN, USA). The test has a range of detection of 3.9–250 pg/mL and the intra- and inter-plate coefficients of variation were less than 10% and 15%, respectively. Urinary liver-type fatty-acid binding protein was measured with an enzyme-linked immunosorbent assay (human uL-FABP
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assay kit 96 test, CMIC holdings Co., Ltd, Tokyo, Japan). The test has a range of detection of 0.3–60 ng/mL and the intra- and inter-plate coefficients of variation were less than 5%.\textsuperscript{52}

Assessment of Dietary Intake

As described elsewhere,\textsuperscript{53} dietary intake was assessed using a validated semi-quantitative food frequency questionnaire (FFQ) developed and updated at Wageningen University.\textsuperscript{54} The questionnaire consisted of 177 food items to record intake during the last month, taking seasonal variations into account. For each item, the frequency was expressed in times per day, week, or month. The number of servings was recorded in natural units (\textit{e.g.}, slice of bread or apple) or household measures (\textit{e.g.}, cup or spoon). The FFQ was self-administered and then checked by a trained researcher on the day of visit to the outpatient clinic. Inconsistent answers were verified with the patients. The results of the FFQ were converted into total energy and nutrient intake per day by using the Dutch Food Composition Table of 2006.

Statistical analyses

The intra-individual coefficient of variation (CV) for plasma cadmium levels in KTR of the TransplantLines Cohort and Biobank Study was calculated using the formula \( CV = (SD/mean) \times 100 \), in which SD is the standard deviation and mean is the mean value for plasma cadmium concentrations as measured in follow-up samples taken at 3-months, 6-months, 1-year, and 2-years post-kidney transplant. Box plots were used to illustrate medians (interquartile range) of plasma cadmium levels at admission for transplantation and at post-transplant follow-up visits. Significance of potential difference between cadmium at admission for transplantation and 3-months post-transplant was tested using the Wilcoxon matched-pairs signed rank test, and significance of potential change during post-kidney transplant follow-up visits was tested using the Kruskal Wallis test.
Prospective analyses

We plotted Martingale residuals against age and kidney function to test which functional form of these covariates best fitted the models. Schoenfeld residuals were calculated to assess whether proportionality assumptions were satisfied. We entered the quadratic and cubic terms of plasma cadmium with the linear term to assess the presence of nonlinear relationships (Supplemental Table 7). A variance inflation factor $<5$ indicates no evidence for collinearity.

Effect-modification analyses

Potential effect-modification by donor age, donor sex, donor type, recipient age, recipient sex, cold ischemia time, history of delayed graft function, eGFR, history of diabetes, systolic blood pressure, use of antihypertensive medication, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and gamma glutamyl transferase were tested by fitting models containing both main effects and their cross-product terms. $P$ value $<0.1$ was considered to indicate effect-modification.\textsuperscript{55} We then performed correction for multiple testing by means of the Bonferroni method. Because we have investigated potential effect-modification for 15 variables, the corrected threshold based on the false discovery rate level of 0.1 was $0.1/15=0.006$. This Bonferroni-adjusted significance threshold ($P_{\text{interaction}}<0.006$) was then considered to indicate the performance of stratified prospective. For these analyses, cut-off points of originally continuous variables were determined to concede clinically meaningful patients' strata.
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SUPPLEMENTAL RESULTS

Description of Extreme Outliers

Out of all outliers, 11 participants (5% of patients pertaining to the highest tertile) were extreme outliers, with widely varying plasma cadmium concentrations (173–3363 ng/L). The highest plasma cadmium concentration was more than 5 times higher than the second highest plasma cadmium concentration. This particular patient, male, 37 years-old at the time of inclusion, was initially transplanted in 1995 because of polycystic kidney disease and had an eGFR of 32 mL/min/1.73 m². He was a current smoker at time of inclusion, and had an extensive graft failure history: after a first transplantation, the patient developed acute rejection, and a re-transplantation was eventually performed in 2003. Thereafter the patient returned to dialysis due to loss of glomerular function in 2010, and underwent a third renal transplantation in 2016. Among the other extreme outliers, 6 had diabetes at inclusion, 3 developed graft failure during follow-up and 2 deceased.
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Supplemental Table 1. Effect-modification analyses on the association of plasma cadmium with graft failure

| Pre-defined potential effect-modifiers | Cadmium per log² (ng/L) | B   | $P_{interaction}$ |
|---------------------------------------|-------------------------|-----|------------------|
| Donor age, years                      |                         | 0.01| 0.18             |
| Donor sex, male, n                    |                         | -0.14| 0.60             |
| Donor type, deceased, n               |                         | -0.22| 0.50             |
| Recipient age, years                  |                         | 0.01| 0.44             |
| Recipient sex, male, n                |                         | 0.23| 0.54             |
| Cold ischemia time, hrs               |                         | 0.004| 0.78             |
| History of delayed graft function, n  |                         | -0.42| 0.54             |
| eGFR, mL/min/1.73 m²                  |                         | -0.01| 0.66             |
| Diabetes, n                           |                         | -0.31| 0.44             |
| Systolic blood pressure, mmHg         |                         | -0.001| 0.93            |
| Use of antihypertensive medication, n |                         | -1.01| 0.23             |
| Aspartate aminotransferase, U/L       |                         | 0.09 | 0.003*          |
| Alanine aminotransferase, U/L         |                         | 0.06 | 0.005*          |
| Alkaline phosphatase, U/L             |                         | 0.01 | 0.22             |
| Gamma glutamyl transferase, U/L       |                         | 0.004| 0.05             |

Effect-modification on death-censored graft failure by pre-defined potential effect-modifiers was tested by fitting models containing both main effects and their cross product terms. $P$ value <0.1 was considered to indicate effect-modification. We then performed correction for multiple testing by means of the Bonferroni method. Because we have investigated potential effect-modification for 15 variables, the corrected threshold based on the false discovery rate level of 0.1 was 0.1/15=0.006. *$P_{interaction}$ below the Bonferroni-adjusted threshold (0.006).
Supplemental Table 2. Past events and outcomes in 672 outpatient kidney transplant recipients

| Event                     | Overall population | Tertile 1 | Tertile 2 | Tertile 3 |
|---------------------------|--------------------|-----------|-----------|-----------|
|                           | N° of events       | N° of events | N° of events | N° of events |
| **Past events**           |                    |           |           |           |
| History of acute rejection| 177                | 53        | 64        | 60        |
| Delayed graft function    | 50                 | 8         | 14        | 28        |
| **Outcomes**              |                    |           |           |           |
| Graft failure             | 78                 | 13        | 26        | 39        |
| Kidney function decline   | 95                 | 22        | 29        | 44        |
| Graft loss                | 190                | 33        | 66        | 91        |
| Death                     | 137                | 21        | 48        | 68        |

Tertile 1: ≤48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: ≥69 ng/L. The primary end-point of this study was graft failure, defined as the requirement of dialysis or re-transplantation. Secondary end-points were kidney function decline (defined as doubling of serum creatinine or graft failure), graft loss (defined as graft failure or death) and all-cause mortality. These endpoints were chosen to adhere to current recommendations and state of the art in the field.\(^{56–59}\) The surveillance system of the outpatient program at our university hospital ensures updated information on patient status and events of graft failure as assessed by a nephrologist. Within this system, patients visit the outpatient clinic with declining frequency, in accordance with the guidelines of the American Society of Transplantation.\(^{510}\) End-points were recorded until September, 2015. General practitioners or referring nephrologists were contacted in case the status of a patient was unknown. No patients were lost to follow-up.
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**Supplemental Table 3.** Association of cadmium with graft loss

| Cadmium per log₂ (ng/L) | Tertile 1 | Tertile 2 | Tertile 3 |
|------------------------|----------|----------|----------|
| Models | HR (95% CI) | P value | HR (95% CI) | HR (95% CI) |
| n=672 | n=224 | n=222 | n=226 |
| Crude | 1.69 (1.41–2.03) <0.001 | 1.00 | 2.19 (1.44–3.33) | 3.12 (2.09–4.64) |
| Model 1 | 1.62 (1.34–1.97) <0.001 | 1.00 | 1.98 (1.30–3.02) | 2.82 (1.87–4.23) |
| Model 2 | 1.23 (0.95–1.61) 0.12 | 1.00 | 1.32 (0.83–2.10) | 1.50 (0.94–2.40) |
| Model 3 | 1.27 (0.84–1.60) 0.13 | 1.00 | 1.32 (0.83–2.10) | 1.49 (0.94–2.39) |
| Model 4 | 1.31 (1.00–1.71) 0.05 | 1.00 | 1.35 (0.84–2.19) | 1.63 (1.01–2.66) |
| Model 5 | 1.60 (1.29–2.00) <0.001 | 1.00 | 1.75 (1.12–2.75) | 2.54 (1.65–3.91) |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with graft loss \(n_{\text{events}}=190\). Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (Tertile 1: ≤48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: ≥69 ng/L). Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary renal disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); and, smoking and alcohol use (model 4). Multivariable model 5 was adjusted for age, sex, and dietary intake of different food groups \(e.g.,\) cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood.)
Supplemental Table 4. Association of cadmium with all-cause mortality

| Models    | Cadmium per log₂ (ng/L) | Tertile 1 HR (95% CI) | P value | Tertile 2 HR (95% CI) | Tertile 3 HR (95% CI) |
|-----------|-------------------------|-----------------------|---------|-----------------------|-----------------------|
| n=672     |                         | Ref.                  |         |                       |                       |
| Crude     | 1.46 (1.23–1.73)        | <0.001                |         | 2.44 (1.46–4.07)      | 3.47 (2.12–5.66)      |
| Model 1   | 1.46 (1.16–1.83)        | 0.001                 |         | 1.87 (1.12–3.13)      | 2.62 (1.60–4.31)      |
| Model 2   | 1.22 (0.92–1.62)        | 0.17                  |         | 1.56 (0.90–2.70)      | 1.81 (1.03–3.16)      |
| Model 3   | 1.21 (0.91–1.61)        | 0.18                  |         | 1.56 (0.90–2.70)      | 1.79 (1.05–3.14)      |
| Model 4   | 1.28 (0.96–1.69)        | 0.09                  |         | 1.62 (0.91–2.88)      | 1.98 (1.11–3.53)      |
| Model 5   | 1.41 (1.07–1.85)        | 0.01                  |         | 1.80 (1.03–3.16)      | 2.39 (1.39–4.11)      |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with all-cause mortality (n<sub>events</sub>=137). Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (Tertile 1: ≤48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: ≥69 ng/L). Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary renal disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); and, smoking and alcohol use (model 4). Multivariable model 5 was adjusted for age, sex, and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood).
**Supplemental Table 5.** Association of cadmium with all-cause mortality, censored at graft failure

| Cadmium per log₂ (ng/L) | Tertile 1 | Tertile 2 | Tertile 3 |
|-------------------------|-----------|-----------|-----------|
| **Models**              | HR (95% CI) | P value | Ref. | HR (95% CI) | HR (95% CI) |
| n=672                   | n=224 | n=222 | n=226 |
| Crude                   | 1.52 (1.17-1.96) | 0.001 | 1.00 | 2.20 (1.28-3.76) | 2.95 (1.76-4.94) |
| Model 1                 | 1.32 (0.99-1.76) | 0.06 | 1.00 | 1.63 (0.95-2.80) | 2.19 (1.30-3.70) |
| Model 2                 | 1.13 (0.80-1.61) | 0.49 | 1.00 | 1.39 (0.78-2.47) | 1.65 (0.91-3.00) |
| Model 3                 | 1.13 (0.79-1.61) | 0.50 | 1.00 | 1.37 (0.77-2.44) | 1.63 (0.90-3.96) |
| Model 4                 | 1.19 (0.83-1.73) | 0.35 | 1.00 | 1.41 (0.77-2.58) | 1.79 (0.96-3.33) |
| Model 5                 | 1.22 (0.88-1.71) | 0.24 | 1.00 | 1.59 (0.88-2.87) | 1.99 (1.12-3.54) |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with all-cause mortality censored at graft failure \( (n_{\text{event}}=112) \). Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution \( (\text{Tertile 1: } \leq 48 \text{ ng/L}; \text{Tertile 2: } 48-68 \text{ ng/L}; \text{Tertile 3: } \geq 69 \text{ ng/L}) \). Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary renal disease, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens mismatches, and donor type (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); and, smoking and alcohol use (model 4). Multivariable model 5 was adjusted for age, sex, and dietary intake of different food groups \( (e.g., \text{cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood}) \).
### Supplemental Table 6. Bias and precision of cadmium measurements

| Cadmium concentration | n  | ng/L | Bias (%) | Inter-assay coef. | SD (ng/L) | CV (%) |
|-----------------------|----|------|----------|-------------------|-----------|--------|
| Low                   | 31 | 75   | −12.0    |                   | 15        | 23     |
| Medium                | 35 | 225  | 3.5      |                   | 26        | 11     |
| High                  | 37 | 450  | −1.5     |                   | 50        | 11     |

CV, coefficient of variation; SD, standard differentiation.
**Supplemental Table 7.** Association of plasma cadmium and risk of graft failure – Verification of linearity

|               | HR (95% CI)     | P value |
|---------------|-----------------|---------|
| **Univariate A** |                 |         |
| \( \log_2 \) cadmium | 1.89 (1.47-2.43) | <0.001  |
| **Univariate B** |                 |         |
| \( \log_2 \) cadmium | 1.88 (0.97-3.65) | 0.06    |
| \( \log_2 \) cadmium\(^2\) | 1.00 (0.90-1.11) | 0.98    |
| **Univariate C** |                 |         |
| \( \log_2 \) cadmium | 2.35 (1.32-4.17) | 0.004   |
| \( \log_2 \) cadmium\(^2\) | 1.40 (0.90-2.17) | 0.14    |
| \( \log_2 \) cadmium\(^3\) | 1.06 (0.98-1.13) | 0.13    |
| **Model 1 A** |                 |         |
| \( \log_2 \) cadmium | 1.96 (1.56-2.47) | <0.001  |
| **Model 1 B** |                 |         |
| \( \log_2 \) cadmium | 1.98 (1.11-3.53) | 0.02    |
| \( \log_2 \) cadmium\(^2\) | 1.39 (0.88-2.19) | 0.16    |
| **Model 1 B** |                 |         |
| \( \log_2 \) cadmium | 1.98 (1.11-3.53) | 0.02    |
| \( \log_2 \) cadmium\(^2\) | 1.39 (0.88-2.19) | 0.16    |
| \( \log_2 \) cadmium\(^3\) | 1.06 (0.99-1.14) | 0.11    |
| **Model 2 A** |                 |         |
| \( \log_2 \) cadmium | 1.89 (1.31-2.74) | 0.001   |
| **Model 2 B** |                 |         |
| \( \log_2 \) cadmium | 2.88 (1.51-5.50) | 0.001   |
| \( \log_2 \) cadmium\(^2\) | 1.09 (0.96-1.23) | 0.18    |
| **Model 2 C** |                 |         |
| \( \log_2 \) cadmium | 3.02 (1.55-5.89) | 0.001   |
| \( \log_2 \) cadmium\(^2\) | 1.24 (0.68-2.27) | 0.49    |
| \( \log_2 \) cadmium\(^3\) | 1.02 (0.93-1.13) | 0.67    |
### Model 3 A

Log<sub>2</sub> cadmium 1.87 (1.28-2.74) 0.001

### Model 3 B

Log<sub>2</sub> cadmium 2.89 (1.51-5.56) 0.001

Log<sub>2</sub> cadmium<sup>2</sup> 1.09 (0.97-1.23) 0.17

### Model 3 C

Log<sub>2</sub> cadmium 3.10 (1.59-6.03) 0.001

Log<sub>2</sub> cadmium<sup>2</sup> 1.34 (0.73-2.45) 0.35

Log<sub>2</sub> cadmium<sup>3</sup> 1.03 (0.94-1.14) 0.50

### Model 4 A

Log<sub>2</sub> cadmium 1.97 (1.37-2.83) <0.001

### Model 4 B

Log<sub>2</sub> cadmium 2.69 (1.41-5.11) 0.003

Log<sub>2</sub> cadmium<sup>2</sup> 1.07 (0.94-1.20) 0.30

### Model 4 C

Log<sub>2</sub> cadmium 2.80 (1.44-5.45) 0.002

Log<sub>2</sub> cadmium<sup>2</sup> 1.21 (0.66-2.21) 0.54

Log<sub>2</sub> cadmium<sup>3</sup> 1.02 (0.93-1.12) 0.68

### Model 5 A

Log<sub>2</sub> cadmium 2.04 (1.56-2.68) <0.001

### Model 5 B

Log<sub>2</sub> cadmium 1.98 (0.95-4.10) 0.07

Log<sub>2</sub> cadmium<sup>2</sup> 0.99 (0.88-1.12) 0.92

### Model 5 C

Log<sub>2</sub> cadmium 2.55 (1.28-5.07) 0.008

Log<sub>2</sub> cadmium<sup>2</sup> 1.48 (0.91-2.40) 0.11

Log<sub>2</sub> cadmium<sup>3</sup> 1.07 (0.99-1.15) 0.10

Model 1: Adjusted for age and sex. Model 2: Model 1 + adjustment for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, human leukocyte antigens mismatches, and donor type. Model 3: Model 2 + adjustment for body mass index, systolic blood pressure, blood glucose, and history of diabetes. Model 4: Model 3 +
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adjustment for smoking and alcohol use. Model 5: Adjusted for age, sex, and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood).
**Supplemental Table 8.** Socioeconomic status and plasma cadmium in 198 kidney transplant recipients.

| Socioeconomic status | Tertiles of plasma cadmium concentrations | $P_{trend}$ |
|----------------------|------------------------------------------|-------------|
|                      | Tertile 1 | Tertile 2 | Tertile 3 |          |
| **Educational status** |          |          |          |          |
| Level 1, n (%)       | 6 (9)     | 5 (8)    | 1 (2)    | 0.04     |
| Level 2, n (%)       | 13 (20)   | 11 (17)  | 6 (9)    |          |
| Level 3, n (%)       | 16 (24)   | 20 (30)  | 18 (27)  |          |
| Level 4, n (%)       | 28 (42)   | 23 (35)  | 33 (50)  |          |
| Level 5, n (%)       | 3 (5)     | 7 (11)   | 8 (12)   |          |
| **Financial status** |          |          |          | 0.02     |
| Paid work, n (%)     | 36 (55)   | 26 (39)  | 23 (35)  |          |
| Work not paid, n (%) | 30 (46)   | 40 (61)  | 41 (62)  |          |

Differences among tertiles of the plasma cadmium distribution (Tertile 1: ≤52 ng/L; Tertile 2: 53–72 ng/L; Tertile 3: ≥73 ng/L), were studied by means of the $\chi^2$ test. Data on financial status was missing in 2 patients. Education levels were bachelor, master or doctorate graduate (level 1), postsecondary or non-tertiary or short-cycle tertiary education (level 2), upper secondary education (level 3), lower secondary education (level 4), and primary or below primary education (level 5), as previously described according to the International Standard Classification of Education (ISCED; UNESCO Institute for Statistics: International Standard Classification of Education, ISCED 2011. Paris, France, UNESCO, 2011).
Supplemental Table 9. Association of cadmium with risk of graft failure in 198 kidney transplant recipients

| Models          | Cadmium per log₂ (ng/L) | HR   | 95% CI       | P value |
|-----------------|-------------------------|------|--------------|---------|
| Death-censored analyses |                        |      |              |         |
| Unadjusted      | 1.64                    | 1.64 | (0.91-2.94)  | 0.102   |
| Model 1         | 1.89                    | 1.89 | (0.99-3.59)  | 0.053   |
| Model 2         | 1.91                    | 1.91 | (1.00-3.66)  | 0.049   |

| Competing risk analyses |                        |      |              |         |
| Unadjusted      | 1.63                    | 1.63 | (0.87-3.04)  | 0.128   |
| Model 1         | 1.92                    | 1.92 | (1.03-3.57)  | 0.041   |
| Model 2         | 1.93                    | 1.93 | (1.03-3.63)  | 0.043   |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with risk of graft failure (n events=28), firstly by accounting for death (with a functioning graft) by censoring at time of death and secondly by performing competing risk analyses according to Fine and Gray. Multivariable model 1 was adjusted for age and sex. Multivariable model 2 was adjusted for age, sex, educational level and financial status.
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**Supplemental Table 10.** Sensitivity analyses of the association of cadmium with risk of graft failure, replacing adjustment of donor type by cold ischemia time

| Models | Cadmium per log2 (ng/L) (n=672) | Tertiles of cadmium | Tertile 1 | Tertile 2 | Tertile 3 | †Tertile 3 | ‡Tertile 3 |
|--------|-------------------------------|---------------------|----------|----------|----------|----------|----------|
|        | HR (95% CI) | P value | Ref. HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) | HR (95% CI) |
| Death-censored analyses | | | | | | | |
| Crude  | 1.89 (1.47–2.43)  | <0.001 | 1.00 | 2.19 (1.13–4.27)  | 3.38 (1.80–6.33)  | 3.17 (1.66–6.05)  | 3.29 (1.74–6.20)  |
| Model 1 | 1.96 (1.56–2.47)  | <0.001 | 1.00 | 2.67 (1.36–5.26)  | 4.31 (2.25–8.22)  | 4.23 (2.16–8.27)  | 4.26 (2.21–8.21)  |
| Model 2 | 1.88 (1.33–2.65)  | <0.001 | 1.00 | 2.53 (1.16–5.56)  | 3.11 (1.41–6.84)  | 3.34 (1.45–7.66)  | 3.12 (1.39–7.02)  |
| Model 3 | 1.87 (1.32–2.64)  | <0.001 | 1.00 | 2.52 (1.15–5.53)  | 3.08 (1.40–6.79)  | 3.29 (1.43–7.56)  | 3.10 (1.38–6.98)  |
| Model 4 | 1.91 (1.37–2.68)  | <0.001 | 1.00 | 2.59 (1.16–5.77)  | 3.33 (1.49–7.45)  | 3.48 (1.49–8.10)  | 3.35 (1.47–6.67)  |
| Model 5 | 1.88 (1.34–2.65)  | <0.001 | 1.00 | 2.55 (1.56–5.62)  | 3.03 (1.37–6.70)  | 3.26 (1.41–7.54)  | 2.99 (1.33–6.76)  |
| Model 6 | 1.82 (1.28–2.60)  | 0.001  | 1.00 | 2.38 (1.04–5.42)  | 2.84 (1.26–6.43)  | 2.98 (1.27–6.95)  | 2.83 (1.23–6.52)  |
| Competing risk analyses | | | | | | | |
| Crude  | 1.90 (1.49–2.42)  | <0.001 | 1.00 | 2.04 (1.05–3.96)  | 3.09 (1.65–5.78)  | 2.83 (1.48–5.41)  | 2.99 (1.59–5.63)  |
| Model 1 | 1.97 (1.66–2.35)  | <0.001 | 1.00 | 2.57 (1.33–4.97)  | 4.13 (2.22–7.69)  | 4.01 (2.11–7.63)  | 4.07 (2.17–7.64)  |
| Model 2 | 1.83 (1.42–2.36)  | <0.001 | 1.00 | 2.19 (1.02–4.73)  | 2.78 (1.31–5.88)  | 2.69 (1.22–5.95)  | 2.69 (1.24–5.84)  |
| Model 3 | 1.82 (1.40–2.37)  | <0.001 | 1.00 | 2.31 (1.00–5.37)  | 2.98 (1.35–6.55)  | 3.00 (1.30–6.89)  | 2.97 (1.31–6.73)  |
| Model 4 | 1.78 (1.41–2.24)  | <0.001 | 1.00 | 2.10 (0.97–4.55)  | 2.67 (1.26–5.65)  | 2.65 (1.20–5.89)  | 2.64 (1.21–5.73)  |
| Model 5 | 1.82 (1.42–2.33)  | <0.001 | 1.00 | 2.11 (0.95–4.66)  | 2.62 (1.23–5.54)  | 2.53 (1.15–5.59)  | 2.54 (1.18–5.48)  |

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| Model | Coefficient | p-value | Coefficient | p-value | Coefficient | p-value | Coefficient | p-value |
|-------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|
|       | 1.99 (1.36–2.91) | <0.001 | 1.00 | 2.01 (0.90–4.51) | 2.66 (1.25–5.66) | 2.96 (1.26–6.96) | 2.63 (1.18–5.84) |

Cox proportional-hazards regression analyses were performed to assess the association of plasma cadmium concentration with risk of graft failure ($n_{events}=78$), accounting for death (with a functioning graft) by censoring at time of death or by performing competing risk analyses according to Fine and Gray.\textsuperscript{33} Associations are shown with plasma cadmium concentration as a continuous variable and according to tertiles of the plasma cadmium distribution (Tertile 1: $\leq$48 ng/L; Tertile 2: 48–68 ng/L; Tertile 3: $\geq$69 ng/L) in the overall population, and without \textsuperscript{†}all ($n=32$) and \textsuperscript{‡}extreme ($n=11$) outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for eGFR, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, cold ischemia time, and human leukocyte antigens mismatches (model 2); body mass index, systolic blood pressure, blood glucose, and history of diabetes (model 3); and, smoking and alcohol use (model 4); induction therapy (anti-thymocyte globulin, IL2 receptor antibody, muromonab-CD3, and rituximab; model 5); and dietary intake of different food groups (e.g., cereals, potatoes, vegetables, fruits, legumes, nuts, meat, milk and dairy products, and fish and seafood; model 6).
Supplemental Figure 1. Plasma cadmium concentrations in 46 KTR of the TransplantLines Prospective Cohort and Biobank Study, at different follow-up visits post-transplantation. Box plots show medians (interquartile range). Significance of potential change during follow-up visits was tested using the Kruskal Wallis test, which indicated no significant change over time (P=0.89). Median (interquartile range) intra-individual coefficient of variation of plasma cadmium concentrations was 2.9% (1.9–4.5%).
Supplemental Figure 2. Plasma cadmium concentrations at admission before transplantation and at 3-months after transplantation in 46 KTR of the TransplantLines Prospective Cohort and Biobank Study.\textsuperscript{34} (A) Plasma cadmium at 3-months post-Tx (transplantation) was significantly different from plasma cadmium at admission for Tx (median (interquartile range), 78 (71–93) and 100 (75–126) ng/L, respectively; \(P<0.001\)). Box plots show medians (interquartile range). Significance of potential difference between Tx and 3 months post-Tx plasma cadmium was tested using the Wilcoxon matched-pairs signed rank test. \(P<0.05\). (B) Plasma cadmium at Tx was significantly associated (Std. \(\beta=0.71, \ P<0.001\)) with plasma cadmium at 3 months post-Tx (\(R^2=0.51\)).
Supplemental Figure 3. Association of whole blood cadmium with plasma cadmium concentration (Std. β=0.52, \( P=0.001 \)) in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study.\(^{34}\)
Supplemental Figure 4. Association of (A) plasma and (B) whole blood cadmium concentrations with eGFR in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study. Plasma cadmium ($R^2=0.03$; Std. $\beta=-0.19$, $P=0.046$), but not whole blood cadmium ($R^2=0.004$; Std. $\beta=0.07$, $P=0.47$) was significantly associated with estimated glomerular filtration rate.
Supplemental Figure 5. Flowchart depicting the phases of inclusion of the study population.
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and CKD in the United States and The Netherlands. *Ci J Am Soc Nephrol.* 2013;8(10):1685-1693.
## Modified STROBE Statement

| Item No | Recommendation | Response |
|---------|----------------|----------|
| 1 & 2   | *(a)* Indicate the study’s design with a commonly used term in the title or the abstract  
        *(b)* Provide in the abstract an informative and balanced summary of what was done and what was found | Yes |
| 3       | Explain the scientific background and rationale for the investigation being reported | Yes |
| 4       | State specific objectives, including any prespecified hypotheses | Yes |
| 5       | Present key elements of study design early in the paper | Yes |
| 6       | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | Yes |
| 7       | *(a)* Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
        Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls  
        Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants | Yes |
| 8       | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | Yes |
| 9 & 10  | For each variable of interest, give sources of data and details of methods of assessment (measurement). | Yes |
| 11      | Describe any efforts to address potential sources of bias | Yes |
| 12      | Explain how the study size was arrived at (if applicable) | Yes |
Quantitative variables 11
Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

Statistical methods 12
(a) Describe all statistical methods, including those used to control for confounding
Yes
(b) Describe any methods used to examine subgroups and interactions
Yes
(c) Explain how missing data were addressed
Yes
(d) **Cohort study**—If applicable, explain how loss to follow-up was addressed
Yes
**Case-control study**—If applicable, explain how matching of cases and controls was addressed
NA
**Cross-sectional study**—If applicable, describe analytical methods taking account of sampling strategy
NA
(e) Describe any sensitivity analyses
Yes

### Results

| Participants | 13* |
|--------------|-----|
| (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed |
| (c) **Use of a flow diagram** |
| Yes |

| Descriptive data | 14* |
|------------------|-----|
| (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders |
| (b) Indicate number of participants with missing data for each variable of interest |
| (c) **Cohort study**—Summarize follow-up time (eg, average and total amount) |
| Yes |

| Outcome data | 15* |
|--------------|-----|
| **Cohort study**—Report numbers of outcome events or summary measures over time |
| **Case-control study**—Report numbers in each exposure category, or summary measures of exposure |
| **Cross-sectional study**—Report numbers of outcome events or summary measures |
| NA |
| Section          | Item | Description                                                                                                                                                                                                 | Yes/No |
|------------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|
| Main results     | 16   | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included.                   |        |
| Other analyses   | 17   | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses.                                                                                                              |        |
| Discussion       |      |                                                                                                                                                                                                              |        |
| Key results      | 18   | Summarize key results with reference to study objectives.                                                                                                                                                      |        |
| Limitations      | 19   | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.                                                        |        |
| Interpretation   | 20   | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.                                          |        |
| Generalizability | 21   | Discuss the generalizability (external validity) of the study results.                                                                                                                                          |        |

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.