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Adverse effects of chronic low level lead exposure on kidney function—a risk group study in children

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Abstract

Background. Children have been considered a risk group for lead (Pb) toxicity, mainly because of neuro-physiological or neuro-cognitive deficits following Pb exposure. Blood Pb levels (b-Pb) of 100 μg/l currently have been defined as the lowest adverse effect level. The aim of this study was to compare, with the help of urinary markers, the kidney function of children with b-Pb just above this threshold with that of unexposed children, to assess from a nephrological point of view whether the current threshold is justified and whether children really are a particularly vulnerable risk group in terms of Pb-induced kidney damage.

Methods. In a cross-sectional study, 112 children, either from unexposed areas (controls, $n=50$) or Pb-contaminated areas ($n=62$), the latter partly with a known history of elevated b-Pb, were examined. Twenty nine urinary or serum markers mostly related to the function or integrity of specific nephron segments were determined (e.g. filtered plasma proteins, tubular enzymes, tubular antigens, eicosanoids).

Results. b-Pb were 39 ± 13 μg/l in controls and 133 ± 62 μg/l in exposed children. The main findings were increased excretion rates of prostaglandins and thromboxane B_2, epidermal growth factor, β_2-microglobulin and Clara cell protein in the exposed children. A relationship between b-Pb and the prevalence of values above the upper reference limits was observed.

Conclusions. With the help of urinary markers, nephron segment-specific effects of chronic low-level Pb exposure could be detected in children. The pattern of effects on glomerular, proximal and distal tubular and interstitial markers was similar to that previously observed in adults. The changes, however, occur at lower b-Pb levels than in adults. The current threshold appears to be justified also from a nephrological point of view, and children can indeed be considered a special risk group.

Key words: children; Clara cell protein; EGF; eicosanoids; glomerular and tubular function; lead exposure

Introduction

Exposure to lead (Pb) can cause considerable toxic effects [1–5]. The blood lead level (b-Pb) is considered a valuable marker of Pb exposure. A variety of factors, such as the use of unleaded petrol, unleaded paint or the restricted use of Pb for water pipes, have led to a reduced exposure of large parts of the population over the last decades. Large surveys showed that the mean b-Pb of, for instance, the German adult population dropped from close to 65 μg/l in the 1980s to 45 μg/l in 1990/92 [6]. On average, children have lower b-Pb than adults. Children of 6–14 years of age screened had a mean b-Pb of 33 μg/l [6]. The trend towards lower Pb exposure means that in terms of effects of Pb, the focus should shift from toxic effects of high level Pb exposure, such as anaemia or encephalopathies, to toxic effects of low level Pb exposure.

Children have been considered a special risk group for Pb exposure; they absorb Pb more readily than adults. Neuro-physiological and neuro-cognitive deficits have been associated with a b-Pb as low as 100–150 μg/l [7–9] and alterations of the haematopoetic system with a b-Pb of 150–300 μg Pb/l [10,11]. However, studies also indicate renal involvement in children: the excretion of retinol-binding protein (RBP) was significantly altered in children with a b-Pb >123 μg/l [12]. Thresholds for interventions were

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defined by different agencies. For children, the CDC (Centers for Disease Control and Prevention, Atlanta, USA) or the German Umweltbundesamt (Federal Environmental Agency) currently recommend b-Pb 100 μg/l as a level of concern [9,13].

In adults, urinary markers related to the function or integrity of different nephron segments have been proven to be useful for detecting effects of Pb. An altered excretion of markers such as α_1-microglobulin, *N*-acetyl-β-D-glucosaminidase, tubular brush border antigens, fibronectin, thromboxane B_2 and 6-keto-prostaglandin$_{1\alpha}$ has been observed [3–5].

The aims of the present study were: (i) to apply a set of mainly urinary markers with a certain specificity for the function or integrity of the nephron segments to describe the effects of chronic low level Pb in children; (ii) to assess for children whether the postulated threshold of 100 μg Pb/l blood appears to be justified on a nephrological basis that takes into account the state of the art of urine analysis; and (iii) to assess whether children are a particularly vulnerable risk group in terms of Pb-induced effects on the kidney.

Subjects and methods

Study population

Subjects examined were children from three schools in the area of Katowice, Poland. Children living in the vicinity of lead-producing factories ($n=62$) were considered exposed. Up to 39 of these children had been screened at least once for Pb in blood during a 3 year period prior to the study reported here. Children from the same province but without Pb emission in their neighbourhood ($n=50$) were considered controls. Table 1 summarizes the group characteristics.

Collection of samples

Spot urines (second of the morning) were collected between 8 and 11 a.m. in polypropylene containers (Sarstedt, Nümbrecht, Germany). The material for blood sampling was certified for trace metal determination (LH-Metallanalytik, Monovetten®, Sarstedt, Nümbrecht, Germany). Aliquots of the urine samples, either native urine, supplemented with preserving solutions, or following gel filtration (Columns PD10, Sephadex® G25M, Pharmacia, Sweden), were frozen at -20°C.

Table 1. Characteristics of groups examined

Variable[a]	Controls	Exposed
Number	50	62
Boys/girls[b]	28/22	44/18
Age (years)[c]	9.9±0.4	10.6±1.2
Height (cm)[c]	141±8	140±10
Weight (kg)[c]	34.8±7.2	34.6±8.2
Body mass index[c]	17.5±2.7	17.4±2.5

Number, mean±SD; [a]no significant differences between the groups examined concerning the variables listed; [b]χ^2 test; [c]*t*-test.

Aliquots for the determination of total protein, the fraction of high molecular weight proteins (HMW, molecular weight greater albumin), the fraction of low molecular weight proteins (LMW, molecular weight less than that of albumin), albumin, transferrin, RBP Clara cell protein, α_1-microglobulin, β_2-microglobulin and the brush border antigens CB7, CG9 and HF5 were supplemented with 0.1 ml of 1 M phosphate buffer, pH 7.6, with 0.2% NaN_3 per ml of urine. For the analysis of the eicosanoids 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), thromboxane B_2 (TXB_2) and prostaglandin E_2 (PGE_2) as well as leukotriene E_4 (LTE_4) 1 μl of lysine acetylsalicylate (1% in H_2O) was added per ml of urine. Fibronectin was quantified in samples containing the protease inhibitor Pefabloc® [4-(2-aminoethyl)-benzolsulfonylfluoride hydrochloride, Merck, Darmstadt, Germany] in a final concentration of 1 mM. Aliquots for the determination of α-glutathione-*S*-transferase (αGST) and π-glutathione-*S*-transferase (πGST) were supplemented with a buffer supplied by the manufacturer of the commercial test kits (Biotrin Nephkit α EIA and Biotrin Nephkit π EIA, Biotrin, Ireland). Analyses of intestinal alkaline phosphatase (IAP) and epidermal growth factor (EGF) were performed in urine aliquots containing per ml of urine 50 μl of 1 M imidazole buffer (pH 7.0), with 2% Triton X-100, 20 mM benzamidine, 2000 U/ml aprotinin, 1% NaN_3. γ-Glutamyltransferase (γGT), alanine aminopeptidase (AAP), *N*-acetyl-β-D-glucosaminidase (NAG_{total}), isoform B of NAG (NAG B), laminin, δ-aminolaevulinic acid and creatinine (Cr) were determined in untreated urines.

Analytical methods

Samples from both cohorts under study were examined within the same runs of the analysis. Total protein in the urine samples was determined by the Coomassie blue binding method [14]. Albumin, HMW and LMW were quantified by densitometry of bands stained with Coommassie blue following protein separation on a gradient SDS–PAGE [15]. Software packages used were Gel-Image1.3® and Gel-Scan XL2.0® (Pharmacia, Uppsala, Sweden). Transferrin, β_2-microglobulin, RBP and Clara cell protein were quantified with a latex immunoassay [16]. Immunoassays were applied for HF5, CB7 and CG9, IAP, EGF, fibronectin and laminin [17–21]. For the determination of eicosanoids and LTE_4, a radioimmunoasssay was applied to samples extracted with Sep-Pak C18 cartridges and high performance liquid chromatography [22]. The methods for determination of NAG_{total}, NAG B, γGT and AAP have been published elsewhere [23–26]. αGST, πGST and α_1-microglobulin were determined with commercially available test kits (Biotrin Nephkit α EIA and π EIA, Biotrin, Ireland; synelisa, α_1-microglobulin, Pharmacia, Freiburg, Germany). Creatinine in urine and serum was quantified with a modified Jaffé reaction (Beckman Creatinine Analyzer2, Beckman Instruments, Germany).

Pb and cadmium (Cd) in urine (u-Pb, u-Cd) and blood (b-Pb, b-Cd) was measured by electrothermal graphite furnace atomic absorption spectrometry using a Perkin-Elmer Zeeman 3030 atomic absorption spectrometer. Standards (serum and urine) were supplied by Nycomed (Garching, Germany). The laboratory performing the heavy metal analysis (Centralnym Laboratorium Toksykologii Metali Ciezkich, Miasteczko Slaskie, Poland) takes part in quality control programmes. Zinc protoporphyrin in erythrocytes was determined with a haematofluorimeter (ZPP Meter 210, AVIV Associates, Lakewood, NJ); values were related to g

of haemoglobin (Hb). δ-Aminolaevulinic acid was quantified with standard methods [27].

Calculations and statistics

Excretion of urinary analytes was related to urinary creatinine to compensate for the influence of urine flow rate on excretion rates. Statistical analysis was performed with SPSS 6.1.3 (Chicago, IL). Variables with a skewed distribution (Kolmogorov–Smirnov test) were compared with the Mann–Whitney U test. In the case of a normal distribution, the *t*-test was applied. The upper limit of normal was defined as the 95th percentile of the control group. Differences in the prevalence of values exceeding the upper limit of normal were compared with χ^2 statistics (Fisher's exact test). Correlation coefficients according to Pearson were calculated. $P \leqslant 0.05$ (two-sided) was considered statistically significant.

Results

Biomarkers of exposure

Table 2 summarizes the results on the biomarkers of exposure. The exposed children had significantly higher b-Pb than the controls (133 ± 62 μg Pb/l *vs* 39 ± 13 μg Pb/l). Figure 1 depicts b-Pb levels of both groups as a cumulative frequency and shows that ~70% of the exposed children had levels above the level of concern defined by the CDC or the Umweltbundesamt. Forty-one exposed children had been screened at least once during the period 1992–1994. Only one measurement for a single child had shown a blood level <100 μg Pb/l. Mean b-Pb levels had remained stable during the whole period covered by the screenings (ANOVA, $P > 0.05$). u-Pb was also elevated. u-Cd was slightly elevated when the excretion was related to g of creatinine. Indicators of haemoglobin metabolism (zinc protoporphyrin, δ-aminolaevulinic acid) did not differ between the two groups studied.

Table 2. Markers of exposure in blood and urine at the time of sampling (1995) and blood lead levels of exposed children from 1992 to 1994

Marker of exposure	Controls ($n=50$)	Exposed ($n=62$)
Blood		
b-Pb [μg/l] 1995	39 ± 13	133 ± 62***
1994		174 ± 54 ($n=39$)[a]
1994		208 ± 89 ($n=10$)
1993		210 ± 43 ($n=21$)
1992		182 ± 26 ($n=21$)
b-Cd (μg/l)	0.55 ± 0.18	0.69 ± 0.23***
Zinc protoporphyrin (μg/g Hb)	1.7 ± 0.5	2.0 ± 1.2
Urine		
u-Pb (μg/l)	7.0 ± 3.7	16.4 ± 15.7***
u-Pb (μg/g Cr)	7.7 ± 3.8	27.6 ± 25.6***
u-Cd (μg/l)	0.40 ± 0.24	0.49 ± 0.35
u-Cd (μg/g Cr)	0.43 ± 0.24	0.88 ± 0.72***
δ-Aminolaevulinic acid (mg/g Cr)	3.2 ± 1.3	3.7 ± 1.8

***$P < 0.001$ *vs* controls, *t*-test; [a]number of children of the exposed group screened in previous years.

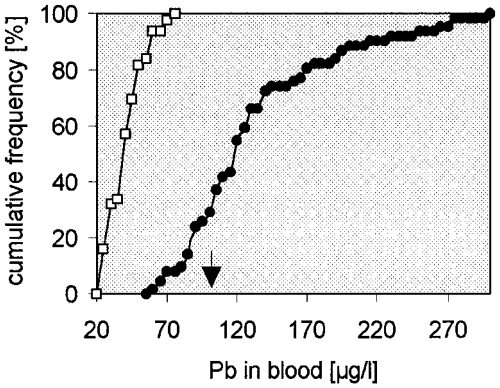


Fig. 1. Cumulative frequency of b-Pb at the time of urine sampling in the exposed (●, $n=62$) and control cohort (□, $n=50$). The arrow indicates the threshold proposed as lowest adverse effect level (100 μg Pb/l) by different health organizations.

Parameters of kidney function determined in serum and general markers of effect in urine

Table 3 shows the parameters determined in serum. Only β_2-microglobulin was elevated in the exposed children. The prevalence of increased results (95th percentile of controls = 3.05 mg/l) was also slightly elevated (16 *vs* 4% among the controls, χ^2 test, $P < 0.05$).The parameters of effect determined in urine (total protein, albumin, laminin, LTE_4) that were not related to a specific nephron segment did not show any differences between the two cohorts (Table 4).

Nephron segment-specific biomarkers

The following markers have a relative—though partly not exclusive—specificity for certain anatomical structures of the nephron and have therefore been called nephron segment specific.

Indicators of glomerular function. 6-Keto-$PGF_{1\alpha}$ and TXB_2 were significantly elevated (Table 4, Figure 2a and b). Both parameters correlated with b-Pb ($r = 0.441$, $P < 0.01$ and $r = 0.225$, $P < 0.025$, respectively). The prevalence of increased results of 6-keto-$PGF_{1\alpha}$ exceeding the upper reference limit of the controls was significantly elevated (24.2%, $P < 0.01$).

When the total exposed population was stratified into three groups according to their b-Pb [<100 μg Pb/l

Table 3. Indicators of kidney function determined in serum

Parameter	Controls ($n=50$)	Exposed ($n=62$)
Creatinine (mg/dl)	0.63 ± 0.10	0.63 ± 0.09
β_2-Microglobulin (mg/l)	1.66 ± 0.80	2.18 ± 0.89**
Clara cell protein (μg/l)	11 (2–107)	11 (5–23)

**$P < 0.01$, *t*-test.

Table 4. Median values (minimum–maximum) of urinary biomarkers

Biomarker	Controls ($n=50$)	Exposed ($n=62$)	P-value[a]
Glomerular markers			
HMW (mg/g Cr)	7 (0–32)	6 (0–52)	n.s.
Transferrin (mg/g Cr)	0.41 (0.07–17.3)	0.83 (0.11–24.3)	<0.05
Fibronectin (μg/g Cr)	220 (0–1243)	189 (45–2316)	n.s.
6-keto-$PGF_{1\alpha}$ (ng/g Cr)	485 (138–1286)	745 (400–3934)	<0.01
TXB_2 (ng/g Cr)	360 (6–847)	471 (120–1307)	<0.01
Proximal tubular markers			
Enzymes			
αGST (μg/g Cr)	6.2 (0.2–58.8)[b]	7.4 (0.5–63.1)	n.s.
AAP (U/g Cr)	11 (5–23)	11 (0–25)	n.s.
γGT (U/g Cr)	47 (16–102)	47 (21–100)	n.s.
NAG_{total} (U/g Cr)	2.1 (0.9–4.8)	1.9 (0.6–8.1)	n.s.
NAG B (U/g Cr)	0.18 (0.03–0.60)	0.13 (0–0.49)	<0.01
IAP (U/g Cr)	0.76 (0.07–2.4)[b]	0.81 (0.13–2.6)[c]	n.s.
Serum-derived proteins			
α_1-Microglobulin (mg/g Cr)	1.1 (0.3–3.1)	1.4 (0.05–17.7)	n.s.
β_2-Microglobulin (μg/g Cr)	37 (4–764)[b]	89 (5–1145)	<0.025
RBP (μg/g Cr)	42 (11–207)	46 (7–183)	n.s.
Clara cell protein (μg/g Cr)	2.8 (1–24.3)[b]	4.2 (1.2–30.2)	<0.025
LMW (mg/g Cr)	2 (0–21)	2 (0–64)	n.s.
Tubular antigens			
CB7 (U/g Cr)	35 (1–128)	37 (1–148)	n.s.
CG9 (U/g Cr)	24 (1–115)	19 (1–151)	n.s.
HF5 (U/g Cr)	32 (1–121)	28 (1–128)	n.s.
Distal tubular markers			
πGST (μg/g Cr)	9.1 (0–151.7)	5.1 (0–534.4)	n.s.
EGF (μg/g Cr)	29 (10–59)[b]	57 (5–160)	<0.001
Collecting duct, interstitial cells			
PGE_2 (ng/g Cr)	242 (10–937)	366 (120–1553)	<0.01
General markers; not primarily related to specific nephron segments			
Total protein (mg/g Cr)	34 (0–127)	34 (0–212)	n.s.
Albumin (mg/g Cr)	6 (0.1–35)	7 (0.3–47)	n.s.
Laminin (μg/g Cr)	0.11 (0–1.09)	0.10 (0–3.08)	n.s.
LTE_4 (ng/g Cr)	317 (14–2627)	366 (34–4210)	n.s.

[a]Mann–Whitney U test; n.s., not significant; [b]$n=49$; [c]$n=61$.

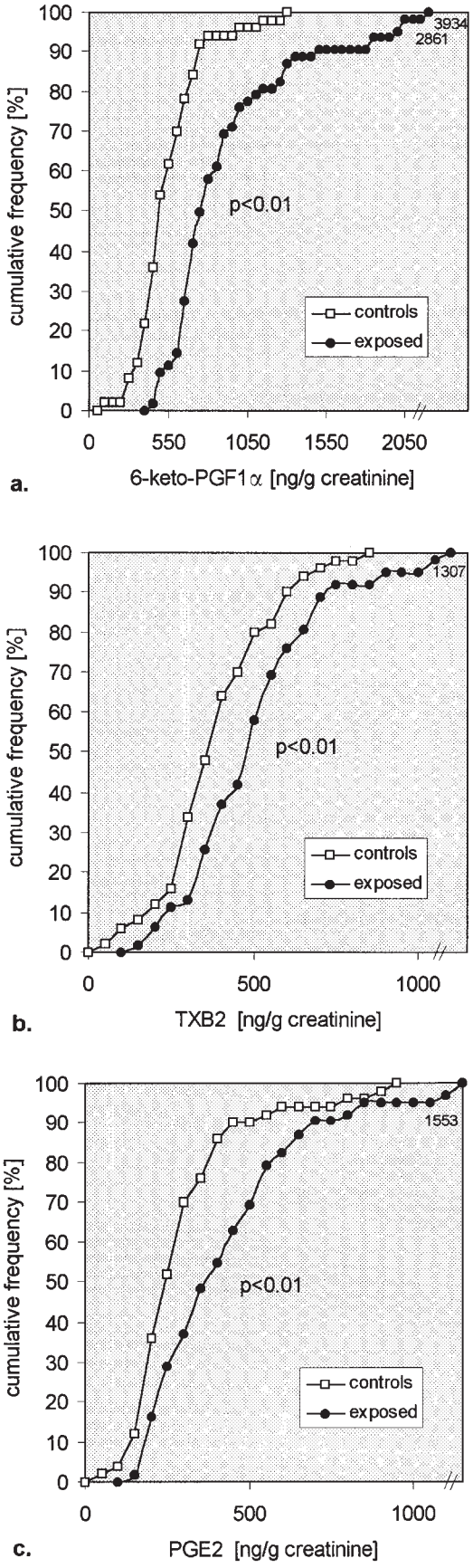
($n=19$), 100–200 μg Pb/l ($n=36$) and >200 μg Pb/l ($n=7$)], an increasing prevalence of abnormal values of 6-keto-$PGF_{1\alpha}$ was observed in the group with higher b-Pb. A corresponding dose-dependent increase could not be observed for TXB_2 (Figure 3).

Transferrin was excreted in higher amounts by the exposed children (Table 4, Figure 4), but no increased prevalence of abnormal values could be observed. There was a weak, though significant correlation with zinc protoporphyrin ($r=0.26$; $P<0.01$). Neither HMW nor fibronectin as glomerular marker appeared to be affected.

Indicators of proximal tubular function. None of the proximal tubular urinary enzymes determined (NAG_{total}, NAG B, αGST, AAP, γGT, IAP) apart from NAG B showed any differences between the two groups studied. NAG B was excreted in lower amounts in the exposed children; no correlation with the parameters of exposure could be observed. CB7, CG9 and HF5 showed no differences among the two groups studied. β_2-Microglobulin and Clara cell protein were elevated in urine from exposed children (Table 4, Figure 5a and b), and both parameters correlated with b-Pb (β_2-microglobulin $r=0.203$, $P<0.05$; Clara cell protein $r=0.261$, $P<0.01$). The prevalences of values exceeding the upper reference limits of controls were elevated for α_1-microglobulin (22.6%, $P<0.01$) and Clara cell protein (21%, $P<0.01$). The other serum-derived low molecular weight proteins were not affected. β_2-Microglobulin is unstable in acid urine [28]. Urinary pH, prior to addition of buffer, was 5.3 ± 0.7 in the controls and 5.4 ± 0.8 in the exposed children. Gender-dependent differences in the excretion of Clara cell protein have been described [29]. There was a higher, though statistically not significant, percentage of boys among the exposed children. However, no influence of gender could be shown with ANOVA and regression procedures.

Indicators of distal tubular function. Excretion of the enzyme πGST was not affected. EGF excretion was significantly ($P<0.001$) elevated in exposed children (Table 4, Figure 6). EGF correlated with b-Pb ($r=0.356$, $P<0.01$), and 59% of the exposed children exceeded the 95th percentile of the controls for EGF ($P<0.001$). A high prevalence of values exceeding the 95th percentile of the controls was found in children with b-Pb from 100 to 200 or >200 μg/l, 72 and 43% respectively (Figure 7).

Indicators of the collecting duct and interstitial cells. PGE_2 as a parameter for the interstitial cells was



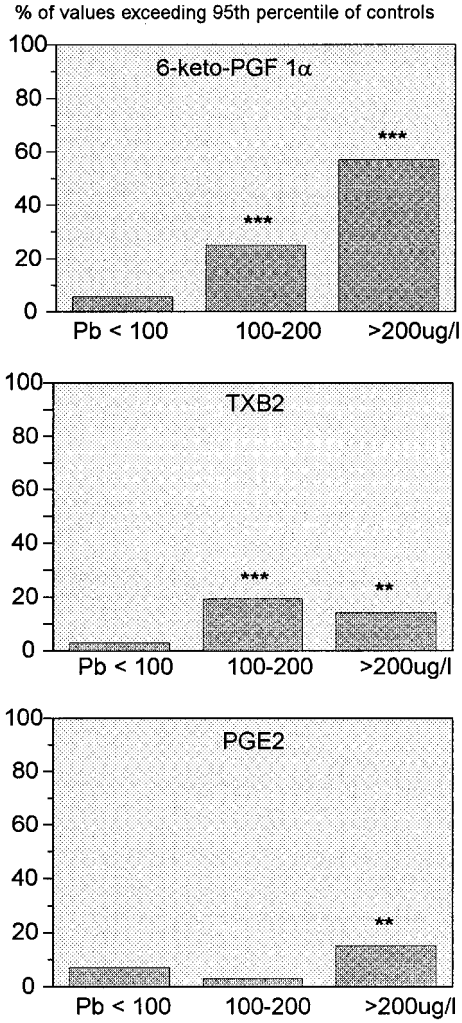


Fig. 3. Prevalence of values exceeding the upper limit of normal of eicosanoids (95th percentiles of the controls: 6-keto-PGF_{1a} 999 ng/g creatinine, TXB_2 684 ng/g creatinine, PGE_2 783 ng/g creatinine) as a function of b-Pb at the time of urine collection. $^{***}P<0.001$, $^{**}P<0.01$, χ^2 test (Fisher's exact test) *vs* group with Pb < 100 µg/l.

elevated (Table 4, Figure 2c). PGE_2 correlated with b-Pb ($r=0.23$, $P<0.05$). In children with b-Pb > 200 µg/l, a significantly higher percentage of children exceeded the 95th percentile of the controls (Figure 3).

Discussion

With the help of urinary biomarkers, the effects of chronic low level Pb exposure could be detected in children. Changes in glomerular and tubular markers (such as eicosanoids, transferrin, Clara cell protein and EGF) were observed. Children showed effects at very

Fig. 2. (**a**–**c**) Cumulative frequency of urinary eicosanoids in Pb-exposed and control children. The *x*-axis has been truncated; figures in the upper right corners denote extreme values that have been cut-off.

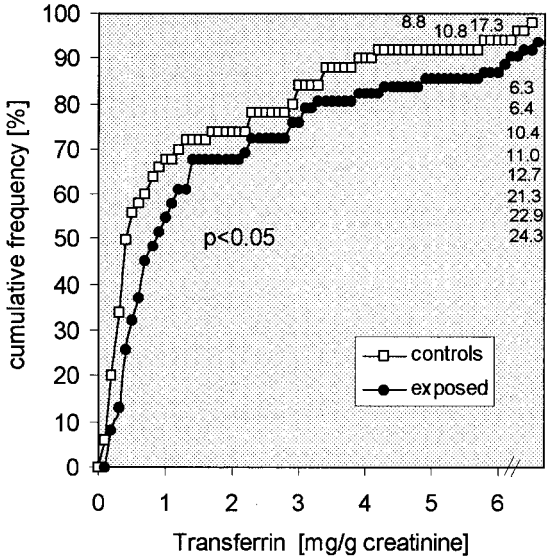


Fig. 4. Cumulative frequency of urinary transferrin in Pb-exposed and control children. The *x*-axis has been truncated; figures in the upper right corners denote extreme values that have been cut-off.

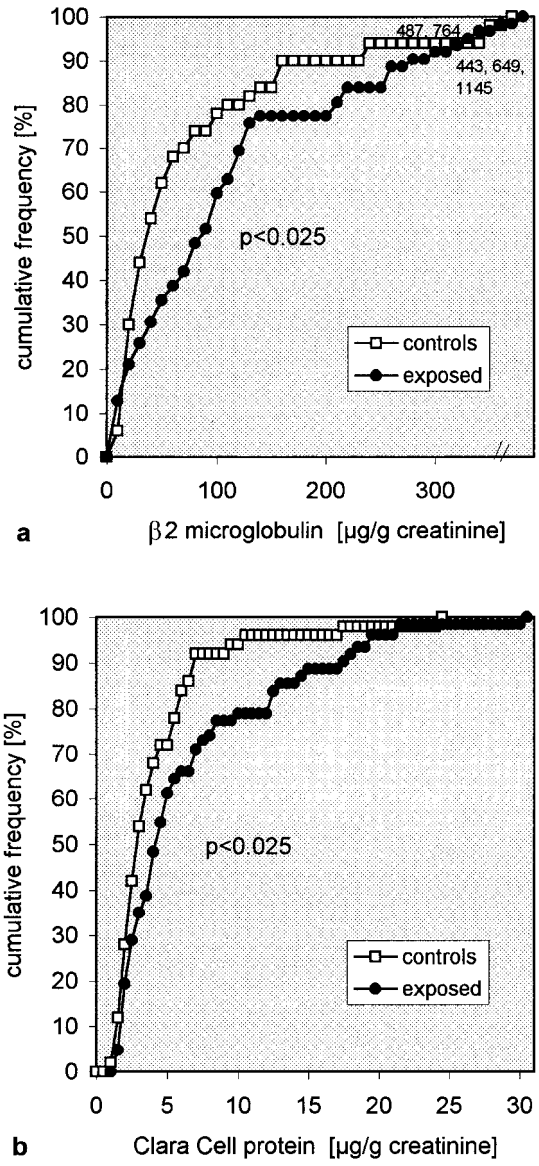


Fig. 5. (**a** and **b**) Cumulative frequency of urinary excretion of β_2-microglobulin and Clara cell protein in Pb-exposed and control children. The *x*-axis has been truncated; figures in the upper right corner denote extreme values that have been cut-off.

low levels of b-Pb. The effects were similar to those previously described for workers with a higher exposure to Pb [3,5]. The observations do not lead to immediate therapeutic interventions, and the relevance of the sub-clinical changes remains a subject of speculation. However, in terms of prevention that attempts to minimize or avoid even subtle effects on kidney function, children can be considered a special risk group, and the current lowest adverse effect level of 100 µg Pb/l blood appears to be justified from a nephrological point of view.

Pb exposure

The exposed children had significant increases in their b-Pb over several years. They also had elevated u-Pb. Pb-induced changes of haemoglobin synthesis could not be observed. This is in accordance with studies showing a threshold for an elevation of zinc protoporphyrin in children at $\sim$250 µg Pb/l blood [30].

b-Cd and u-Cd were modestly, but significantly elevated in the exposed children. b-Pb significantly correlated ($P<0.001$) with b-Cd ($r=0.309$), u-Cd ($r=0.561$) and u-Pb ($r=0.768$). Synergistic effects of Pb and Cd cannot be excluded.

Evaluation of kidney function with markers of effect in serum and urine

Creatinine in serum and total protein were not affected, indicating once more that these parameters—important in clinical routine—are not sensitive enough to reflect subtle changes in kidney function in environmental medicine.

Eicosanoids as urinary markers. As in previous studies on Pb-exposed adults, eicosanoids as urinary markers showed an altered excretion[3,5]. PGE_2, 6-keto-$PGF_{1\alpha}$ and TXB_2, the latter two being stable products of prostacyclin and TXA_2, were elevated. LTE_4, a lipooxygenase metabolite of arachidonic acid, however, was not affected. In the kidney, 6-keto-$PGF_{1\alpha}$ is synthesized mainly in glomeruli and PGE_2 mainly in medullary collecting duct cells [31]. Excretion of 6-keto-$PGF_{1\alpha}$, TXB_2 and PGE_2 was 30–54% higher in the exposed children than in the controls. A higher prevalence of increased results as a function of b-Pb could also be observed (Figure 3).

Among the biological effects of eicosanoids are vasoconstriction and vasodilatation of afferent and efferent blood vessels, and contraction of smooth muscle cells and mesangial cells [32–34]. Due to their short half-life (TXA_2, for example, is metabolized

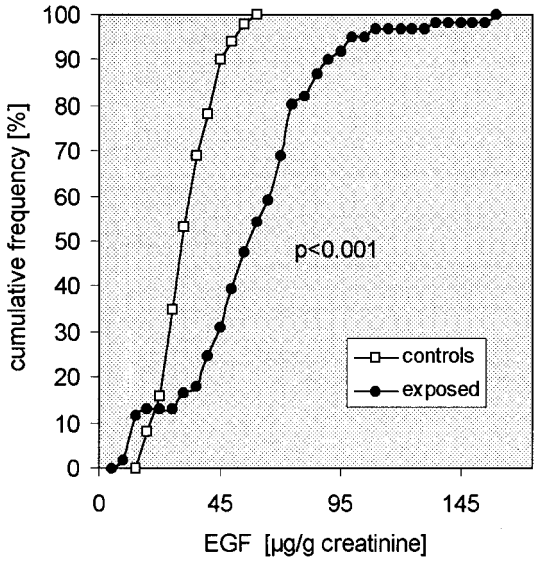


Fig. 6. Cumulative frequency of urinary EGF in Pb-exposed and control children.

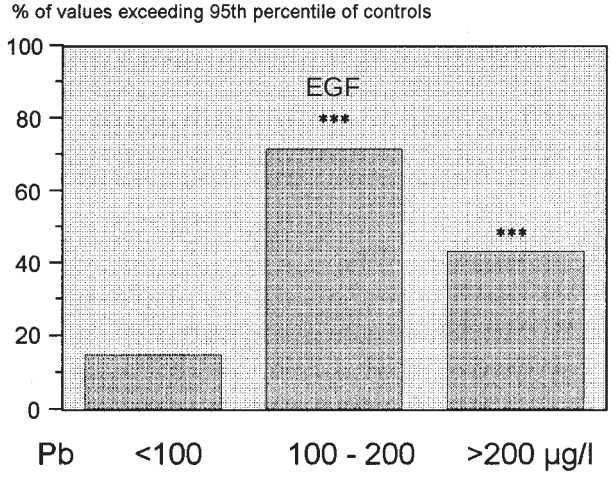


Fig. 7. Prevalence of values exceeding the upper limit of normal of EGF (95th percentile of the controls of 51 µg/g creatinine) as a function of b-Pb at the time of urine collection. ***$P<0.001$, χ^2 test (Fisher's exact test) *vs* group with Pb < 100 µg/l.

within 30s [34]), eicosanoids can be considered to be active mainly at their site of secretion. Under physiological conditions, extra-renal TXB_2 seems be excreted in urine in a modified form [34]. Under pathophysiological conditions, such as hypertension, higher urinary levels of TXB_2 were found in urine [35]. An association of Pb exposure and blood pressure has been discussed [36]. Among the children examined, there were no known cases of hypertension.

Enzymes and renal antigens as markers of proximal tubular function. Neither αGST, AAP, γGT, NAG_{total} nor IAP as markers of proximal tubular function showed any difference between the two groups studied. A recently published study on Pb-exposed children in Romania showed that NAG_{total} and AAP were affected [37]. However, mean b-Pb in those children was 342 µg/l, almost three times higher than in the Polish children.

The isoform NAG B has been considered to be more specific for the proximal tubule; in kidney disease there is an exponential increase in NAG B as part of NAG_{total} [38]. In workers, NAB B was elevated even following low cadmium exposure; no threshold for an increase could be defined [39]. In the Pb-exposed Polish children, NAG B was lower than in the controls (Table 4), but NAG B as a percentage of NAG_{total} was about the same in both groups.

Monoclonal antibodies directed against antigens of the tubular brush border (CB7, CG9, HF5) have been proposed as markers of toxic damage to the kidney of children [40], but did not show alterations in this study. In experimental studies on rats exposed to high doses of Pb, these markers were elevated, together with GST and NAG [41].

Proteins as markers of glomerular and proximal tubular function. β_2-Microglobulin, Clara cell protein and α_1-microglobulin in urine were either significantly elevated or showed a higher prevalence of values exceeding the upper reference limits of the controls (Table 4, Figure 5b and c). β_2-Microglobulin in serum was also elevated in the exposed children (Table 3). Possibly a selective induction of β_2-microglobulin synthesis could explain its increase in Pb-exposed children without any changes in glomerular filtration rate. In this case, β_2-microglobulin in urine would reflect synthetic activity rather than tubular function. In toxicological studies, Clara cell protein (synonym CC16 or protein 1) has been used mainly as a marker of damage to the respiratory tract. Secreted into the blood, Clara cell protein is handled by the kidney like other proteins of low molecular weight. Increases in urinary Clara cell protein concentrations and fractional excretion indicate an impairment of proximal tubular protein reabsorption. Clara cell protein has been considered a more sensitive marker of proximal tubular function than β_2- and α_1-microglobulin and RBP in previous studies on cadmium-induced proteinuria and Chinese herb nephropathy [29].

The protein RBP and LMW as the total amount of proteins with a molecular weight less than that of albumin were not different between the two groups. That RBP was not affected is at variance with a study in Czech children [12] but in accordance with the aforementioned study in Romanian children [37].

The proteins fibronectin and laminin are structural

Table 3. Indicators of kidney function determined in serum

Parameter	Controls ($n=50$)	Exposed ($n=62$)
Creatinine (mg/dl)	0.63 ± 0.10	0.63 ± 0.09
β_2-Microglobulin (mg/l)	1.66 ± 0.80	2.18 ± 0.89**
Clara cell protein (µg/l)	11 (2–107)	11 (5–23)

**$P<0.01$, *t*-test.

proteins that can be of renal origin. In Pb-exposed adults, fibronectin excretion was shown to be reduced [5]. No such effect could be observed in the children.

Markers of distal tubular function. EGF was excreted by the exposed children in significantly higher amounts than by the controls. EGF expression is most pronounced in the distal tubule [42]. Receptors for EGF have been identified in the glomeruli and along the tubules [42,43]. A variety of effects have been attributed to EGF: changes in glomerular haemodynamics, and alteration of water permeability and sodium reabsorption of the distal nephron and cortical collecting duct [42]. EGF induces the synthesis of eicosanoids [44]. Therefore, altered excretion of eicosanoids could be a secondary effect of an elevated EGF release. Previous studies showed decreased excretion rates of EGF following acute kidney failure or kidney transplantation, probably due to ischaemia or drugs, and higher excretion rates in the adaptive phase following uninephrectomy [45,46]. πGST is expressed in the distal tubule [47] and was elevated during acute rejections of transplanted kidneys, whereas the isoform αGST of the proximal tubule was not affected [48].

In the children, about the same pattern of markers (6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2, transferrin, α_1- and β_2-microglobulin, Clara cell protein, EGF) was affected as previously reported for Pb-exposed adults [3–5]. However, the exposed adults had mean Pb levels of 420 or 480 μg/l, their corresponding controls had 70 or 167 μg/l. The exposed children described here had a mean b-Pb of 133 μg/l, their controls 39 μg/l. Higher body burdens of Pb in the so-called unexposed adults can be considered a consequence of a longer duration of Pb accumulation. When the unexposed adults described by Pergande *et al*. [4] and Fels *et al*. [5] were divided into two subgroups, one with low b-Pb one with high b-Pb, no changes in urinary markers comparable with those observed in the children were found.

We conclude that early urinary markers can help to detect subtle alterations in kidney integrity in children. Chronic exposure is followed by changes in all nephron segments. The pattern of changes observed is similar to that of adults, but seems to occur at lower exposure levels. Children, therefore, appear to be a particularly vulnerable risk group, and the threshold of 100 μg Pb/l blood can be considered to be justified not only from a neurological but also from a nephrological point of view.

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