Renal function and hyperfiltration capacity in lead smelter workers with high bone lead

Harry Roels, Robert Lauwerys, Jozef Konings, Jean-Pierre Buchet, Alfred Bernard, Stuart Green, David Bradley, Wyn Morgan, David Chettle

Abstract

Objective—The study was undertaken to assess whether the changes in urinary excretion of eicosanoids (a decrease of 6-keto-PGF_{1α} and PGF₂ and an increase of thromboxane) previously found in lead (Pb) exposed workers may decrease the renal haemodynamic response to an acute oral protein load.

Methods—The renal haemodynamic response was estimated by determining the capacity of the kidney to increase the glomerular filtration rate (in terms of creatinine clearance) after an acute consumption of cooked red meat (400 g). A cross sectional study was carried out in 76 male Pb workers (age range 30 to 60 years) and 68 controls matched for age, sex, socioeconomic state, general environment (residence), and workshift characteristics.

Results—The Pb workers had been exposed to lead on average for 18 (range 6–36) years and showed a threefold higher body burden of Pb than the controls as estimated by in vivo measurements of tibial Pb concentration (Pb-T) (geometric mean 66 v 21 µg Pb/g bone mineral). The geometric mean concentrations of Pb in blood (Pb-B) and Pb in urine (Pb-U) were also significantly higher in the Pb group (Pb-B: 430 v 141 µg Pb/l; Pb-U: 40 v 7.5 µg Pb/g creatinine). These conditions of chronic exposure to Pb did not entail any significant changes in the concentration of blood borne and urinary markers of nephrotoxicity, such as urinary low and high molecular weight plasma derived proteins (β₂-microglobulin, retinol binding protein, albumin, transferrin), urinary activities of N-acetyl-β-D-glucosaminidase and kallikrein, and serum concentrations of creatinine, β₂-microglobulin, urea, and uric acid. All participants also had normal baseline creatinine clearances (>80 ml/min/1.73 m²) amounting on average to 115.5 in the controls v 121.3 ml/min/1.73 m² in the Pb group. Both control and Pb exposed workers showed a significant increment in creatinine clearance (on average 15%) after oral protein load suggesting that the previously found changes in secretion of urinary eicosanoids apparently has no deleterious effect on renal haemodynamics in the examined Pb workers.

Conclusion—The finding that both base-

line and stimulated creatinine clearance rates were not only significantly higher in the Pb workers but also positively correlated with Pb-T, suggests that moderate exposure to Pb may be associated with a slight hyperfiltration state, which has been found to attenuate the age related decline in baseline creatinine clearance by a factor of two. Although the relevance of this effect for the worker's health is unknown, it can be concluded that adverse renal changes are unlikely to occur in most adult male Pb workers when their blood Pb concentration is regularly kept below 700 µg Pb/l. One should, however, be cautious in extrapolating this conclusion to the general population because of pre-employment screening of the Pb workers for the absence of renal risk factors.

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Longstanding occupational exposure to lead (Pb) may cause chronic nephrotoxic effects consisting mainly in a decline of the glomerular filtration rate possibly leading to end stage renal insufficiency.^{1–4} The intensity of exposure to Pb without adverse effects on the kidney is still uncertain because incipient Pb nephropathy is difficult to diagnose. Most cross sectional studies conducted on Pb exposed workers have rarely reported renal changes among subjects whose lead in blood (Pb-B) did not regularly exceed 600 µg/l (see Gennart *et al*⁵ for review). These findings were recently confirmed by Cárdenas *et al*⁶ who applied a battery of more than 20 potential markers of renal changes to a cohort of 50 workers moderately exposed to Pb (Pb-B ranging from 360 to 650 µg/l; mean duration of exposure 14 years). In this last study, however, changes in the urinary excretion of eicosanoids (decreased urinary excretion of 6-keto-PGF_{1α} and PGE₂—two vasodilators—and an enhanced excretion of thromboxane—a vasoconstrictor) were found to be associated with Pb-B or zinc-protoporphyrin concentration in blood (ZPP-B). As the changes in the urinary excretion of eicosanoids likely reflect a disturbance of their synthesis in the kidney, these results raise the suggestion that moderate exposure to Pb might influence renal haemodynamics. The present study was undertaken to test this hypothesis.

It is known that acute protein consumption

Unit of Industrial Toxicology and Occupational Medicine, Medical School, Catholic University of Louvain, Brussels, Belgium

H Roels
R Lauwerys
J Konings
J-P Buchet
A Bernard

Department of Medical Physics and Biomedical Engineering, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, UK
S Green
D Bradley
W Morgan

Department of Physics and Astronomy, McMaster University, Hamilton, Ontario, Canada
D Chettle

Correspondence to: Professor R Lauwerys, Université Catholique de Louvain, Unité de Toxicologie Industrielle et Médecine du Travail, Clos Chapelle-aux-Champs 30.54, B-1200 Bruxelles, Belgium.

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increases renal perfusion in normal humans leading to a transient hyperfiltration (so-called renal functional reserve).⁷ The mechanism of the increased glomerular filtration rate after an acute protein load has not yet been completely established, but renal production of vasodilatory prostanooids is suspected of playing a part.⁸⁻¹⁰ Therefore we have assessed whether in adult male workers moderately exposed to Pb, the haemodynamic response of the kidney to an oral protein load was different from that found in age matched control subjects. Because examination of the workers had to be carried out in the medical department of a factory, short term measurement of creatinine clearance (Ccr) was considered to be the most practical method for assessing the change in the glomerular filtration rate after acute protein consumption (haemodynamic response = peak (P) Ccr-baseline (B) Ccr). In the context of chronic Pb exposure current Pb-B hardly provides adequate information on the cumulative dose of Pb to the kidneys. Therefore, the Pb body burden of the exposed and control workers was assessed by measuring the concentration of Pb in the tibia (Pb-T) by x ray fluorescence.

Subjects and methods

STUDY POPULATION

Workers exposed to Pb and controls (age range 30 to 60 years) were recruited from the male workforce of a large Pb smelter in Belgium. Exposed workers had to be occupationally exposed to Pb for at least five years and their historical Pb-B should regularly exceed 300 µg Pb/l. Control workers were selected from the same plant but should never have been directly occupationally exposed to Pb. Current or past Pb exposure of some control workers might be higher within this plant, however, than that prevailing in the general environment. Also, the occupational history of eligible participants should not show any excessive exposure to cadmium (Cd), mercury (Hg), or other known nephrotoxins. Furthermore, their medical history should not disclose previous EDTA chelation treatment, analgesic misuse, chronic medication for gout, or any other pathological condition (for example, diabetes, past or present renal disease) that might interfere with renal function, except hypertension (subjects on angiotensin converting enzyme inhibitor treatment were excluded).

In view of the possible intricate relation between hypertension, Pb exposure, and renal function we deliberately included in the study a certain number of Pb workers with a history of hypertension. Care was taken to ensure that both control and Pb exposed groups were age matched and had similar socioeconomic state (education, salary), general environment (place of residence), and workshift characteristics. The final database was composed of 68 control and 76 Pb exposed workers, who may be subdivided into normotensive and hypertensive subgroups. Subjects whose diastolic blood pressure over the past four years never

exceeded 90 mm Hg were classified as normotensive (55 controls and 47 Pb exposed workers), whereas hypertension was defined as having a diastolic pressure in excess of 90 mm Hg or as being on antihypertensive treatment regardless of the measured blood pressure (13 controls and 29 Pb exposed workers). Five control and six Pb exposed workers took antihypertensive drugs mainly consisting of β blockers.

METHODS

Protocol for creatinine clearance and protein induced hyperfiltration measurements

The subjects fasted from 2200 the day before the investigation. In each subject, the Ccr was determined before and after a single oral protein load according to a protocol adapted from Bosch *et al.*¹¹ At 0830 the subject drank 400 ml of water and then 200 ml of water every 30 minutes until 1500; timed urine specimens were collected during three periods: from 0900 to 1100 (baseline Ccr) and after protein load, from 1200 to 1330, and from 1330 to 1500 (first and second period of challenged Ccr); a blood sample was taken in the middle of each of these three periods for the determination of serum creatinine; 400 g of cooked meat (lean beef) was eaten between 1115 and 1145. For each subject the higher of the two postmeat Ccr values was called the peak Ccr, which may occur in either the first or the second period. This protocol was carried out in the medical department of the plant under the supervision of the same investigator. The subjects remained seated and refrained from smoking during the period of examination.

Blood and urine samples

The collection, handling, and storage of the blood samples and the spot urine samples (about 100 ml urine obtained just before 0900) were carried out exactly as described previously.¹² The morning blood sample (at 1000) served also to determine the packed cell volume and the concentrations of ZPP in blood (ZPP-B), Cd in blood (Cb-B) and Pb-B, and to obtain serum. An aliquot of 5 ml spot urine was frozen as soon as possible at -20°C for the assay of kallikrein activity; another aliquot (4 ml) was immediately buffered (for analysis of low and high molecular weight proteins), and the remainder of the spot urine was utilised to determine Cd-U, Hg-U, Pb-U, δ -aminolevulinic acid (δ -ALA), and the activity of N-acetyl- β -D-glucosaminidase (NAG-U).

Biological analyses

Creatinine concentrations in serum and urine were measured with a Technicon RA1000 (Tarrytown, USA). The concentrations of β_2 -microglobulin in urine and serum, and the urinary concentrations of retinol binding protein (RBP), albumin, and transferrin were determined by assays relying on latex particle agglutination.¹³ The determinations of Cd-B and Pb-B and Cd-U were carried out with a Perkin-Elmer Zeeman 3030 atomic absorption spectrometer. More precise information

on the analytical procedures for these analyses and the implementation of internal and external quality control programmes have been described elsewhere.¹⁴ The Pb-U was determined according to the APDC/MIBK extraction method of Zinterhofer *et al*¹⁵ modified for flameless atomic absorption analysis with a Perkin-Elmer 5000 spectrometer equipped with a deuterium background corrector. Total Hg-U was analysed with an automated cold vapour atomic absorption technique¹⁶ involving SnCl₂ for generating elemental Hg. The procedure for the internal quality control of the Pb-U analysis has been detailed previously,¹⁷ and both internal and external quality control programmes were implemented for the analysis of Hg-U.¹⁷⁻¹⁹ The specific measures taken to minimise contamination at the stages of sampling and the analytical procedures have been extensively detailed elsewhere.²⁰ Urinary δ -ALA was quantified by the method of Lauwerys *et al*²¹ and urinary NAG activity (NAG-U) was assayed fluorimetrically.²² Urinary kallikrein activity was determined by a colorimetric method with the chromogenic tripeptide S-2266 (Kabi Vitrum; Stockholm, Sweden).²³ ZPP-B was measured with a haematofluorimeter (Aviv Associates, Lakewood, NJ, USA). The serum γ -glutamyl transpeptidase (γ -GT) activity and the activity of urea and uric acid in serum were measured by automated techniques with the analysis system ABA-100 (Abbott Laboratories, Irving TX, USA).

In vivo measurement of Pb in the tibia

The concentration of tibial Pb (Pb-T) was determined with the K shell x ray fluorescence (XRF) approach developed by the group at Birmingham University and the Queen Elizabeth Medical Centre, UK.²⁴⁻²⁶ The individual measurement precision attained for the examined cohort (control and exposed workers) ranged from ± 4.9 to ± 14.2 $\mu\text{g Pb/g}$ bone mineral at one standard deviation (SD) with a mean SD of ± 7.6 ($n = 131$); hence the practical detection threshold (2 SD) of the measurement system was 15.2 $\mu\text{g Pb/g}$ bone mineral. The measurements were carried out on control and Pb exposed workers who were scheduled every 30 minutes in an alternating order of sequence. Informed written consent was requested for this measurement from control and Pb exposed workers of whom 61 and 70 respectively, agreed to participate. The study protocol was approved by the medical ethics committee of the Catholic University of Louvain.

Data processing and statistical analysis

The creatinine clearances were normalised for 1.73 m² of body surface area. The difference in Ccr value ($\Delta\text{Ccr} = \text{haemodynamic response}$) between the postmeat PCcr and the BCcr was calculated; the relative increase of Ccr was expressed in % of BCcr or as the ratio PCcr/BCcr.

The intrinsic uncertainty associated with the present in vivo tibial Pb measurement system did not allow precise determinations

below 15.2 $\mu\text{g Pb/g}$ bone mineral (see earlier); therefore the Pb-T results recorded less than this value in 13 control workers (between -9.2 and 14.7 $\mu\text{g Pb/g}$ bone mineral) were replaced for statistical calculations by the variance weighted mean (Pb-T) of these 13 values, being 5.4 $\mu\text{g Pb/g}$ bone mineral ($\text{Pb-T} = \sum_{i=1}^n (\text{Pb-T}_i)/\sigma_i^2 / \sum_{i=1}^n (1/\sigma_i^2)$; where Pb-T_i = individual tibia Pb concentration, σ_i = uncertainty associated with Pb-T_i, $n = 13$).

The Statistical Analysis System (SAS Institute, Cary, NC, USA) for database management and statistical analysis was used. The blood, serum, urine (except urinary flow rate), and Pb-T measurements, and also the Ccr values showed skewed distributions; they were normalised by logarithmic transformation before application of parametric statistics (Student's unpaired *t* test, paired *t* test, Yates' χ^2 test, analysis of variance and Duncan's multiple range test between subgroups, and simple regression analysis). Multivariate regression analyses were performed to identify Pb exposure variables (predictor variables) that correlate with the concentrations of renal markers in serum and urine or with the Ccr measurements (dependent variables). The Pb-B, Pb-U, Pb-T, and ZPP-B were entered separately in the model together with potential covariates such as age, the concentration of Cd-U and serum γ -GT (as an index of alcohol intake), the presence of hypertension (absence 0; presence 1), smoking habits (never smokers 0; others 1), cohort code (control 0; exposed 1), and the first order interaction terms between the presence of hypertension and the concentrations of Cd-U or the four Pb exposure variables. Significant predictors and covariates were traced by a stepwise regression procedure that ended when all the regression coefficients in the model were significant at the 5% level of probability. To avoid collinearities between age and other independent variables, age was centered for 45 years. The level of $p = 0.05$ was considered as statistically significant.

Results

CHARACTERISTICS OF THE WORKERS

Table 1 summarises the general characteristics of the different groups. Control and Pb workers had similar smoking habits: 26% and 24% respectively were never smokers, the others were either current or ex-smokers. At the time of the study the total Pb group showed Pb-B and Pb-U that were on average three to five times higher than those measured in the total control group. The moderate but significant increases of ZPP-B and δ -ALA in urine reflect an interference of Pb with the haem-biosynthetic pathway of the Pb workers. The body burden of Pb estimated by the measurement of Pb-T was on average also about three times higher in the Pb workers than in their controls (table 1). The striking difference between both groups is illustrated by the cumulative frequency distributions of Pb-T (fig 1). The values for Pb-T found for the

Table 1 General characteristics of control and Pb exposed workers

	Controls			Pb Exposed		
	Normotensive (n = 55)§	Hypertensive (n = 13)§	Total (n = 68)§	Normotensive (n = 47)	Hypertensive (n = 29)	Total (n = 76)
Age (y)†	43.0 (9.1) (28.5–60.2)	45.1 (8.7) (30.0–56.4)	43.4 (9.0) (28.5–60.2)	42.3 (8.1) (29.4–55.8)	45.7 (6.8) (29.4–55.3)	43.6 (7.8) (29.4–55.8)
Years of Pb exposure†	—	—	—	15.9 (6.8) (5.9–35.9)	20.8 (7.3) (8.9–36.3)	17.7 (7.3) (5.9–36.3)
Years of employment†	20.8 (9.1) (5.9–40.8)	20.2 (7.1) (6.9–31.5)	20.7 (8.7) (5.9–40.8)	—	—	—
Height (cm)†	175.5 (6.1) (165.0–195.0)	175.3 (6.8) (164.0–186.0)	175.5 (6.2) (164.0–195.0)	175.0 (5.3) (163.5–188.5)	174.4 (6.2) (163.0–188.0)	174.8 (5.6) (163.0–188.5)
Weight (kg)†	76 (11) (54–104)	92 (14) (74–118)	79 (13) (54–118)	76.5 (12) (55–100)	84 (12) (66–113)	79 (12) (55–113)
Body surface area (m ²)†	1.91 (0.14) (1.62–2.31)	2.07 (0.15) (1.90–2.37)	1.95 (0.16) (1.62–2.37)	1.91 (0.15) (1.63–2.26)	1.98 (0.15) (1.74–2.32)	1.94 (0.15) (1.63–2.32)
Blood variables:‡						
Packed cell volume (%)	46.0 (40.2–52.2)	45.7 (41.5–50.8)	45.9 (40.2–52.2)	45.7 (41.2–51.3)	45.7 (40.8–50.8)	45.7 (40.8–51.3)
Pb (µg/l)	139 (63–261)	148 (77–279)	141 (63–279)	466*** (342–679)	378*** (259–566)	430*** (259–679)
Cd (µg/l)	0.6 (0.2–2.1)	0.5 (0.1–2.6)	0.6 (0.1–2.6)	1.4*** (0.4–5.9)	1.0** (0.3–3.3)	1.2*** (0.3–5.9)
ZPP (µg/g haemoglobin)	1.1 (0.7–2.1)	1.1 (0.8–1.4)	1.1 (0.7–2.1)	3.3*** (1.2–11.9)	2.5*** (1.0–17.9)	3.0*** (1.0–17.9)
Serum γ GT (IU/l)	23 (8–135)	39 (14–305)	25 (8–305)	27 (9–352)	34 (13–87)	29 (9–352)
Urine variables:‡						
Pb (µg/g creatinine)	7.2 (2.8–26.2)	9.0 (4.1–18.4)	7.5 (2.8–26.2)	43.9*** (10.0–113.5)	33.5*** (15.3–104.5)	39.6*** (10.0–113.5)
Cd (µg/g creatinine)	0.52 (0.16–1.23)	0.57 (0.19–1.51)	0.53 (0.16–1.51)	1.10*** (0.29–2.54)	0.95** (0.37–1.98)	1.04*** (0.29–2.54)
δ-ALA (mg/g creatinine)	3.0 (1.5–5.3)	2.8 (2.1–4.6)	3.0 (1.5–5.3)	4.8*** (2.1–19.4)	3.6* (2.0–7.8)	4.3*** (2.0–19.4)
Pb-T (µg/g bone mineral)‡	21.7 (<15.2–69.3)	20.2 (<15.2–52.9)	21.4 (<15.2–69.3)	64.0*** (19.6–167.1)	69.0*** (21.7–162.3)	65.8*** (19.6–167.1)

*p < 0.05; **p < 0.01; ***p < 0.001 v respective control group (Student's *t* test).

†Arithmetic mean (SD) (range) and ‡geometric mean (range) are shown.

§Serum γ-GT: normotensive n = 42, hypertensive n = 10, total n = 52.

||Pb-T: controls (normotensive n = 49, hypertensive n = 12, total total n = 61); Pb exposed (normotensive n = 44, hypertensive n = 26, total n = 70).

exposed group were generally higher than those encountered in other cross sectional surveys of industrially exposed populations.²⁶ As a result, this population is well suited to the investigation of the effects of Pb on renal function.

Biological indices of exposure to Hg or Cd indicated that none of the workers (either control or Pb exposed) was ever excessively exposed to these metals. For Cd, a slightly higher exposure to this metal was found in the

Pb workers as reflected by their concentrations of Cd-U and Cd-B. Their values were, however, within the limits of distributions usually found for groups from the population at large in Belgium. Hg-U never exceeded 10 µg Hg/g creatinine.

MARKERS OF NEPHROTOXICITY

The status of renal function was evaluated just before the protein induced hyperfiltration protocol started. Except for NAG-U the mean results for the nine other renal markers did not differ significantly between the exposed and control workers (table 2). Fasting serum concentrations of creatinine and β₂-microglobulin were normal (<13 mg/l or <2 mg/l respectively) in all subjects; the serum concentrations of urea and uric acid were also normal (<481 mg/l and <75 mg/l, respectively) apart from a few marginally increased results. No significant difference was found between the four subgroups for serum urea, but serum uric acid tended to be somewhat higher in the hypertensive subgroups possibly due to the combined effect of diuretic consumption and a reduced renal excretion of uric acid in hypertension.²⁷ The prevalences of increased urinary values of high (albumin, transferrin) and low (β₂-microglobulin, RBP) molecular weight proteins did not differ significantly between the Pb exposed and control group (χ², p > 0.05) (table 2). The four subgroups did not differ as to their mean urinary excretion of β₂-microglobulin, RBP, and transferrin. Albuminuria in the hypertensive control subgroup was, however, significantly higher than that in the other three subgroups. NAG-U was significantly higher in

Figure 1 Cumulative frequency distributions of Pb in tibia (Pb-T) of control and Pb exposed workers. Thirteen control workers had Pb-T concentrations below the minimal detectable concentration (MDC) of 15.2 µg Pb/g bone mineral.

Table 2 Bloodborne and urinary markers of renal function in controls and Pb exposed workers

	Controls			Pb Exposed		
	Normotensive (n = 55)†	Hypertensive (n = 13)‡	Total (n = 68)‡	Normotensive (n = 47)	Hypertensive (n = 29)	Total (n = 76)
Serum†						
β_2 -Microglobulin (mg/l)	1.31 (0.92–1.95)	1.23 (1.01–1.96)	1.29 (0.92–1.96)	1.27 (0.97–1.86)	1.32 (1.00–1.87)	1.29 (0.97–1.87)
Creatinine (mg/l)	9.7 (7.8–12.8)	9.0 (8.1–10.0)	9.5 (7.8–12.8)	9.1 (6.9–10.7)**	9.4 (7.3–11.5)	9.2 (6.9–11.5)
Urea (mg/l)	324 (233–486)	316 (248–414)	322 (233–486)	297 (159–503)	336 (208–464)	311 (159–503)
Uric acid (mg/l)	54 (38–81)	61 (43–78)	55 (38–81)	51 (33–82)	57 (40–90)	53 (33–90)
Urine†						
Albumin (mg/g creatinine)	4.9 (0.9–21.3)	9.2 (2.0–350.4)	5.6 (0.9–350.4)§	4.8 (1.5–33.0)	5.8 (1.4–25.4)	5.2 (1.4–33.0)§
Transferrin (μ g/g creatinine)	451 (137–3768)	648 (65–25080)	483 (65–25080)	405 (35–7978)	472 (38–3093)	429 (35–7978)
β_2 -Microglobulin (μ g/g creatinine)	74 (9–342)	87 (20–840)	77 (9–840)	62 (10–195)	75 (6–192)	67 (6–195)
RBP (μ g/g creatinine)	66 (16–185)	83 (42–376)	69 (16–376)††	71 (13–354)	63 (21–132)	68 (13–354)††
NAG (IU/g creatinine)	1.07 (0.30–2.84)	1.15 (0.37–3.16)	1.09 (0.30–3.16)	1.38 (0.57–4.91)**	1.16 (0.6–3.47)	1.29 (0.57–4.91)**
Kallikrein (U/g creatinine)	0.58 (0.12–1.83)	0.49 (0.11–1.16)	0.56 (0.11–1.83)	0.53 (0.12–1.96)	0.60 (0.15–2.72)	0.55 (0.12–2.72)

*p < 0.05; **p < 0.01 v the respective control group (Student's *t* test).

†Geometric mean (range) is shown.

‡Urea in serum and uric acid in serum: normotensive n = 42, hypertensive n = 10, total n = 52.

§Control/exposed: 7/6 values > 15 mg/g creatinine.

|| C/E: 5/7 values > 1750 μ g/g creatinine.

¶ C/E: 2/0 values > 300 μ g/g creatinine.

†† C/E: 1/1 values > 300 μ g/g creatinine.

the normotensive Pb subgroup and in the total Pb group, whereas the prevalence of increased NAG-U values (>2.20 IU/g creatinine) was not significantly different between the control (4/68) and exposed groups (11/76). Neither the activity of urinary kallikrein (an enzymic marker of the distal tubule) nor the prevalence of abnormally low values of activity were different between the groups. The results did not change when two control and three Pb exposed workers with low natriuria (<35 mmol/g creatinine) were ignored (low sodium intake may cause a pronounced increase of this enzyme).

Stepwise multiple regression analysis on merged subcohorts (controls + exposed) showed that none of these 10 renal markers was related to any Pb exposure variable and that Cd-U was the only predictor variable that

significantly correlated with NAG-U (n = 128, *r* = 0.44, *p* < 0.0001). Cohort code did not emerge as significant covariate of NAG-U, indicating that NAG-U was only associated with the renal burden of Cd (reflected as Cd-U) irrespective of whether they were controls or Pb workers. Similar correlation characteristics between NAG-U and Cd-U were found for the control group (n = 68, *r* = 0.41, slope = 0.331, *p* < 0.005) and the Pb exposed group (n = 76, *r* = 0.35, slope = 0.401; *p* < 0.001).

CREATININE CLEARANCE AND PROTEIN INDUCED HYPERFILTRATION

Table 3 summarises the results of the protein induced hyperfiltration measurements. During the whole examination period the diuresis was maintained at about 6–7 ml/min on average and was not significantly different between controls and Pb exposed subjects (results not shown). In each subject the serum creatinine concentration slightly increased after the oral protein load. At PCcr it amounted on average to 10.4 and 10.1 mg/l in the control and Pb workers respectively. All the subjects had a normal BCcr (all values >80 ml/min/1.73 m²). On a group basis (total), the Pb workers had a mean BCcr slightly higher than that of their controls (121.3 v 115.5 ml/min/1.73 m²; *p* = 0.04) and their mean PCcr was 9 ml/min/1.73 m² higher (140.6 v 131.6; *p* = 0.004). Likewise, the normotensive Pb workers showed significantly higher BCcr and PCcr than their controls, whereas in the hypertensive workers no difference was found between the control and Pb exposed subgroups. Whatever the group of control or Pb exposed workers, the paired *t* test (on log values) between log PCcr and log BCcr invariably showed a highly significant (*p* < 0.0001) relative increase in Ccr after oral

Table 3 Creatinine clearance rates in control and Pb exposed workers: measurements under baseline conditions and after an acute oral protein load (400 g cooked meat)

	Creatinine clearance† (ml/min/1.73 m ²)			
	Controls		Pb exposed	
Normotensive	n = 55		n = 47	
Baseline	114.2	(81–156)	123.5**	(97–177)
Protein load:				
1st period	126.0	(101–191)	136.8**	(102–182)
2nd period	125.4	(101–194)	135.6**	(99–198)
Peak period	130.2†	(105–194)	143.0†***	(111–198)
Hypertensive	n = 13		n = 29	
Baseline	121.2	(106–156)	117.8	(88–155)
Protein load:				
1st period	135.1	(112–175)	131.9	(96–189)
2nd period	131.0	(110–159)	127.2	(95–157)
Peak period	137.5†	(120–175)	136.8†	(96–189)
Total	n = 68		n = 76	
Baseline	115.5	(81–156)	121.3*	(88–177)
Protein load:				
1st period	127.7	(101–191)	134.9*	(96–189)
2nd period	126.5	(101–194)	132.3*	(95–198)
Peak period	131.6†	(105–194)	140.6†***	(96–198)

*p < 0.05; **p < 0.01; ***p < 0.001 v the control group (Student's *t* test).

†p < 0.0001 v baseline (paired *t* test: log PCcr – log BCcr – that is, log PCcr/BCcr).

‡Geometric mean (range) is shown.

protein load. Neither this relative increase (on average from 13 to 16%) nor the haemodynamic response (on average from 16.0 to 19.6 ml/min/1.73 m²) after protein load were significantly different, however, when comparing the control workers (normotensive, hypertensive, total) with the corresponding Pb exposed workers (table 4).

Table 4 Haemodynamic response and relative increase of creatinine clearance after an acute oral protein load (400 g cooked meat) in control and Pb exposed workers

	Controls		Pb exposed	
Normotensive	n = 55		n = 47	
Δ Ccr(ml/min/1.73 m ²)*	16.0 (11.3)	(-8 to 50)	19.6 (15.9)‡	(-1 to 78)
Ratio PCcr/BCcr†	1.14	(0.95-1.62)	1.16‡	(0.99-1.75)
Hypertensive	n = 13		n = 29	
Δ Ccr(ml/min/1.73 m ²)	16.3 (10.9)	(-5 to 31)	19.1 (16.4)‡	(-9 to 83)
Ratio PCcr/BCcr	1.13	(0.96-1.25)	1.16‡	(0.93-1.78)
Total	n = 68		n = 76	
Δ Ccr(ml/min/1.73 m ²)	16.1 (11.2)	(-8 to 50)	19.4 (16.0)‡	(-9 to 83)
Ratio PCcr/BCcr	1.14	(0.95-1.62)	1.16‡	(0.93-1.78)

*Haemodynamic response = difference between peak creatinine clearance after oral protein load and baseline creatinine clearance; arithmetic mean (SD) (range) is shown.

†Ratio between peak creatinine clearance (PCcr) and baseline creatinine clearance (BCcr); geometric mean (range) is shown.

‡Not significantly different from the control group (Student's *t* test).

Figure 2 Age related decline of baseline creatinine clearance and peak creatinine clearance after acute protein load in control and Pb exposed workers (y axis: log scale).

Table 5 Determinants of creatinine clearance rates under baseline conditions and after protein induced hyperfiltration

Variable†	Baseline creatinine clearance (log value)		Peak creatinine clearance (log value)	
	Relation with		Relation with	
	Pb-B (n = 128)	Pb-T (n = 117)	Pb-B (n = 128)	Pb-T (n = 117)
R ²	0.066	0.126	0.151	0.217
Intercept (antilog)	119	104	138	102
Partial regression coefficient (partial r):				
Log Pb-B (μg/l)	NS	—	NS	—
Log Pb-T (μg/g bone mineral)	—	0.0319 (0.19)*	—	0.0405 (0.24)**
Age - 45 (y)	-0.0019 (-0.26)**	-0.0027 (-0.30)***	-0.0031 (-0.32)***	-0.0035 (-0.35)***
Log Cd-U (μg/g creatinine)	NS	NS	NS	NS
Log serum γ-GP (IU/l)	NS	NS	NS	NS
Presence of hypertension	NS	NS	NS	NS
Smoking habits	NS	NS	NS	NS
Cohort code	NS	NS	NS	NS
First interaction factor				
Hypertension . log Pb-B	NS	—	NS	—
Hypertension . log Pb-T	—	NS	—	NS
Hypertension . log Cd-U	NS	NS	NS	NS

*p < 0.05; **p < 0.01; ***p < 0.001 for partial r.

†Details on variables: see data processing and statistical analysis.

Stepwise multiple regression analysis was performed on merged subcohorts (controls and exposed, distinguished by the categorical variable "cohort code") to identify significant determinants of the BCcr or the PCcr after an oral protein load (table 5). Pb exposure variables (Pb-B, Pb-T, Pb-U, or ZPP-B) were considered separately in the model. Whatever the model, age was, as expected, negatively associated with both Ccr measurements, whereas the haemodynamic response and the ratio PCcr/BCcr were independent of age (results not shown).

The close age matching of the controls and lead workers allowed a sound and direct comparison between both subcohorts for the effect of age on BCcr or PCcr. Simple linear regression analysis showed significant inverse relation between age and BCcr (control: *r* = -0.45, *p* < 0.001; Pb: *r* = -0.23, *p* = 0.05) or PCcr (control: *r* = -0.50, *p* < 0.001; Pb: *r* = -0.27, *p* < 0.05) (fig 2). These age related declines were less pronounced in the Pb subcohort compared with the controls; however, the slope was significantly less negative in the Pb workers only for BCcr (*t* test on slope, *p* < 0.05). Their age related decrease in BCcr over a lifespan of 30 years (from 30 to 60 years of age) was on average half that found in the control workers (0.41 v 0.84 ml/min/1.73 m² per year).

With regard to the Pb exposure variables the stepwise multiple regression analysis showed the same results with Pb-B, Pb-U, and ZPP-B—that is, none of these variables emerged as a predictor of BCcr or PCcr; however, the results were different when Pb-T was introduced as Pb exposure variable. This showed a modest but positive and statistically significant association with BCcr or PCcr. Depending on the biological exposure variables (Pb-B or Pb-T), Cd-U or serum γ-GT was also positively associated with PCcr. Cohort code did not emerge as a significant covariate of BCcr or PCcr, indicating that the association with Pb-T or the lack of association with Pb-B, Pb-U, or ZPP-B are irrespective of whether they were controls or Pb workers.

Discussion

The cohort of Pb workers examined in the present study had a significantly increased internal dose of Pb. Measurements of Pb-T suggest that on average their body burden was three times higher than that of the control subjects. Their current Pb-B was also about three times higher but did not exceed 700 $\mu\text{g/l}$. From the historical records of urinary δ -ALA and Pb-B it may also be inferred that Pb exposure in this smelter only rarely gave rise to Pb-B above 700 $\mu\text{g/l}$ during the past 25 years. We have confirmed our previous findings^{5,6,28} and those of other cross sectional studies²⁹⁻³³ that in adult male workers with long term moderate occupational exposure to Pb, no significant change in the urinary excretion of low or high molecular weight proteins is detected and that serum concentrations of creatinine, β_2 -microglobulin, and uric acid were not affected. It is interesting to note, however, that the Pb-T (median) is on average five times higher in the present group of active Pb smelter workers than in the group studied at a Swedish Pb smelter.³³ The increased mean NAG-U found in the Pb workers is likely caused only by their concomitant slight exposure to Cd as it was already noted in our previous study.⁶ The increased NAG-U shown by other investigators^{29,32} in Pb exposed workers should be interpreted with caution as potential, albeit slight, Cd exposure has usually not been considered as a possible covariate.

The examined population was thus suitable to assess whether in asymptomatic workers, an increased Pb body burden may decrease the renal haemodynamic response to an acute oral protein load. No such effect was detected. This indicates that the changes in urinary prostaglandin secretion detected during our previous study on workers exposed to Pb apparently has no deleterious consequences for renal haemodynamics. In fact, the most intriguing finding in the present study is that both the BCcr and stimulated Ccr were not only significantly increased in Pb exposed workers but also positively correlated with the Pb body burden estimated on the basis of Pb-T. It is unlikely that this finding is fortuitous or due to an uncontrolled bias. Both subgroups of Pb workers (normotensive and hypertensive) were indeed well matched with their corresponding controls for all factors that may exert an influence on renal function and the study was carried out with a protocol that guaranteed exactly the same procedures for all workers. Also, multiple regression analysis on the separate subcohorts supports the contention that the relations between the creatinine clearance rates and Pb-T as shown in table 5 are real and not a product of an artifact produced by merging the control and Pb subcohorts. The data reported here are not the first to show an increased renal filtration capacity in Pb workers. Significantly higher Ccr values have been reported in other studies conducted on moderately exposed male Pb workers^{28,33} or on middle aged adults with documented childhood Pb poisoning.³⁴ A

recent experimental study has also shown that in rats given chronically high doses of Pb, the glomerular filtration rate progressively rose until month 9 then decreased from month 12 when tubulointerstitial nephritis was present.³⁵

One might also argue that Pb-T still represents a potential source of endogenous Pb exposure due to Pb release during bone turnover (for example, the age related rate of radial bone loss in midlife men amounts to 0.45% per year³⁶). The elimination of Pb from the skeleton after occupational exposure to Pb, however, seems to occur rather slowly in retired Pb workers—namely, biological half life of 16 years for finger bone³⁷ or calcaneum and 27 years for the tibia.³⁸ On the other hand, the study by Wittmers *et al*³⁹ showed that Pb concentrations in compact and trabecular bone sites increase until 70 years of age and that beyond this age the cortical bone Pb values seemed to continue to rise, whereas those of trabecular bone declined. In view of this evidence, it seems unlikely that in the absence of other risk factors the age related kinetics of bone Pb may mobilise a sufficient amount of Pb to induce a delayed Pb nephropathy in subjects whose historical occupational exposure can be qualified as moderate. This hypothesis is supported by the finding by Gerhardsson *et al*³³ that the BCcr in a group of 30 retired lead workers (on average: age, 68 years; tibia and calcaneum Pb, 39 and 100 $\mu\text{g Pb/g bone mineral}$; Pb-B, 100 $\mu\text{g Pb/l}$) seven years after cessation of Pb exposure is similar to that of age matched controls whose concentrations of Pb-T, Pb in calcaneum, and Pb-B were three times lower.

In conclusion, the present study suggests that, contrary to our expectation based on the previously noted changes in the urinary excretion of eicosanoids, a moderate exposure to Pb may be associated with a slight hyperfiltration state. This phenomenon significantly attenuates the age related decline of the BCcr in the present Pb cohort (on average by a factor of two) compared with that in the control cohort showing on average a yearly decrease of 0.8 ml/min/1.73 m² for the age range from 30 to 60 years (fig 2). It does not seem likely that this difference between control and Pb workers may be attributable to a potential selection bias operating on the level of the subcohorts examined. Indeed, the pre-employment medical criteria and routine health surveillance programmes were the same for both control and Pb workers. Although the health significance of the Pb induced hyperfiltration effect is unknown, it can be concluded that the results of this study support our previous suggestion that compliance with the European Commission Directive⁴⁰ on Pb exposure (Pb-B < 700 $\mu\text{g/l}$) prevents the occurrence of adverse renal changes in most adult male workers. As male workers are usually screened for the absence of renal risk factors at pre-employment medical examination, however, this conclusion may not necessarily be extrapolated to the general population, in which other factors might increase the renal susceptibility to Pb.⁴¹

Appendix

Conversion of units:

Pb 1 µg	= 4.83 nmol
Cd 1 µg	= 8.90 nmol
Hg 1 µg	= 4.99 nmol
Creatinine 1 g	= 8.84 mmol

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