Assessment of the filtration reserve capacity of the kidney in workers exposed to cadmium

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Abstract

It has been assessed whether an internal dose of cadmium (Cd), as reflected by a Cd concentration in urine not yet sufficient to induce a significantly increased urinary excretion of various plasma proteins (microproteinuria defined as β_2 -microglobulin in urine $> 300 \mu\text{g/g}$ creatinine, or retinol-binding protein in urine $> 300 \mu\text{g/g}$ creatinine, or albumin in urine $> 15 \text{ mg/g}$ creatinine, or a combination of these), may affect the filtration reserve capacity of the kidney. The last was determined by measuring the difference between the baseline creatinine clearance and the maximal creatinine clearance after an acute oral load of protein (400 g of cooked red meat). In total 215 men were examined of whom eventually 87 Cd exposed workers (concentration of Cd in urine $> 2 \mu\text{g/g}$ creatinine) from zinc/cadmium smelters and 92 control workers (concentration of Cd in urine $< 2 \mu\text{g/g}$ creatinine, absence of microproteinuria, normal fasting serum creatinine) were retained for data analysis performed separately for workers aged less or more than 50 years. Microproteinuria was present in 20 Cd workers, all older than 50. This study confirmed the previous observation that the age related decline of the baseline glomerular filtration rate (GFR) is accelerated in male workers with Cd induced microproteinuria; the same observation was made for the maximal GFR. It was found, however, that a renal Cd burden that had not yet caused microproteinuria did not impair the filtration reserve capacity of the kidney. This study therefore validates the previous estimate of the threshold effect concentration of Cd in urine ($10 \mu\text{g/g}$ creatinine) that is intended to prevent the occurrence of microproteinuria in male Cd workers. It should be kept in mind, however,

that because of the likely interference of the healthy worker effect, this conclusion may not be directly extrapolated to the general population.

In chronic occupational exposure to cadmium (Cd) the kidney (cortex) constitutes the critical organ. The early stage of interference of Cd with renal function is usually characterised by an increased excretion of low molecular weight proteins (for example, β_2 -microglobulin, retinol-binding protein, α_1 -microglobulin) due to tubular dysfunction; in some subjects an increased excretion of high molecular weight proteins (for example albumin and transferrin) because of increased glomerular permeability may be seen.^{1,2} We have previously shown, through several cross sectional studies in adult male workers, that the concentration of Cd in urine (which reflects the renal burden of Cd) above which the risk of occurrence of such microproteinuria significantly increases, is around $10 \mu\text{g/g}$ creatinine.³⁻⁶ We have also shown that Cd induced microproteinuria is irreversible^{7,8} and may lead to an exacerbation of the age related decline in the glomerular filtration rate (GFR).⁸ This explains why some elderly retired Cd workers are suffering from renal insufficiency requiring haemodialysis (unpublished observations).

An important question that remains to be answered is whether an internal dose of Cd, not yet sufficient to induce microproteinuria, may affect the filtration reserve capacity of the kidney. Because of the functional reserve of the kidney, the measurement of the basal GFR might not be sensitive enough to detect renal changes as structural and functional adaptation of nephrons may occur. In other words, adaptive increase in single nephron glomerular filtration rate after total or partial loss of damaged nephrons tends to obscure injury until a considerable amount of kidney parenchyma is irreversibly lost.⁹ This implies that, under normal conditions, the basal GFR is submaximal. If, as has been suggested, glomerulotubular balance is very tightly maintained,⁹ one may postulate that reduction of tubular function (for example, Cd induced tubular proteinuria) may have repercussions on the glomerular level and inversely. Because early changes in glomerular function cannot be detected by the

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measurement of basal GFR, only the application of a test which assesses the filtration reserve capacity of the kidney might allow us to evaluate whether the early renal changes induced by a chemical have any significance for health. Functional (or filtration) reserve of the kidney can be defined as the increment of GFR above the baseline GFR induced by an adequate stimulus (for example, an acute oral load of proteins or infusion of amino acids, glucagon, or dopamine); the maximal GFR obtained during such stimulation represents the maximum filtration capacity.¹⁰⁻¹⁴ For example, in kidney donors and in patients who had had a post-streptococcal glomerulonephritis but whose baseline creatinine clearances were in the normal range, the increased creatinine clearance after a protein load was less than in control subjects; this result was interpreted as an indication of a reduced filtration reserve capacity in the kidney donor and patient groups.¹³ Studies performed on volunteers have shown that the rise in GFR after ingestion of proteins is primarily due to an increased renal blood or plasma flow resulting from a fall in renal vascular resistance.¹⁵⁻¹⁷ The mechanism responsible for this change in renal haemodynamics has not yet been completely elucidated. Several extrarenal and intrarenal mediators (insulin, glucagon, growth hormone, atrial natriuretic peptide, renin, renal prostaglandins) have been considered.^{14,18} It seems, however, that the renal prostaglandins (mainly PGE₂ and 6-keto-PGF_{1α}) play an important part.¹⁹⁻²¹ The local release of prostaglandins may be mediated by renal kallikrein and the kinins generated by it.²² As we have previously shown that Cd workers may exhibit decreased kallikrein activity in urine,²³ it was considered important to assess whether an increased renal Cd burden (as reflected by Cd concentrations in urine), not yet sufficient to modify the urinary excretion of plasma proteins, may already impair the renal filtration reserve capacity. Therefore, in the present investigation the GFR has been estimated in control and Cd workers by measuring the creatinine clearance under baseline conditions and after an acute oral protein load.

Subjects and methods

STUDY POPULATION

The investigation was carried out on 215 male volunteers (107 control and 108 Cd exposed workers) who were currently employed or who had been occupied in two zinc/cadmium smelters in Belgium. The control workers were closely matched for age with the exposed workers and care was taken to see that both groups had similar socioeconomic (education, salary) and environmental (place of residence) characteristics. A detailed occupational and medical questionnaire was given to each participant and

information was also provided on dietary habits during the week before the study. To be included in the final database, participants should have complied scrupulously with the study protocol, they should not regularly take analgesics, and their medical history should not show any pathological condition that might have influenced renal function. Also control workers should never have been occupationally exposed to nephrotoxins, their current concentrations of Cd in urine should be below 2 µg/g creatinine, and they should not exhibit microproteinuria, which is defined as β₂-microglobulin in urine > 300 µg/g creatinine, or retinol-binding protein in urine > 300 µg/g creatinine, or albumin in urine > 15 mg/g creatinine, or a combination of these. The Cd workers should have been uninterruptedly exposed to Cd for at least one year, they should excrete more than 2 µg Cd/g creatinine, and they should not have been exposed to other known nephrotoxins. At the time of the examination the concentration of mercury (Hg) in urine and the concentrations of lead (Pb) and zinc-protoporphyrin in blood ought to be lower than 5 µg Hg/g creatinine, 400 µg Pb/l, and 2.5 µg zinc-protoporphyrin/g haemoglobin (Hb) in both the controls and the Cd exposed workers. In total 36 subjects did not meet these selection criteria (no compliance with the protocol, n = 6; medical history, n = 9; current or previous excessive exposure to lead, n = 10; and subjects intermittently exposed to Cd whose concentration of Cd in urine was < 2 µg/g creatinine, n = 11) so that, eventually, 87 Cd and 92 control workers were retained for data analysis.

The Cd workers were subdivided, on the basis of their age (younger and older than 50) and an assessment of various renal parameters at the time of the survey, into four subgroups—namely, (1) 36 subjects (group E1: mean duration of exposure to Cd 7.8 years) younger than 50, without microproteinuria, and with normal fasting serum creatinine (< 13 mg/l); (2) 31 subjects (group E2a: mean duration of exposure to Cd 14.9 years) older than 50, without microproteinuria, and with normal fasting serum creatinine; (3) 12 subjects (group E2b: mean duration of exposure to Cd 22.3 years) also older than 50, with microproteinuria, but with normal fasting serum creatinine; and (4) eight retired subjects (group E3: mean 12.3 years of removal from Cd exposure after mean duration of exposure to Cd 28.5 years) older than 50, with both microproteinuria and some degree of renal insufficiency (fasting serum creatinine between 13 and 25 mg/l). The control subjects were divided into three subgroups—namely, 47 subjects younger than 50 (group C1), 35 subjects older than 50 (group C2), and 10 subjects (group C3) closely matched for age with group E3. The subjects of subgroup C3 were also retired employees from the plants concerned.

STUDY PROTOCOL

The subjects were expected to be in a fasting state since 2200 the day before the investigation. In each subject, the creatinine clearance was determined before and after a single oral protein load (400 g of cooked lean beef) according to the following protocol adapted from Bosch *et al*¹⁰: at 0830 drinking of 400 ml of water (hydration from 0900 until 1500; 200 ml of water every 30 minutes); at 0900 emptying of bladder and start of the baseline two hour urine collection; at 1000 blood sampling; at 1100 emptying of bladder. Between 1115 and 1145 ingestion of the cooked meat. At noon emptying of bladder and start of the first 90 minute urine collection; at 1245 blood sampling; at 1330 emptying of bladder (end of the first and start of the second 90 minute urine collection); at 1415 blood sampling; at 1500 emptying of bladder (end of the second 90 minute urine collection). This protocol was carried out in the medical departments of the plants and was supervised by the same investigator. Three control and three Cd exposed workers were examined simultaneously. The subjects remained seated and refrained from smoking during the period of examination.

BLOOD AND URINE SAMPLES

Syringes, tubes, and urine containers were previously checked for lack of contamination with heavy metals. The first blood sample (20 ml) was divided as follows: 5 ml was placed in a polypropylene tube (containing 50 µl ethylene diaminetetra-acetic acid Na₂, 10% w/v) for the analysis of Cd and Pb. The remainder was kept in a plain tube for determination of basal serum creatinine. The second and third blood samples (each 10 ml) served to determine the serum creatinine concentrations after the intake of meat. The blood

and serum samples were analysed within 24 hours and subsequently kept at 4°C and -20°C respectively. During the 30 minutes before the baseline test a spot urine sample (100 ml) was collected. An aliquot of 4 ml was immediately transferred to a tube containing 0.4 ml phosphate buffer (1 mol/l, pH 7.6 supplemented with 0.2% NaN₃) and kept at -20°C until the analysis of β₂-microglobulin, retinol-binding protein, and albumin. The remainder of the urine was kept at 4°C for subsequent analysis of Cd and Hg.

BIOLOGICAL ANALYSES

Urinary creatinine concentration was measured by Jaffe's picrate method²⁴ and serum creatinine concentration was determined according to the method of Heinegård and Tiderström²⁵ slightly modified for automation. The urinary concentrations of β₂-microglobulin, retinol-binding protein, and albumin were determined by automated assays relying on latex particle agglutination.²⁶ The determinations of Cd and Pb in whole blood or Cd in urine were carried out by electrothermal atomic absorption spectrometry using STPF techniques coupled with a Zeeman-effect background correction system (Perkin-Elmer Zeeman 3030). The method of external standard line in whole blood matrix was used for the analysis of blood, and for urine the method of standard addition was used. Total Hg in urine was analysed with an automated cold vapour atomic absorption technique.²⁷ Zinc-protoporphyrin concentration in blood was measured with a haemato-fluorimeter (Aviv Associates, Lakewood, NJ).

DATA PROCESSING AND STATISTICAL ANALYSIS

For each subject, three creatinine clearances (baseline, and first and second periods of 90 minutes

Table 1 General characteristics and biological measurements of control (C1) and Cd exposed (E1) workers younger than 50

	Group C1* (n = 47)		Group E1* (n = 36)	
	Mean	Range	Mean	Range
Age (y)	38.6	21.2-49.8	42.5	23.4-49.9
Years of exposure to Cd	—	—	7.8	1.1-14.0
Height (cm)	175.9	165-190	174.4	168-188
Weight (kg)	77.3	57-97	77.2	61-108
Body surface area (m ²)	1.93	1.64-2.14	1.91	1.74-2.26
Blood concentrations:				
Cd (µg/l)†	0.88	0.2-3.1	4.33	1.2-14.7
Pb (µg/l)	137	54-285	189	82-374
Zinc-protoporphyrin (µg/g Hb)	1.1	0.6-2.3	1.2	0.6-1.7
Serum concentration:				
Creatinine (mg/l)	10.0	7.5-12.5	9.8	7.4-12.0
Urine concentrations:				
Cd (µg/g creatinine)†	0.71	0.1-1.76	4.97	2.13-14.69
β ₂ -Microglobulin (µg/g creatinine)†	57	4-214	64	22-185
Retinol-binding protein (µg/g creatinine)†	63	11-145	68	21-178
Albumin (mg/g creatinine)†	3.9	1.9-12.2	5.0	2.0-14.5

*Groups C1 and E1; normal fasting serum creatinine and no microproteinuria.

†Geometric mean.

Table 2 General characteristics and biological measurements of control (C2) and Cd exposed (E2a and E2b) workers older than 50

	Group C2* (n = 35)		Group E2a* (n = 31)		Group E2b† (n = 12)	
	Mean	Range	Mean	Range	Mean	Range
Age (y)	53.5	50.2-60.8	54.0	50.1-63.0	55.5	50.5-63.9
Years of exposure to Cd	—	—	14.9	6.0-31.9	22.3	6.0-36.4
Height (cm)	170.6	161-182	172.9	160-184	171.1	161-185
Weight (kg)	75.9	56-88	79.0	65-98	75.8	66-85
Body surface area (m ²)	1.87	1.59-2.12	1.92	1.76-2.16	1.88	1.75-2.07
Blood concentrations:						
Cd (µg/l)‡	1.03	0.4-2.6	3.16	1.2-8.8	7.51	3.1-18.3
Pb (µg/l)	134	65-298	211	87-366	221	124-375
Zinc-protoporphyrin (µg/g Hb)	1.1	0.6-1.8	1.3	0.7-2.4	1.2	0.7-1.6
Serum concentration:						
Creatinine (mg/l)	10.1	7.4-12.3	10.1	7.7-12.5	9.9	8.2-11.7
Urine concentrations:						
Cd (µg/g creatinine)‡	1.10	0.36-1.93	4.69	2.07-8.81	11.10	5.80-21.66
β ₂ -Microglobulin (µg/g creatinine)‡	67	7-143	63	15-204	298	31-35170
Retinol-binding protein (µg/g creatinine)‡	66	18-188	70	18-228	467	107-7600
Albumin (mg/g creatinine)‡	5.3	2.5-14.5	4.4	2.0-10.0	31.5	4.6-396.1

*Groups C2 and E2a; normal fasting serum creatinine and no microproteinuria.

†Group E2b; normal fasting serum creatinine and microproteinuria.

‡Geometric mean.

after protein load) were computed and normalised for a 1.73 m² body surface area. The filtration reserve capacity of the kidney was estimated by measuring the difference between the maximum creatinine clearance value after protein load (peak period) (PCC) and the baseline creatinine clearance value (BCC); the relative increase of creatinine clearance was expressed in per cent of the baseline value or as the ratio PCC/BCC.

SAS procedures²⁸ were used for statistical analysis. Effects on group means were evaluated by analysis of variance and differences between subgroups were assessed with Duncan's multiple range test. Student's *t* test (two tailed) for unpaired data was

used to determine the significance of differences between subgroup means. A paired *t* test was applied to assess the difference between the baseline creatinine clearance value and the maximum value after protein load within each subgroup. Measurements not normally distributed (Cd in blood, and Cd, β₂-microglobulin, retinol-binding protein, and albumin in urine) were log transformed before application of parametric statistics. Multivariate regression analysis was performed on the total population to trace the determinants significantly affecting the creatinine clearance measurements. The level of *p* = 0.05 was considered as statistically significant.

Table 3 General characteristics and biological measurements of retired Cd exposed workers (E3) and their controls (C3)

	Group C3* (n = 10)		Group E3† (n = 8)	
	Mean	Range	Mean	Range
Age (y)	62.8	53.3-71.9	62.6	53.2-71.8
Years of exposure to Cd	—	—	28.5	19.9-41.7
Height (cm)	171.4	164-179	168.8	161-177
Weight (kg)	79.5	53-99	78.7	64-113
Body surface area (m ²)	1.92	1.57-2.17	1.88	1.74-2.22
Blood concentrations:				
Cd (µg/l)‡	1.61	0.8-2.6	8.21	3.6-16.5
Pb (µg/l)	108	54-171	228	115-377
Zinc-protoporphyrin (µg/g Hb)	1.1	0.6-1.5	1.3	0.8-2.4
Serum concentration:				
Creatinine (mg/l)	10.5	8.9-12.9	16.5	13.4-23.6
Urine concentrations:				
Cd (µg/g creatinine)‡	1.72	1.01-3.65	10.87	6.30-16.68
β ₂ -Microglobulin (µg/g creatinine)‡	45	10-133	7447	485-40260
Retinol-binding protein (µg/g creatinine)‡	48	23-63	4657	773-17624
Albumin (mg/g creatinine)‡	5.0	3.0-13.3	48.6	3.9-209.0

*Group C3; normal fasting serum creatinine and no microproteinuria.

†Group E3; increased fasting serum creatinine and microproteinuria.

‡Geometric mean.

Table 4 Renal filtration reserve capacity measurements in control (C1) and Cd exposed (E1) workers younger than 50

	Serum creatinine (mg/l)		Diuresis (ml/min)		Creatinine clearance (ml/min/1.73 m ²)	
	Mean	Range	Mean	Range	Mean	Range
Group C1 (n = 47)†						
Baseline	10.0	7.5–12.5	7.8	3.5–12.3	127.8**	95–182
Protein load:						
First period	12.2	8.6–15.2	6.4	3.2–10.4	131.9	102–208
Second period	12.0	8.9–15.2	5.8	2.0–10.4	127.3	95–206
Peak period‡	11.9	8.6–15.2	6.2	3.2–10.4	134.7**	104–208
Group E1 (n = 36)†						
Baseline	9.8	7.4–12.0	7.9	3.9–11.8	132.1*	101–169
Protein load:						
First period	11.8	9.3–15.6	6.3	1.5–11.5	135.9	97–192
Second period	11.4	8.3–15.8	5.2	0.9– 7.8	130.3	90–178
Peak period	11.4	8.5–15.6	6.2	1.5–10.9	139.9*	101–192

*p < 0.05; **p < 0.005; paired *t* test between baseline and peak period creatinine clearances.

†Groups C1 and E1: normal fasting serum creatinine and no microproteinuria.

‡Peak period refers to either first or second 90 minute period after protein load in which maximal creatinine clearance is reached.

Results

Tables 1, 2, and 3 give the general characteristics and the results of several biological measurements in the control and the Cd subgroups before the protein load. Analysis of variance did not show significant differences between the Cd subgroups and the corresponding control subgroups with regard to age, height, weight, and body surface area. Although none of the current Pb and zinc-protoporphyrin concentrations in blood exceeded 400 µg/l or 2.5 µg/g Hb, respectively, slight but statistically significant (p < 0.001) increases in concentration of Pb in blood were found in the Cd subgroups in comparison with their corresponding control subgroups, whereas for zinc-protoporphyrin only subgroup E2a showed

slightly higher values than control subgroup C2. Therefore, the possible confounding effect of Pb on the creatinine clearance measurements was taken into account in the multivariate regression analysis (see below). As expected, concentration of Cd in urine, which mainly reflects the body (renal) burden of Cd, increased with age in the control subgroups. Cadmium in blood showed the same trend. Due to environmental contamination by Cd (mainly in the past) of the areas where the zinc/cadmium smelters are in operation, two subjects belonging to subgroup C3 had concentrations of Cd in urine above 2 µg/g creatinine. These subjects, however, had never been occupationally exposed to Cd. As expected, the blood and urinary concentrations of Cd in the Cd sub-

Table 5 Renal filtration reserve capacity measurements in control (C2) and Cd exposed (E2a and E2b) workers older than 50

	Serum creatinine (mg/l)		Diuresis (ml/min)		Creatinine clearance (ml/min/1.73 m ²)	
	Mean	Range	Mean	Range	Mean	Range
Group C2 (n = 35)†						
Baseline	10.1	7.4–12.3	8.1	2.4–14.5	121.8 (NS)	90–186
Protein load:						
First period	12.3	9.9–16.2	6.8	3.3– 9.9	122.3	98–148
Second period	12.0	9.9–16.6	5.4	2.6– 8.8	116.5	90–156
Peak period§	12.1	9.9–16.2	6.4	2.6– 9.9	124.8 (NS)	98–156
Group E2a (n = 31)†						
Baseline	10.1	7.7–12.5	6.7	1.3–10.3	115.9**	89–144
Protein load:						
First period	12.0	9.6–14.8	6.2	2.4–11.4	118.2	83–147
Second period	11.6	8.0–14.1	5.4	2.4– 8.6	118.5	91–177
Peak period	11.5	8.0–14.2	5.8	2.4– 8.8	124.4**	96–177
Group E2b (n = 12)‡						
Baseline	9.9	8.2–11.7	7.9	4.8–12.3	116.3 (NS)	72–151
Protein load:						
First period	12.7	10.7–16.3	6.2	1.8–11.6	110.3	78–137
Second period	12.7	10.7–16.8	5.5	4.1– 7.2	110.2	70–137
Peak period	12.6	10.7–16.3	5.9	4.1– 8.6	113.8 (NS)	78–137

**p < 0.005; paired *t* test between baseline and peak period creatinine clearances. NS = Non-significant.

†Groups C2 and E2a: normal fasting serum creatinine and no microproteinuria.

‡Group E2b: normal fasting serum creatinine and microproteinuria.

§Peak period: see table 4.

Table 6 Renal filtration reserve capacity measurements in retired Cd exposed workers (E3) and their controls (C3)

	Serum creatinine (mg/l)		Diuresis (ml/min)		Creatinine clearance (ml/min/1.73 m ²)	
	Mean	Range	Mean	Range	Mean	Range
Group C3 (n = 10)†						
Baseline	10.5	8.9–12.9	6.3	4.6–10.3	97.2 (NS)	80–120
Protein load:						
First period	12.3	10.0–15.1	7.3	4.2–11.1	98.7	85–115
Second period	11.8	9.5–14.8	5.7	3.9–7.0	99.7	85–119
Peak period§	12.1	10.0–15.1	6.5	3.9–11.1	100.7 (NS)	88–119
Group E3 (n = 8)‡						
Baseline	16.5	13.4–23.6	4.7	2.2–8.0	67.0 (NS)	39–91
Protein load:						
First period	19.7	15.3–28.7	4.9	2.9–6.1	63.8	41–80
Second period	19.6	15.7–28.7	4.1	3.3–5.2	64.5	43–84
Peak period	19.5	15.7–28.7	4.8	3.3–6.1	66.3 (NS)	43–84

Paired *t* test between baseline and peak period creatinine clearances. NS = non-significant.

†Group C3: normal fasting serum creatinine and no microproteinuria.

‡Group E3: increased fasting serum creatinine and microproteinuria.

§Peak period: see table 4.

groups were appreciably increased compared with those of the control subgroups. The mean urinary Cd concentrations in subgroups E1 and E2a (both without microproteinuria) amounted to about 5.0 µg/g creatinine whereas those in subgroups E2b and E3 (both with microproteinuria) exceeded 10 µg/g creatinine. On average, the microproteinuria of subgroup E2b can be considered as moderate, whereas that of subgroup E3 is much more pronounced (tables 1–3).

The results of the renal filtration reserve capacity measurements are summarised in tables 4–6. In each subject the serum creatinine concentration increased after oral protein load. On average, the hydration was maintained at an adequate level throughout the whole examination period as shown by the mean diuresis which exceeded 5 ml/minute in all the subgroups, except in subgroup E3 (with some degree of renal insufficiency) who showed a somewhat lower diuresis. Figure 1 shows the mean values (SEM) of baseline and peak period creatinine clearances for each subgroup. In each age group the Cd workers without microproteinuria and with normal fasting serum creatinine (subgroups E1 and E2a) showed a significant increment of the creatinine clearance after oral protein load (tables 4 and 5). Their relative increase in creatinine clearance amounted on average to 6.9 and 7.6%, respectively, and that of the corresponding control subgroups C1 and C2 5.9 and 3.5% (fig 1). Microproteinuria was found in 20 Cd workers aged more than 50 years. Those presenting with moderate microproteinuria and normal fasting serum creatinine (subgroup E2b) still exhibited a normal baseline creatinine clearance (mean 116.3 ml/min/1.73 m²); however, no rise in creatinine clearance could be found after oral protein load (mean peak period creatinine clearance 113.8 ml/min/1.73 m²) (table 5; fig 1). No increment in

creatinine clearance was found in the retired Cd workers (subgroup E3) presenting with pronounced microproteinuria and some degree of renal insufficiency (mean baseline and peak period creatinine clearances 67 and 66.3 ml/min/1.73 m² respectively). The age matched retired control workers (subgroup C3) had a baseline creatinine clearance still in the normal range (on average 97.2 ml/min/1.73 m²) and their renal filtration could still be slightly stimulated by the protein load (table 6; fig 1).

To identify the determinants which influence the baseline creatinine clearance (BCC), the peak creatinine clearance (PCC), or the ratio PCC/BCC, a stepwise multiple regression analysis was carried out on the total study population (n = 179) with the independent variables: age, concentration of Cd in urine, concentration of Pb or zinc-protoporphyrin in blood, microproteinuria (coded 0 when absent, 1 when present), and the first order interaction terms between age and concentration of Cd in urine, and age and microproteinuria. Table 7 lists all the determinants that statistically correlated with BCC, PCC, or the ratio PCC/BCC, and their partial correlation (R²) and regression coefficients. The results indicate that the slightly different exposure to Pb between the control and Cd exposed workers (especially evidenced by Pb concentration in blood) was not a confounding factor. Baseline and peak creatinine clearances were significantly (*p* ≤ 0.0004) negatively associated with two determinants—namely, age and the interaction term between age and microproteinuria. The ratio PCC/BCC only correlated with this interaction term. Age explained 20 and 28% of the variance of baseline and peak creatinine clearances respectively; the interaction between age and microproteinuria explained 5.5, 8.3, and 2.5% of the variance of BCC, PCC, and the ratio PCC/BCC.

Figure 1 Mean (SEM) of baseline creatinine clearance (open columns) and peak creatinine clearance after protein load (hatched columns) in different subgroups of control and Cd exposed workers. Statistically significant increments in creatinine clearance after protein load are indicated by an asterisk (paired *t* test). Arithmetic mean ($\bar{X}$) for age, geometric mean ($\bar{X}_g$) and range for Cd concentration in urine (Cd-U), - and + for absence and presence of microproteinuria.

Discussion

Because the workers had to be examined in the medical services of the plants, a study protocol had to be developed in which procedures were not too invasive but which was reliable enough to reach the objectives of the study. Experimental conditions that were considered to represent the best compromise were indirect assessment of the GFR through short term measurement of the creatinine clearance in well hydrated subjects and the use of an oral protein load to produce GFR stimulation. The interest and limitations of creatinine clearance to assess the GFR have been extensively discussed.²⁹⁻³⁴ Short term measurement of endogenous creatinine clearance under adequate hydration can be considered as a reliable test to estimate baseline GFR.³³⁻³⁷ It might be

argued that an exogenous load of creatinine via cooked meat could result in an increased tubular secretion of creatinine, which could lead to an overestimation of the postprandial GFR. Studies by Boyle *et al*³⁸ have shown that acute intake of red meat protein does cause significant increments in creatinine clearance by the same percentage whether or not tubular secretion of creatinine is blocked with trimethoprim. Furthermore, several well conducted studies on healthy subjects, in whom short term measurements of inulin and creatinine clearances were performed simultaneously after an acute protein load, showed that the pre and postprandial creatinine clearances agreed very well with the respective inulin clearances.^{10 33 34 39} This evidence supports the methodology we have chosen to assess

Table 7 Multiple regression analysis on total study population (control and Cd subgroups combined ($n = 179$))

Dependent variable	Independent variable	Partial regression coefficient	Partial R^2	p Value
Baseline creatinine clearance (BCC)	Age	-0.8000	0.2012	0.0001
	Age. Microproteinuria	-0.3132	0.0549	0.0004
Peak creatinine clearance (PCC)	Age	-1.0032	0.2826	0.0001
	Age. Microproteinuria	-0.4123	0.0834	0.0001
Ratio PCC/BCC	Age. Microproteinuria	-0.0011	0.0248	0.0352

Age in years; creatinine clearance in ml/min/1.73 m²; microproteinuria coded 0 when absent, 1 when present.

the functional reserve capacity of the kidney in healthy control and Cd exposed workers.

The increments in creatinine clearance after protein load (expressed as % increase) found in the present investigation seem slightly lower than those reported elsewhere. This might be because the present population was composed mainly of workers

who regularly had a high protein intake (as inferred from dietary habits). Experimental studies⁴⁰ and controlled investigations in man^{9 10 12} have shown that regular protein intake may lead to an increased baseline GFR.

To facilitate the discussion, fig 2 shows the baseline and peak creatinine clearances of the different control

Cd-U (μ g/g creatinine) $\bar{X}_g$:	0.7	5.0	1.1	4.7	11.1	1.7	10.9
β_2 -m-U (μ g/g creatinine) $\bar{X}_g$:	57	64	67	63	298	45	7447
RBP-U (μ g/g creatinine) $\bar{X}_g$:	63	68	66	70	467	48	4657
Years of Cd exposure $\bar{X}$:	—	7.8	—	14.9	22.3	—	28.5

Figure 2 Mean (SEM) of baseline creatinine clearance and peak creatinine clearance after protein load as a function of mean age for different subgroups of control (C) and Cd exposed (E) workers. Geometric mean ($\bar{X}_g$) for Cd-U, β_2 -microglobulin (β_2 -m-U), and retinol-binding protein (RBP-U) in urine; arithmetic mean ($\bar{X}$) for duration of exposure.

and Cd subgroups separately as a function of age (group mean) along with the main characteristics of the subgroups. In the subjects younger than 50 the baseline and peak creatinine clearances were not significantly different between the control (C1) and Cd exposed (E1) subgroups. This suggests that the filtration reserve capacity of the kidney is not affected in the last subgroup (without microproteinuria) although its average Cd concentration in urine amounted to 5 µg/g creatinine (average duration of exposure about eight years). The results for the subgroups older than 50 (C2, C3, E2a, E2b, E3) clearly show that the age related decline of renal function not only affects the baseline creatinine clearance (this is well known⁴¹), but also the peak creatinine clearance after protein load. The subgroups with a mean age of about 55 (C2, E2a, E2b) are particularly interesting when comparing their baseline and peak creatinine clearances. The Cd subgroup E2a without microproteinuria and a mean concentration of Cd in urine of 4.7 µg/g creatinine (average duration of exposure about 15 years) showed a slightly lower baseline creatinine clearance than the control subgroup C2. Its peak creatinine clearance, however, after protein load reached the same value as that of the control subgroup, thus showing that renal functional reserve still exists in the Cd subgroup E2a. The creatinine clearance response to protein load was different in the Cd subgroup E2b that presented with moderate microproteinuria (mean β_2 -microglobulin and retinol-binding protein concentration in urine < 500 µg/g creatinine) and a mean concentration of Cd in urine of 11.1 µg/g creatinine (mean duration of exposure about 22 years). The Cd subgroup E2b had a baseline creatinine clearance similar to that of the Cd subgroup E2a, but after protein load its creatinine clearance failed to rise and remained significantly lower than that of subgroup E2a without microproteinuria, showing the absence of any functional renal reserve in Cd subgroup E2b. That the Cd workers of subgroup E2b did not yet show a decrease in baseline creatinine clearance does not contradict our previous results from a five year follow up study on ex-Cd workers.⁸ At the start of that longitudinal investigation the ex-Cd workers were, compared with the present subgroup E2b, somewhat older (mean age 59), their body burden of Cd was much higher (mean current concentration of Cd in urine = 22 µg/g creatinine; duration of Cd exposure = 25 years), and their microproteinuria was much more pronounced (mean β_2 -microglobulin and retinol-binding protein concentration in urine > 1500 µg/g creatinine). The characteristics of the subgroup of retired Cd workers (subgroup E3: mean age about 63) resembled more or less those of the ex-Cd workers investigated previously.⁸ They exhibited an average Cd concentration in urine of 10.9 µg/g

creatinine (mean duration of exposure about 29 years), a persistently pronounced microproteinuria (mean β_2 -microglobulin and retinol-binding protein concentration in urine at least 10 times that of subgroup E2b) already associated with some degree of renal insufficiency. As expected, the retired Cd workers of subgroup E3 showed a significantly decreased baseline creatinine clearance compared with that of their matched controls (subgroup C3) and they exhibited no more renal reserve. The multiple regression analysis showed that the age related decline of the baseline creatinine clearance is exacerbated in the presence of Cd induced microproteinuria (table 7). This result agrees with our previous finding.⁸ Furthermore, the present study also shows the same trend for the peak creatinine clearance after protein load.

We have recently reported that urinary kallikrein activity was lower in Cd exposed workers than in control subjects and this reduction was more pronounced in workers with microproteinuria than in those without signs of renal dysfunction.²³ If, as suggested by the study of Bolin *et al.*,²² the transient increase in the GFR after an acute oral protein load results from the increased production of kallikrein by the kidney, the present results indicate that the ability to increase kallikrein production after a protein stress remains sufficient in Cd workers whose increased renal Cd burden did not yet cause microproteinuria. This mechanism might be impaired when microproteinuria has occurred.

In conclusion, our study indicates that as shown previously for the baseline GFR,⁸ the age related decline of the maximal GFR is exacerbated in the presence of Cd induced microproteinuria. The investigation supports our previous estimate of the threshold effect concentration of Cd in urine (10 µg/g creatinine), which is intended to prevent the occurrence of microproteinuria in Cd exposed male workers. This conclusion, however, may not be extrapolated to the general population because we have indications that in an occupationally active male population, the influence of the healthy worker effect may lead to an underestimation of the risk of Cd for other groups of the general population.⁴²

Appendix

CONVERSION UNITS

Cd	1 µg = 8.90 nmol
Pb	1 µg = 4.83 nmol
Creatinine	1 g = 8.84 mmol

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