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

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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 $\log_2$ 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.

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Kidney transplantation is the criterion 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 in 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 nonimmune mechanisms that—over a background of donor and recipient risk factors—enclose potential hazards for adverse graft end points.[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 tubular 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 higher urinary excretion of kidney damage biomarkers and with increased risk of ESKD and renal replacement treatment.[7,14,18–23]

Better detection techniques allowing the 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—nonoccupational—exposure to heavy metals. Cadmium, in particular, has been associated with increased risk of CKD even at low levels of exposure.[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 nontoxic.[24–26] Kidney transplant recipients (KTRs) are chronically exposed to oxidative stress due

Table 1 | Baseline characteristics of 672 kidney transplant recipients

Characteristic	Tertiles of plasma cadmium concentrations			P_{trend}
	Tertile 1 (≤48 ng/l) (n = 224)	Tertile 2 (48–68 ng/l) (n = 222)	Tertile 3 (≥69 ng/l) (n = 226)	
Demographics and anthropometrics				
Age, yr	48 ± 14	54 ± 12	56 ± 11	<0.001
Sex, male	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	101 (45)	94 (42)	72 (32)	
Former	90 (40)	88 (40)	107 (47)	
Current	21 (9)	27 (12)	32 (14)	
Alcohol use				0.32
0 g/d	18 (8)	27 (12)	30 (13)	
0–10 g/d	123 (55)	127 (57)	119 (53)	
10–30 g/d	43 (19)	44 (20)	44 (20)	
>30 g/d	15 (7)	5 (2)	10 (4)	
Systolic blood pressure, mm Hg	134 ± 17	136 ± 16	137 ± 19	0.18
Diastolic blood pressure, mm Hg	83 ± 11	83 ± 11	82 ± 11	0.60
Use of antihypertensive medication	187 (84)	197 (89)	208 (92)	0.02
Dietary intake				
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	111 (70–146)	118 (73–166)	122 (76–173)	0.29
Vegetables, g/d	80 (56–116)	80 (48–124)	75 (53–107)	0.54
Fruits, g/d	100 (48–189)	110 (53–197)	104 (39–186)	0.22
Legumes, g/d	29 (14–48)	30 (18–45)	31 (17–43)	0.88
Nuts, g/d	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	13 (7–21)	16 (6–23)	13 (6–24)	0.85
Kidney function and transplant history				
eGFR, ml/min per 1.73 m^2	60 ± 19	52 ± 18	45 ± 19	<0.001
Proteinuria	43 (19)	50 (23)	57 (25)	0.31
Urinary protein excretion, g/24 h	0.15 (0.02–0.28)	0.19 (0.02–0.35)	0.21 (0.02–0.45)	0.01
Dialysis vintage, mo	20 (5–43)	25 (10–48)	30 (11–55)	0.001
Transplant vintage, yr	7 (3–13)	5 (1–12)	5 (1–10)	0.003
Acute rejection	53 (24)	64 (29)	60 (27)	0.46
Cold ischemia time, h	13 (2–21)	16 (3–21)	15 (3–21)	0.09
Warm ischemia time, min	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	133 (59)	150 (68)	158 (70)	0.05
Primary kidney disease				0.40
Glomerulosclerosis	70 (31)	61 (28)	60 (27)	
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	110 (49)	136 (61)	139 (62)	0.01
Use of proliferation inhibitor	196 (88)	180 (81)	184 (81)	0.12
Corticosteroid dose <10 mg/24 h	95 (42)	97 (44)	84 (37)	0.35
Liver function parameters				
ASAT, U/l	21 (18–26)	22 (19–27)	22 (18–27)	0.09
ALAT, U/l	19 (14–26)	19 (14–26)	18 (14–26)	0.93
Alkaline phosphatase, U/l	66 (51–81)	67 (55–85)	68 (54–91)	0.06
GGT, U/l	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	1.7 (1.2–2.2)	1.6 (1.2–2.1)	1.8 (1.3–2.7)	0.08

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Table 1 | (Continued)

Characteristic	Tertiles of plasma cadmium concentrations			P_{trend}
	Tertile 1 (≤48 ng/l) (n = 224)	Tertile 2 (48–68 ng/l) (n = 222)	Tertile 3 (≥69 ng/l) (n = 226)	
Diabetes and glucose homeostasis				
Diabetes	41 (18)	58 (26)	64 (28)	0.03
Glucose, mmol/l	5.2 (4.7–5.8)	5.2 (4.8–6.1)	5.3 (4.8–6.2)	0.09
HbA_{1C}, %	5.9 ± 0.7	6.0 ± 1.0	6.1 ± 0.8	0.04
Markers of tubular toxicity				
uEGF, ng/ml	5.45 (2.99–8.13)	3.99 (2.16–7.21)	3.57 (1.47–7.26)	<0.001
uLFABP, ng/ml	0.65 (0.27–2.11)	0.91 (0.43–3.13)	1.21 (0.50–5.90)	<0.001

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; HbA_{1C}, glycated hemoglobin; HDL, high-density lipoprotein; HLA, human leukocyte antigen; LDL, low-density lipoprotein; uEGF, urinary epidermal growth factor; uLFABP, urinary liver-type fatty acid binding protein.
Values are presented as mean ± SD, median (interquartile range), or n (%). 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 using the analysis of variance or the linear regression test for continuous variables and using the chi-square test for categorical variables.

to maintenance immunosuppressive therapy, decreased kidney clearance, and other, often co-occurring, prooxidant conditions, such as aging, hypertension, and diabetes.[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,[28] which makes the TransplantLines Food and Nutrition Biobank and Cohort Study[29] ideal for epidemiological studies evaluating whether cadmium—even at relatively low levels—associates with increased risk of adverse long-term kidney graft end points. With a strong body of evidence suggesting that hazardous exposure to cadmium may be susceptible to clinical monitoring and modifiable by nontoxic therapeutic interventions, assessment and characterization of cadmium-associated risk may provide rationale for the development of novel risk management strategies post–kidney transplantation.[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 be exposed to plasma-containing cadmium via diffusion from red blood cells not only 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.[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 (mean age, 53 ± 13 years; 387 [58%] male). The mean estimated glomerular filtration rate (eGFR) was 43 ± 20 ml/min per 1.73 m^2. The median cadmium concentration was 58 ng/l (interquartile range [IQR], 43–75 ng/l). Using cutoff points of 500 and 1500 ng/l for hazardous and toxic concentrations, respectively, a single study subject was observed in each of such categories.[32] A detailed description of baseline characteristics by tertiles of the study population according to plasma cadmium distribution (tertile 1: ≤48 ng/l; tertile 2: 48–68 ng/l; tertile 3: ≥69 ng/l) is given in Table 1.

Cadmium and risk of late graft failure

During a median follow-up of 4.9 years (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 (hazard ratio [HR], 1.89; 95% confidence interval [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) than patients in the lowest tertile (reference group). In multivariable-adjusted analyses, these findings remained materially unchanged (Table 2 and Figure 2).[33]

Effect modification and stratified analyses

Effect modification of the association between plasma cadmium and risk of graft failure is presented in Supplementary Table S1. Aspartate aminotransferase and alanine aminotransferase were found to be significant effect modifiers ($P_{interaction} = 0.003$ and $P_{interaction} = 0.005$, respectively). In subsequent stratified analyses (cutoff point, 25 U/l), we found that the association of plasma cadmium with risk of graft

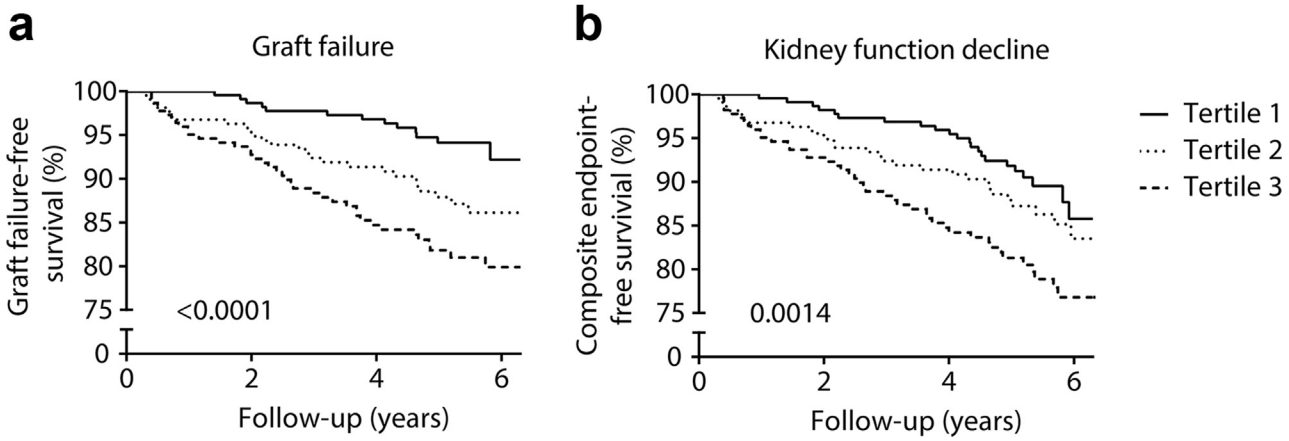

Figure 1 | Kaplan-Meier curve for (a) death-censored graft failure (n_{events} = 78) and (b) kidney function decline (n_{events} = 95) according to tertiles of plasma cadmium distribution. Tertile 1: ≤48 ng/l; tertile 2: 48–68 ng/l; tertile 3: ≥69 ng/l. *P* values were calculated using the log-rank test. *Graft failure* was defined as return to dialysis or retransplantation. *Kidney function decline* was defined as doubling of serum creatinine or graft failure.

failure was significant across both patients' strata; however, KTR with levels of liver enzymes higher than 25 U/l were at a particularly increased risk of graft failure (Figure 3).

Description of extreme outliers

A description of clinical characteristics of extreme outliers is given in Supplementary 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 HR, 3.29; 95% CI, 1.74–6.20, respectively). This finding remained materially unchanged in further multivariable-adjusted

Table 2 | Association of cadmium with risk of graft failure

				Tertiles of cadmium					
	Cadmium per log_2 (ng/l)			Tertile 1	Tertile 2	Tertile 3	Tertile 3[b]	Tertile 3[c]	
	(*n* = 672)			(*n* = 224)	(*n* = 222)	(*n* = 226)	(*n* = 194)	(*n* = 215)	
Model	HR (95% CI)	*P*	e values[c]	Reference	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	e values[c]
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 (1.14–5.41)	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	1.00	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	1.00	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	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

CI, confidence interval; HR, hazard ratio.
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 the 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: ≤48 ng/l; tertile 2: 48–68 ng/l; tertile 3: ≥69 ng/l) in the overall population and without [a]all (*n* = 32) and [b]extreme (*n* = 11) outliers.
[c]e values are calculated for the association estimate (HR) and the limit of the CI closest to the null per doubling of plasma cadmium and for patients in the third tertile of plasma cadmium distribution after the exclusion of extreme outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for estimated glomerular filtration rate, 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, interleukin-2 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).

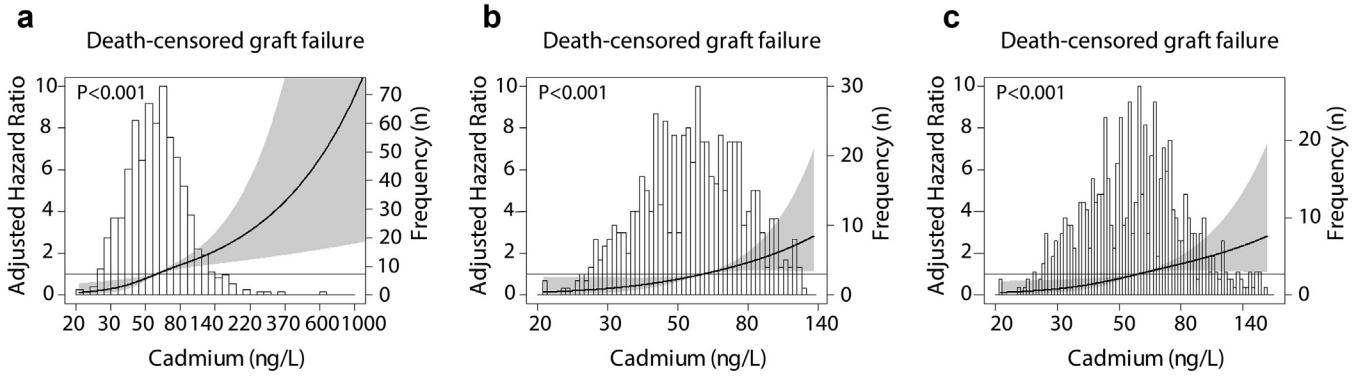

Figure 2 | Association of plasma cadmium concentration with risk of death-censored graft failure in the (a) overall study population, (b) with exclusion of all outliers, and (c) with exclusion of extreme outliers. Data were fitted by Cox proportional hazards regression using median plasma cadmium concentration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

analyses. Table 2 provides e values for the observed coefficient estimate and lower limit of the CI in death-censored and competing risk analyses of graft failure per doubling of plasma cadmium and for patients in the third tertile after the exclusion of extreme outliers. Supplementary Table S8 describes socioeconomic status in a sample population of consecutively enrolled 198 KTR, according to tertiles of plasma cadmium distribution. The association of plasma cadmium with risk of graft failure was independent of socioeconomic status (Supplementary Table S9).

Cadmium and risk of kidney function decline, graft loss, and all-cause mortality

During a median follow-up of 4.9 years (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. Supplementary Table S2 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 the exclusion of outliers (Table 3). Plasma cadmium was also independently associated with graft loss (Supplementary Table S3). The association with all-cause mortality was mainly driven by graft failure (Supplementary Tables S4 and S5).

Serial plasma cadmium levels in a sample population of the TransplantLines Cohort and Biobank Study

In Supplementary Figure S1,[34] we show box plots with the median (IQR) plasma cadmium concentrations of 46 KTRs (mean age, 52 ± 14 years; eGFR, 43 ± 28 ml/min per 1.73 m^2) from the TransplantLines Prospective Cohort and Biobank Study. The 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. The median (IQR)

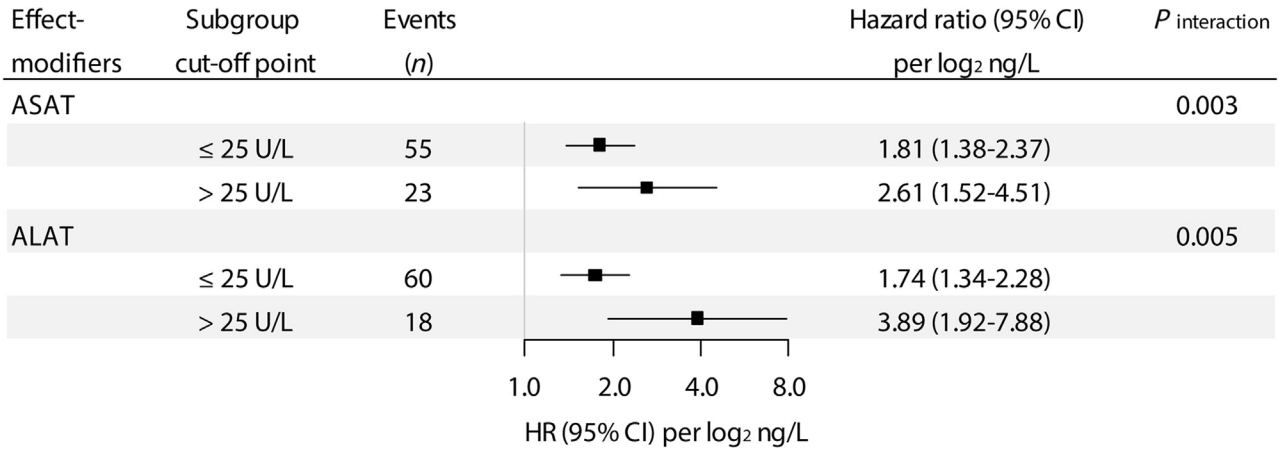

Figure 3 | Stratified analyses of the association of plasma cadmium with risk of graft failure. $P_{interaction}$ was calculated by fitting models that contain both main effects and their cross-product term. The Bonferroni-adjusted significance threshold $P_{interaction} < 0.01$ was considered to indicate the performance of stratified analyses shown hereby. Cutoff points of originally continuous variables were determined to concede clinically meaningful patients' strata. Within each subgroup, hazard ratios (HRs) (95% confidence intervals [CIs]) were calculated per $\log_2$ (ng/l) change in plasma cadmium and adjusted for age and sex. ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase.

Table 3 | Association of cadmium with kidney function decline

	Cadmium per $\log_2$ (ng/l)		Tertiles of cadmium				
			Tertile 1	Tertile 2	Tertile 3	Tertile 3[a]	Tertile 3[b]
	(n = 672)		(n = 224)	(n = 222)	(n = 226)	(n = 194)	(n = 215)
Model	HR (95% CI)	P	Reference	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Crude	1.66 (1.29–2.15)	<0.001	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)	<0.001	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)	0.003	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)	0.004	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)	0.001	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)	0.003	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)	0.006	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)

CI, confidence interval; HR, hazard ratio.
Cox proportional hazards regression analyses were performed to assess the association of plasma cadmium concentration with kidney function decline (n_{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 [a]all (n = 32) and [b]extreme (n = 11) outliers. Multivariable model 1 was adjusted for age and sex. Subsequently, additive adjustment was performed for estimated glomerular filtration rate, proteinuria, primary kidney disease, dialysis vintage, transplant vintage, acute rejection, human leukocyte antigen 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, interleukin-2 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).

intraindividual 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 Supplementary Figure S2,[34] we show that (panel A) plasma cadmium at 3 months post-transplantation was significantly different from plasma cadmium at admission for transplantation (median [IQR], 78 [71–93] and 100 [75–126] ng/l, respectively; $P < 0.001$) and that (panel B) plasma cadmium at transplantation was significantly associated (standardized β = 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 Supplementary Figure S3, we show the association of whole blood cadmium concentration with plasma cadmium concentration (standardized $\beta = 0.52$; $P = 0.001$) in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study. In Supplementary Figure S4, we show the association of (panel A) plasma (standardized $\beta = -0.19$; $P = 0.046$) and (panel B) whole blood (standardized β = 0.07; P = 0.47) cadmium concentrations with eGFR. Plasma but not blood cadmium was significantly associated with eGFR. In further analyses with adjustment for hematocrit, the association between plasma cadmium and eGFR became stronger (standardized $\beta = -0.24$; $P = 0.01$) and the association between whole blood cadmium and eGFR changed toward a nonsignificant inverse association (standardized $\beta = -0.02$; 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 the 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 patients with CKD.[3,4] Particularly in the outpatient kidney transplantation setting, this is the first clinical study describing a prospective association of cadmium with adverse long-term end points. The present 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 enzyme levels. Our results point toward 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 nontoxic 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 present study is the first to investigate the association of plasma cadmium with clinical end points. Most of the previous studies on mammals have measured cadmium in urine or whole blood samples.[15] Our findings 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.[5] After ingestion or inhalation, cadmium is temporarily stored in the liver bound to metallothionein.[5,24] 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.[25] With a kidney half-life of up to 45 years, a buildup of cadmium in the proximal tubule will ensue.[9] 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., nuclear factor κB, activator protein 1 [AP-1], and nuclear factor erythroid 2 [Nrf2]), which play a major role in cadmium-associated kidney pathophysiolology[24] through activation of cell death pathways involving p53, thus linking long-term cadmium exposure with proximal tubular cell apoptosis (human kidney-2 cells)[35] 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-β-D-glucosaminidase, retinol-binding protein, and α_1- and β_2-microglobulin. It is thought that as tubular injury progresses, more generalized tubular dysfunction occurs.[25] Prozialeck *et al.* recently showed that kidney injury molecule-1 outperforms classic biomarkers of cadmium-induced nephrotoxicity.[36] Further studies, and particularly human studies, have shown that urinary kidney injury molecule-1 displays a better dose-response association with long-term low-dose cadmium exposure.[37–40] Although in the present study we show that plasma cadmium strongly correlates with urinary excretion of 2 novel tubular damage biomarkers, that is, epidermal growth factor and liver-type fatty acid–binding protein,[41] future investigations in KTR are warranted to investigate the association of plasma cadmium with urinary excretion of other low-molecular-weight proteins and kidney injury molecule-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.[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.[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 present study is etiological in nature, which needs to be separated from prediction research.[48] Whereas the latter is a distinct field of epidemiological 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.[48] This differentiation is relevant because in both scientific and clinical practice, the 2 kinds of analyses are often confused, reportedly resulting in poor-quality publications with limited interpretability and applicability. We remark that although its observational design does not allow causality assumptions, the present 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 an increased risk of graft failure in KTR.

Previous cohort studies performed in the general population have shown that cadmium is adversely associated with survival.[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. In contrast, 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 underscore 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 in air.[50] The lifelong Dutch dietary intake of cadmium is below the European Food Safety Authority tolerable weekly cadmium intake of 2500 ng/kg body weight.[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.[18] Mining and metal industry countries, for example, China—which is the world's leading country on cadmium production since 2014—have markedly increased patients' cadmium exposure.[51–53] Because of the dose-dependent effect suggested by the results of the present and previous studies, consequences of cadmium-associated kidney tissue injury may likely be more hazardous in such populations,[7,18,28,50,51,53] yet we emphasize that heavy metal exposure–associated CKD risk has been reported across all geographic regions.[54]

Taken together, these findings underscore that clinical monitoring of bodily cadmium concentrations, reduction of environmental exposure, and nontoxic 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, for example, of an *in vivo* X-ray fluorescence technique that using plane polarized X-rays allows a noninvasive 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.[55] Chelation therapy, used in heavy metal poisoning and iron overload syndrome, could henceforth offer an otherwise underestimated therapeutic approach. Lin and coworkers have repeatedly shown that the excretion of lead, a heavy metal with comparable nephrotoxicity to cadmium, can be increased by using calcium ethylenediaminetetraacetic acid chelation, which has been shown to slow progression of ESKD.[56–62] 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 who were sequentially recruited during outpatient visits at our university hospital and then closely monitored by regular checkup 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 present 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 were 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 socioeconomic status (SES) in the whole study population, we provide the results of sensitivity analyses in a sample population of consecutively enrolled 198 KTR to ponder the notion that the association of cadmium with risk of graft failure is independent of SES in Dutch KTR (Supplementary Table S9), which may also be in line with the 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.[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,[34] in which we found low intraindividual variability, indicative of relatively stable plasma cadmium levels over time post-transplantation. This finding additionally underscores that even at low levels, nephrotoxic exposure to cadmium may represent an overlooked hazard for preserved graft functioning. We also acknowledge that our predominantly White 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 in 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 a stronger association 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] Although 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 reverse 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 those 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 before 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 regard to SES and environmental determinants of cadmium exposure.

In conclusion, the present 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 of a particularly strong association between plasma cadmium and risk of kidney graft failure in patients with relatively higher liver enzyme levels may contribute with pathophysiological support to our findings and be clinically relevant to aid in 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 regard to exposure. Whether clinical monitoring of bodily cadmium concentrations, reduction of environmental exposure, and nontoxic 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 at ≥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 eligible KTR (87%) signed informed consent. Patients with a pancreas transplant ($n = 1$) and patients missing plasma cadmium measurements ($n = 34$) were excluded from the present analysis, resulting in 672 KTR, of whom data are hereby presented (a flowchart is shown in Supplementary Figure S5). Additional information can be found in the Supplementary Methods. 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 acute rejection. According to a strict protocol, all patients were asked to collect a 24-hour urine collection sample during the day before to their visit at the outpatient clinic. Blood was drawn in the morning after the completion of 24-hour urine collection. The measurement of clinical and laboratory parameters has been described in the Supplementary Methods and in detail elsewhere.[68] Blood and plasma cadmium concentrations (Supplementary Table S6) were determined with an inductively coupled plasma mass spectrometer (Varian 820-MS, Varian, Palo Alto, CA) using a validated method for the measurement of heavy metals in plasma as detailed in the Supplementary Methods. Information on alcohol consumption and smoking behavior was obtained by using a questionnaire.[69] *Diabetes* was defined as the use of antidiabetics or a fasting blood glucose of ≥7.0 mmol/l. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[70] In the first 198 consecutively enrolled KTR, SES was investigated using a self-report questionnaire at inclusion, categorizing education as described elsewhere[71] according to the International Standard Classification of Education: bachelor, master, or doctorate graduate (level 1); postsecondary or nontertiary 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 from 4 possible categories: short, enough, good, or excellent monthly budget.

As described elsewhere,[72] dietary intake was assessed using a 177-food item–validated semiquantitative food frequency questionnaire developed and updated at Wageningen University.[69] Further information on the food frequency questionnaire can be found in the Supplementary Methods.

Clinical end points

The primary end point of this study was *graft failure*, defined as the requirement of dialysis or retransplantation. 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 end points were chosen to adhere to current recommendations and state of the art in the field.[73–76] For the analyses of graft failure, kidney function decline, and graft loss, patients who died with a functioning graft were censored at the 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.[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 of 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.[34] Cadmium plasma concentrations were determined using inductively coupled plasma mass spectrometry, as described in detail in the Supplementary 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 years (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-section between cadmium concentration in each of these samples and eGFR.

Statistical analyses

Data analyses were performed using SPSS 23.0 for Windows (IBM Corporation, Chicago, IL), GraphPad Prism 7.02 software (GraphPad Software Inc., San Diego, CA), and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). The baseline characteristics of study subjects were described by subgroups of patients according to tertiles of plasma cadmium distribution. Normally distributed variables are expressed as mean ± SD and skewed variables as median (IQR). Categorical variables are

expressed as *n* (number) with percentage (%). Differences were studied using the chi-square test for categorical variables and using linear regression analyses for continuous variables. Variables with skewed distribution, that is, transplant vintage, cold ischemia time, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, low-density lipoprotein cholesterol, triglycerides, and blood glucose, were natural log transformed. A 2-sided *P* value of <0.05 was considered significant.

Analyses for testing difference and calculating intraindividual coefficient of variation for follow-up plasma cadmium levels in KTR of the TransplantLines Cohort and Biobank Study can be found in the Supplementary Methods.

Prospective analyses

In prospective analyses of the primary end point graft failure, a Kaplan-Meier curve and a log-rank test were used 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 the Supplementary Methods and Supplementary Table S7), in which plasma cadmium was $\log_2$ 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 the time of death and by performing competing risk analyses according to Fine and Gray.[33] To illustrate the association of plasma cadmium ($\log_2$ 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 antigen 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; lifestyle-related risk factors (i.e., smoking status and alcohol consumption) in model 4; induction therapy (anti–thymocyte globulin, interleukin-2 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 was 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 the Supplementary Methods) was considered to indicate the performance of stratified prospective analyses. For these analyses, cutoff 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 the Supplementary Methods)[78] 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 the 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.[67] 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.

DISCLOSURE

All the authors declared no competing interests.

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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.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Results.

Table S1. Effect-modification analyses on the association of plasma cadmium with graft failure.

Table S2. Past events and outcomes in 672 outpatient kidney transplant recipients.

Table S3. Association of cadmium with graft loss.

Table S4. Association of cadmium with all-cause mortality.

Table S5. Association of cadmium with all-cause mortality, censored at graft failure.

Table S6. Bias and precision of cadmium measurements.

Table S7. Association of plasma cadmium and risk of graft failure – verification of linearity.

Table S8. Socioeconomic status and plasma cadmium in 198 kidney transplant recipients.

Table S9. Association of cadmium with risk of graft failure in 198 kidney transplant recipients.

Table S10. Sensitivity analyses of the association of cadmium with risk of graft failure, replacing adjustment of donor type by cold ischemia time.

Figure S1. Plasma cadmium concentration of 46 KTR from the TransplantLines Prospective Cohort and Biobank Study,[34] at different follow-up visits post-transplantation.

Figure S2. (**A**) Description and (**B**) linear regression analyses of the association between plasma cadmium at admission for transplantation and at 3-months post-transplantation in 46 KTR from the TransplantLines Prospective Cohort and Biobank Study.[34]

Figure S3. Association of whole blood cadmium with plasma cadmium concentration in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study.[34]
Figure S4. Association of (**A**) plasma and (**B**) total blood cadmium concentrations with eGFR in 116 KTR of the TransplantLines Prospective Cohort and Biobank Study.[34]
Figure S5. Flowchart depicting the phases of inclusion of the study population.

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