Measurement of vitamin D_3 metabolites in smelter workers exposed to lead and cadmium

S R Chalkley, J Richmond, D Barltrop

Abstract

***Objectives*—To investigate the effects of lead and cadmium on the metabolic pathway of vitamin D_3.**

***Methods*—Blood and urinary cadmium and urinary total proteins were measured in 59 smelter workers occupationally exposed to lead and cadmium. In 19 of these workers, the plasma vitamin D_3 metabolites, (25-hydroxycholecalciferol (25 OHD_3), 24*R*, 25-dihydroxycholecalciferol (24*R*,25$(OH)_2D_3$) and 1α, 25-dihydroxycholecalciferol (1α,25$(OH)_2D_3$)) were measured together with blood lead. Vitamin D_3 metabolites were measured by radioimmunoassay, (RIA), lead and cadmium by atomic absorption spectrophotometry, and total proteins with a test kit.**

***Results*—Ranges for plasma 25$(OH)D_3$, 24*R*,25$(OH)_2D_3$ and 1α,25$(OH)_2D_3$ were 1.0–51.9 ng/ml, 0.6–5.8 ng/ml, and 0.1–75.7 pg/ml, respectively. Ranges for blood lead were 1–3.7 µmol/l, (21–76 µg/dl), blood cadmium 6–145 nmol/l, and urinary cadmium 3–161 nmol/l. Total proteins in random urine samples were 2.1–32.6 mg/dl. Concentrations of lead and cadmium in blood showed no correlation (correlation coefficient -0.265) but there was a highly significant correlation between blood and urinary cadmium. Concentrations for 24*R*,25$(OH)_2D_3$ were depressed below the normal range as blood and urinary cadmium increased, irrespective of lead concentrations. High cadmium concentrations were associated with decreased plasma 1α,25$(OH)_2D_3$ when lead concentrations were <1.9 µmol/l and with above normal plasma 1α,25$(OH)_2D_3$ when lead concentrations were >1.9 µmol/l, Kruskal-Wallis analysis of variance (K-W ANOVA) χ^2=10.3, p=0.006. Plasma 25$(OH)D_3$ was negatively correlated with both urinary total proteins and urinary cadmium, but showed no correlation with plasma 24*R*,25$(OH)_2D_3$, 1α,25$(OH)_2D_3$, blood lead, or blood cadmium.**

***Conclusion*—Continuous long term exposure to cadmium may result in a state of equilibrium between blood and urinary cadmium. Cadmium concentrations in blood could be predicted from the cadmium concentration of the urine, (regression coefficient +0.35 SE 0.077). Exposure to cadmium alone decreased the concentrations of 1α,25$(OH)_2D_3$ and 24*R*,25$(OH)_2D_3$, whereas exposure to both cadmium and lead increased the concentrations of 1α,25$(OH)_2D_3$. It has been suggested that cadmium and lead interact with renal mitochondrial hydroxylases of the vitamin D_3 endocrine complex. Perturbation of the vitamin D metabolic pathway by cadmium may result in health effects, such as osteoporosis or osteomalacia, risks which are possibly increased in the presence of lead.**

(*Occup Environ Med* 1998;**55**:446–452)

Keywords: vitamin D_3 metabolites; lead; cadmium; total proteins; blood; urine; smelter workers

Department of Academic Child Health, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London
S R Chalkley
J Richmond
D Barltrop

Correspondence to:
Professor D Barltrop, Academic Child Health, Imperial College School of Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London SW10 9NH.
Tel: 0044 181 746 8775; fax: 0181 746 8770.

Accepted 12 January 1998

This study sought to investigate the effect of exposure to the heavy metals lead and cadmium on the plasma concentrations of vitamin D_3 metabolites and to determine the effects of dual exposure. The metabolic pathway of vitamin D_3, cholecalciferol, involves the gut, liver, kidneys, and bones, all of which are affected by these metals.[1 2] The liver and kidneys are target organs for lead and cadmium, so that a potential biochemical effect might be found in the vitamin D_3 pathway to explain the changes in bone metabolism found after exposure to lead or cadmium.[3–6] Damage caused by cadmium to the renal tubules[7 8] was considered especially relevant to the formation of the more active dihydroxy metabolites, 24*R*, 25-dihydroxycholecalciferol (24*R*,25$(OH)_2D_3$) and 1α, 25-dihydroxycholecalciferol, (1α,25$(OH)_2D_3$).

In the human, vitamin D_3 is obtained either from the diet[9 10] or is metabolised from 7-dehydrocholesterol by the epidermis in the presence of sunlight or ultraviolet light.[11 12] The enzymatic conversion of vitamin D_3 to 25-hydroxycholecalciferol, 25$(OH)D_3$, takes place in the liver,[13] whereas the second hydroxylation of 25$(OH)D_3$ to form the dihydroxy metabolites, 24*R*,25-$(OH)_2D_3$ and 1α,25$(OH)_2D_3$ occurs mainly in the kidney.[14] It has been suggested that this second hydroxylation is catalysed by a single enzyme[15]; however, earlier studies identified two membrane bound multiple enzyme complexes in the mitochondria of cells in the proximal convoluted and straight tubular sections of the nephron.[16 17] Other sites postulated for this hydroxylation include the placenta[18] and the epidermis, especially in the keratinocytes,[19] which are extremely active in psoriatic patients.[20]

Absorption of calcium from the gut is increased by 1α,25$(OH)_2D_3$,[21–23] whereas it has been shown that 24*R*,25$(OH)_2D_3$ does not affect this regulation of calcium uptake from the small intestine.[24] Both metabolites are implicated in the turnover of calcium in bone, with 1α,25$(OH)_2D_3$ considered more

important in the control of osteoclasts,[25 26] and 24*R*,25$(OH)_2D_3$ more effective on the osteoblasts.[27]

Dietary lead intake affects the absorption of calcium from the gut[28] and interferes with bone formation.[29 30] Calcium and lead compete for binding sites on mucosal proteins in the intestine,[31] although more recent work by these authors suggested that the primary effect was on the cholecalciferol endocrine system, rather than the interaction of calcium and lead at the intestinal level.[28] In children with increased lead absorption, a reduction in 1α,25 $(OH)_2D_3$ concentrations has been reported,[32] which returned to normal once blood lead decreased to <30 μg/dl (1.4 μmol/l). It has been suggested that chronic cadmium exposure is linked with the subsequent appearance of osteomalacia and osteoporosis.[33]

In smelter workers with a known exposure to both lead and cadmium there was a possibility that if the vitamin D_3 metabolic pathway was affected, this should be apparent in the plasma concentrations of the active dihydroxy metabolites. The effects of exposure to a combination of lead and cadmium were also investigated. In this study, the plasma concentrations of three metabolites of vitamin D_3, 25$(OH)_2D_3$, 24*R*,25$(OH)_2D_3$, and 1α,25 $(OH)_2D_3$, blood concentrations of lead and cadmium, and urinary concentrations of cadmium and total proteins were measured.

Materials and methods

BLOOD AND URINARY SAMPLES

During their routine health check, male workers in a large non-ferrous metal smelter took part in a survey of the effects of long term low level cadmium exposure.[34] In the five year follow up study[35] 59 of these workers were available for retesting (follow up group). Blood samples from 19 of these participants collected consecutively, (study group), were stored in conditions suitable for analysing vitamin D_3 metabolites. These blood samples were collected into tubes containing lithium heparin anticoagulant, mixed, and stored at 4°C in light proof containers. Samples were divided into two; one batch was stored at –20°C until analysed for lead and cadmium, whereas the other was centrifuged and the plasma separated and stored in glass vials at –20°C, protected from light, until assayed for vitamin D_3 metabolites. The urine samples were stored in acid washed plastic vials at –20 °C. These were spot urine samples, not 24 hour collections, thus all urinary calculations are based on concentration rather than excretion rates. Apart from the difficulty of obtaining collections of 24 hour urine from these volunteers, the probability of cadmium contamination was a deciding factor in collecting spot samples.

CHEMICALS

Tritiated (^3H) vitamin D_3 metabolites were obtained from Amersham International, Little Chalfont, Bucks, UK, Sephadex LH20 was from Pharmacia Biotech, St Albans, Herts, UK, 25$(OH)D_3$ binding globulin was prepared in our laboratory from normal rat serum, 1α,25$(OH)_2$ D_3 binding protein was kindly donated by Dr S Edelstein (Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel), and the control samples for metals in blood and urine were from Behring Diagnostics, Hounslow, Middlesex, UK). All other chemicals and reagents were from Merck BDH, Lutterworth, Leics, UK and were of AnalaR grade unless stated otherwise.

VITAMIN D_3 METABOLITES

Figure 1 shows the analytical procedures, which were based on the methods developed by Edelstein (personal communication). Extraction methods were based on the procedure described by Lidor *et al*.[36] Trace amounts of all three tritiated metabolites were added to all samples to monitor the recovery of the metabolites throughout the procedure. An adjustable rack supplied by Ilacon, Tonbridge, Kent, UK, that held the vials in a water bath at 35°C while a stream of nitrogen was directed at each individual vial, was used to evaporate all samples to dryness when changing solvents or reducing sample volumes. For high pressure liquid chromatography (HPLC), a Waters M45 HPLC solvent delivery system was used with a μPorasil 27477 column, Waters, Watford, Herts, UK, calibrated with ^3H-25$(OH)D_3$, ^3H-24*R*,25$(OH)_2D_3$, and ^3H-1α,25$(OH)_2D_3$, run separately to establish the positions of the three peaks. The eluting solvent was isopropanol-:hexane (1:9), degassed under vacuum.

The radioimmunoassay (RIA) was a modified protein binding method, with activated dextran/charcoal separation and ^3H -25$(OH)D_3$ or ^3H 1α,25$(OH)_2D_3$.[36] For 25$(OH)D_3$ and 24*R*,25 $(OH)_2D_3$ the binding protein was vitamin D_3 binding globulin from rat serum[37] and for 1α,25$(OH)_2D_3$ cytosol from the duodenum of rachitic chicks was used. Samples, standards, controls, and blanks were dissolved in absolute ethanol, each sample was assayed in duplicate, and each standard in quadruplet. Barbitone buffer, pH 8.6, was used in the assay for 25$(OH)D_3$ or 24*R*,25$(OH)_2D_3$ and TRIS-molybdate buffer, pH 7.4, for the 1α,25 $(OH)_2D_3$. Assays were left overnight at 4°C, dextran/charcoal suspension was added and after 30 minutes on ice, the assay samples were filtered into counting vials with an Ilacon filter unit. Radioactivity was measured with a Packard Tricarb β-counter, Packard Instruments, Caversham, Berks, UK. Standard curves were calculated with log/logit transformation, the sample concentrations were calculated, and the values were adjusted for percentage recovery. Matched volumes of a plasma pool used as a laboratory quality control were included in each run. The RIA for vitamin D_3 metabolites had minimum detection limits of 0.5 ng/tube for 25$(OH)D_3$ and 24*R*,25$(OH)_2D_3$ and 0.5 pg/tube for 1α,25 $(OH)_2$ D_3. The working concentration ranges were 30–200 ng/ml for 25$(OH)D_3$ and 24*R*, 25$(OH)_2D_3$ and 20–300 pg/ml for 1α,25 $(OH)_2$ D_3 calculated from the relevant precision profile. Control ranges for vitamin D_3 metabolites measured in this laboratory in adults with no known exposure to lead or cadmium were as follows, 25$(OH)D_3$ 12.2–26.2 ng/ml, 24*R*,

Figure 1 Procedures in the analysis of vitamin D_3 metabolites.

$25(OH)_2D_3$ 1.3–2.6 ng/ml, and $1\alpha,25(OH)_2$ D_3 34.2–42.5 pg/ml.

BLOOD LEAD, AND BLOOD AND URINARY CADMIUM

Samples were analysed for lead and cadmium with an IL Video 11 spectrophotometer and IL 655 furnace atomiser with Fastac II autosampler, Thermo Electron, Warrington, Cheshire, UK. A single matrix matched calibration curve was used to determine concentrations and all samples were analysed in duplicate with two replicates per sample; reproducibility was <10% CV. Control blood samples for metals were used at two concentrations, lead 15 and 40 μg/dl, and cadmium 4.5 and 57.9 nmol/l, control urine samples for cadmium were used at three concentrations 30, 180, and 410 nmol/l. The laboratory participated in external quality assurance schemes for blood lead and for blood and urinary cadmium. The results obtained were as follows, Blood Lead Proficiency Testing, Centers for Disease Control, Atlanta, Georgia, State Laboratory of Hygiene, all concentrations were within target ranges; UK National External Quality Assessment Scheme (NEQAS) for lead and cadmium in blood, Queen Elizabeth Hospital, Birmingham, the mean running variance index score, (MRVIS) for lead was MRVIS=24, (MRVIS=44 for all participants), for cadmium MRVIS=23 (MRVIS=57 for all participants); trace element quality assessment scheme (TEQAS), Analytical Unit, Robens Institute, University of Surrey, UK, for blood lead and blood and urinary cadmium, all the cumulative scores (CUSUM) were above the acceptable concentration of CUSUM=20, blood lead CUSUM=85.8, blood cadmium CUSUM=71, urinary cadmium CUSUM=36.4.

Table 1 Vitamin D_3 metabolites, lead, cadmium, and total proteins in study and follow up groups

	Study group (n=19)			*Follow up group (n=55)*			
	AM (range)	*Median*	*Skewness*	*AM (range)*	*Median*	*Skewness*	*Normal values*
Plasma 25 $(OH)D_3$ (ng/ml)	28.6 (1.0–51.9)	28.4	−0.194				8–45 *
Plasma 24*R*,25- $(OH)_2D_3$ (ng/ml)	1.7 (0.6–5.8)	1.3	2.218				2–5 *
Plasma 1∝,25- $(OH)_2D_3$ (pg/ml)	32.2 (0.1–75.7)	35.0	−1.686				15–40 *
Blood lead (μmol/l)	2.2 (1.0–3.6)	2.1	0.235				<1.4 †
(μg/dl)	47 (21–76)	40.0	0.235				<25 †
Blood cadmium (nmol/l)	37 (11–145)	32.0	3.324	30 (6–74)	27.0	3.386	<27 ‡
Random urinary cadmium (nmol/l)	32 (6–160)	15.0	2.499	30 (3–161)	18.0	2.567	<8 ‡
Random urinary total protein (mg/dl)	9.7 (3.2–21.7)	9.2	1.239	10.2 (2.1–32.6)	8.9	1.459	<200 mg/24 h§ (~12.5–20 mg/dl)

Sources quoted for normal values were as follows: vitamin D metabolites* Rosen *et al*[40]; blood lead† Brody *et al*[41]; blood and urinary cadmium‡ Staessen *et al*[42]; urinary total proteins§ Macart *et al.*[39]

URINARY TOTAL PROTEINS

These were measured with a BioRad total protein test kit, BioRad Laboratories, Watford, Herts, UK, based on Macart's modification of the Bradford method[38 39] in which protein in the urine is bound to an acidified dye reagent (Coomassie brilliant blue (G250) dye). The observed change in absorbance was proportional to the protein concentration and the test was linear up to 150 mg/dl with a sensitivity of 1.1 mg/dl. Assays were carried out at room temperature in glass tubes and all measurements were in duplicate. Saline solution, 0.9%, was used as the reagent blank and extra control standards were prepared with a mixture of human albumin and globulin in a 1:1 ratio. Dye/protein colour was stable for 60 minutes and all samples were read within that time with a Varian Techtron spectrophotometer, series 634, Varian, Walton on Thames, Surrey, KT12 2QF, UK.

STATISTICAL ANALYSIS

The statistical package SPSS for Windows was used for all calculations. Frequency distributions were measured for all variables and log transformations used for skew variables. Correlation coefficients and regression analyses were calculated. The non-parametric Kruskal-Wallis one way analysis of variance (K-W one way ANOVA) was used to measure the relation between 1α,25$(OH)_2$ D_3 and cadmium with lead.

Results

Table 1 shows the concentrations obtained for plasma 25$(OH)D_3$, 24*R*,25$(OH)_2D_3$, and 1α,25$(OH)_2D_3$, blood lead, blood and urinary cadmium, and urinary total proteins. For the follow up group, results for blood and urinary cadmium and urinary total proteins were obtained in 55 of the 59 cases. In the study group, a complete set of concentrations was obtained in all 19 cases. Table 2 shows a correlation matrix with significance levels for all variables.

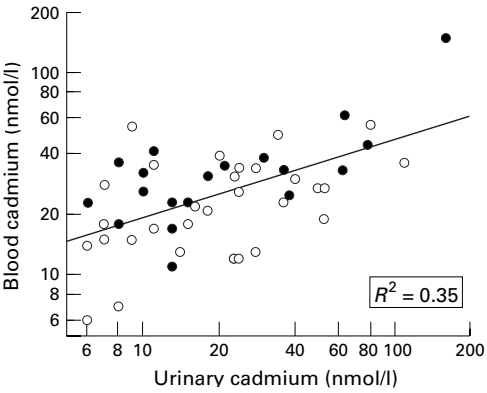

Figure 2 Relation between cadmium concentrations in blood and spot urine samples. Shown as log scales for both axes. ● study group; ○ follow up group.

Figure 2 shows the regression line for log blood cadmium and log urinary cadmium, the regression coefficients being +0.35 (SE 0.077) and the residual SD (in logs to the base 10) was 0.20, corresponding to a 95% prediction width of 2.5-fold. Of the 19 concentrations, 16 (84%) plasma 25$(OH)D_3$ results were in the normal range, but only six (21%) of the 24R,25$(OH)_2$ D_3 and six (31%)of the 1α,25$(OH)_2D_3$ results.

The K-W one way ANOVA showed significant differences in the plasma 1α,25$(OH)_2D_3$ concentrations, χ^2=10.3, df 2, p=0.006, (fig 3), when these were grouped according to the following combination of blood lead and blood or urinary cadmium concentrations: group 1, low Pb/high Cd, blood lead <1.9 μmol/l with urinary cadmium >8 nmol/l or blood cadmium >27 nmol/l; group 2, raised Pb/low Cd, blood lead >1.4 μmol/l with urinary cadmium < 8 nmol or blood cadmium <27 nmol/l; group 3, high Pb/high Cd, blood lead >1.9 μmol/l with

Table 2 Correlation matrix for all variables

	Log blood cadmium	*Log urinary cadmium*	*Blood lead*	*Log*			*Log urinary proteins*
				25(OH)D_3	*24R,25(OH)$_2D_3$*	*1∝,25(OH)$_2D_3$*	
Log blood cadmium	1.0000						
Log urinary cadmium	0.695**	1.0000					
Blood lead	−0.265	−0.338	1.000				
25$(OH)D_3$	0.108	0.388	0.063	1.000			
Log 24*R*,25$(OH)_2D_3$	−0.348	−0.496*	0.154	0.379	1.000		
1∝,25$(OH)_2D_3$	0.165	0.337	0.378	−0.086	−0.176	1.000	
Log urinary proteins	−0.183	0.044	−0.075	−0.547*	−0.009	−0.126	1.000

*p<0.05; **p<0.01.

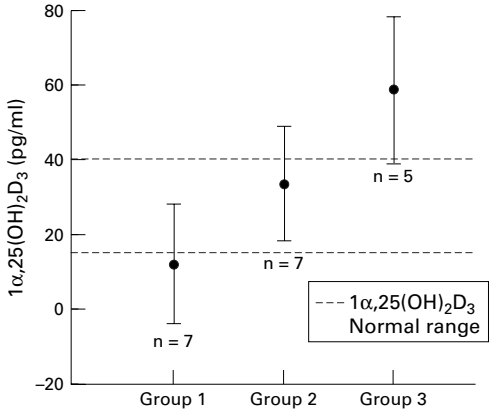

Figure 3 The effect of lead and cadmium on plasma 1∝,25(OH)$_2$D$_3$ concentrations. Error bars show mean and 95% confidence interval (95% CI). Group 1=blood lead <1.9 µmol/l with urinary cadmium >8 nmol/l or blood cadmium >27 nmol/l; group 2=blood lead >1.4 µmol/l with urinary cadmium <8 nmol or blood cadmium <27 nmol/l; group 3=blood lead>1.9 µmol/l with urinary cadmium >8 nmol/l or blood cadmium >27 nmol/l.

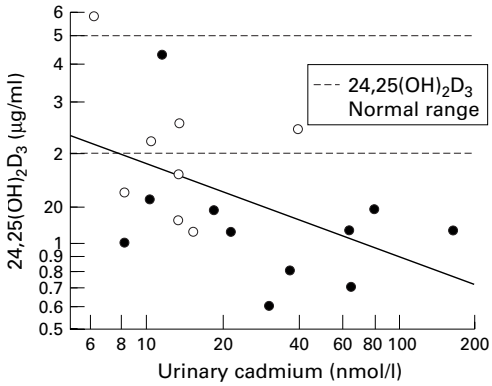

Figure 4 The effect of cadmium on plasma 24R, 25 (OH)$_2$D$_3$ concentrations, shown as log scales for both axes. ● Blood CD >27 nmol/l; ○ Blood CD <27 nmol/l..

urinary cadmium > 8 nmol/l or blood cadmium >27 nmol/l.

Figure 4 shows the effect of urinary cadmium concentrations >8 nmol/l and blood cadmium concentrations >27 nmol/l on plasma 24*R*,25(OH)$_2$D$_3$. There is a decrease in the plasma 24*R*,25(OH)$_2$D$_3$ concentrations as the cadmium concentrations increase.

Discussion

These results suggest that increased blood and urinary cadmium and blood lead cause perturbation of the conversion of 25(OH)D$_3$ to 24*R*,25(OH)$_2$D$_3$ and 1α,25(OH)$_2$D$_3$. In the presence of cadmium, lead seems to be associated with increased conversion of 25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$. Unlike the effect in children,[32] in whom the conversion of 25(OH)D$_3$ to 1α,25(OH)$_2$D$_3$ may be reduced when the blood lead concentration is >1.4 µmol/l (>30 µg/dl), in these adults the conversion seemed to be enhanced as blood lead concentrations increased. The negative correlation found in children between blood lead and 1α,25(OH)$_2$D$_3$[43] was not found in these adults.

The membrane bound enzyme complexes that catalyse the formation of 24*R*,25(OH)$_2$D$_3$ and 1α,25(OH)$_2$D$_3$ from 25(OH)D$_3$ are termed 25OH-D$_3$-24R-hydroxylase (24R-OHase) and OHD$_3$-1α-hydroxylase (1α-OHase) respectively.[44] They are found in the mitochondria of the cells lining the proximal convoluted tubules and the straight tubular segments of the nephron. Phosphorylation of an inactive phosphatase is necessary for the activation of the 1α-OHase, which catalyses the formation of 1α,25(OH)$_2$D$_3$ and a calcium dependant protein kinase converts this enzyme complex to the fully active 24R-OHase. It has been suggested that lead interferes with phosphate linked reactions and that perturbations of calcium mediated processes are an early effect of lead toxicity at the cellular level.[45] Cadmium induces osteomalacia by a direct action on bone through abnormal calcium homeostasis[46] and has an effect on bone repair by reduction of alkaline phosphatase activity in osteoblastic cells.[47]

Lead is initially accumulated in renal tissue and may be taken up by the renal tubular epithelial cells from the basolateral side by active transport as the free ion.[48] Both the structure and function of mitochondria are very sensitive to lead, which is rapidly taken up by these organelles by an active transport process that may be comparable with the transport process associated with calcium. Granules found in mitochondria exposed to lead are thought to be precipitates of both calcium and lead phosphates.[49 50] Lead may therefore interfere with both calcium and phosphate metabolism and thus affect enzymatic function in the vitamin D$_3$ pathway. The kidney matures gradually from infancy and thus these membrane bound enzyme complexes that catalyse the formation of 24*R*,25(OH)$_2$D$_3$ and 1α,25(OH)$_2$D$_3$ from 25(OH)D$_3$ may be more susceptible to the effects of lead in children as opposed to adults. The whole enzyme complex may be affected, inhibiting the 1α-OHase and the 24*R*-OHase so that both 1α,25(OH)$_2$D$_3$ and 24*R*,25(OH)$_2$D$_3$ are low in children with raised lead concentrations. Cadmium damage to the renal tubules may affect the folding, cleavage, and reorientation of the active sites of these enzymes.[44] Therefore cadmium and lead could have a combined effect on this enzyme complex inhibiting the inactivation of the 1α-OHase and its conversion to partially active 24*R*-OHase. This would subsequently prevent the formation of fully active 24*R*-OHase, resulting in raised concentrations of 1α,25(OH)$_2$D$_3$ and depleted concentrations of 24*R*,25(OH)$_2$D$_3$.

Concentrations of lead and cadmium in blood are indicators of present or recent exposure to these metals, whereas the concentrations in urine indicate previous or long term exposure.[42 51] However, a significant correlation has been shown between cadmium concentrations in blood and urine in this study, which suggests that continuous long term exposure to cadmium produces a state of equilibrium. The interrelation between blood and urinary cadmium concentrations and the decrease in 24*R*,25(OH)$_2$D$_3$ may have appeared after exposure to cadmium of as yet unknown duration. As these workers were still exposed to

cadmium and were taking part in a five year follow up study, it is possible to state that they had been continuously exposed to cadmium for at least five years. The preferential incorporation of lead rather than calcium into bone and the osteomalacia and osteoporosis found after cadmium ingestion may be linked with decreased 24*R*,25$(OH)_2D_3$ concentrations, but this remains unproved. Epidemiological and clinical studies in Japan since 1962 have shown that bone is affected only after very high exposure to cadmium.[6] However, where there is exposure to both lead and cadmium, as in this report, the effects on bone, mediated through the vitamin D_3 pathway, may possibly be found at lower exposures.

The negative correlation of 25$(OH)D_3$ with total urinary proteins agrees with that found by Saha,[52] who also found that patients with heavy proteinuria had abnormal regulation of 1α,25$(OH)_2D_3$ and a dependency on 25$(OH)D_3$. Chronic renal failure from other causes did not seem to affect 25$(OH)D_3$ concentrations.[53] Nogawa *et al* found that cadmium initially disturbed the hydroxylation of 25$(OH)D_3$ to 24*R*,25$(OH)_2D_3$ but that at higher blood cadmium concentrations the hydroxylation of 1α,25$(OH)_2D_3$ was affected, in participants with poor renal function.[54]

Conclusions

The highly significant correlation between the cadmium concentrations in blood and urine suggested that continuous long term exposure to cadmium can result in an equilibrium between blood and urinary concentrations. In this situation, it is possible to estimate cadmium blood concentrations from the analysis of spot urine samples. Cadmium and lead have an effect on vitamin D_3 metabolism at blood and urinary concentrations below those at which there is clinical evidence of toxicity. Cadmium decreased the concentrations of 1α,25$(OH)_2D_3$ and 24*R*,25$(OH)_2D_3$, whereas cadmium and lead increased the concentrations of 1α,25$(OH)_2D_3$. Biochemical changes may occur that could have long term health implications. Although many sources of lead and cadmium have been removed, exposure in industrial situations is still possible. The simultaneous exposure to more than one contaminant may lower threshold levels for the appearance of health effects and this should be taken into account in routine monitoring.

We thank Professor MJR Healy for his statistical advice regarding distributions of data, regression analysis, and correlation coefficients, Dr CD Strehlow for his invaluable help and advice, Ms J Wells for organising the sample collection as part of an epidemiological study initiated by Professor G Kazantzis, and Ms M Thompson for the atomic absorption spectrophotometry. The financial support of the Westminster Children's Research Trust is gratefully acknowledged.

1 Lockitch G. Perspectives on lead toxicity [review]. *Clin Biochem* 1993;**26**:371–81.
2 Tandon SK, Khandelwal S, Jain VK, *et al*. Influence of dietary iron deficiency on acute metal intoxication. *Biometals* 1993;**6**:133–8
3 Barry PS, Mossman DB. Lead concentrations in human tissues. *Br J Ind Med* 1970;**27**:339–51.
4 Hicks DG, O'Keefe RJ, Reynolds KJ, *et al*. Effects of lead on growth-plate chondrocyte phenotype. *Toxicol Appl Pharmacol* 1996;**140**:164–72.
5 Borjesson J, Mattsson S, Stromberg U, *et al*. Lead in fingerbone: a tool for retrospective exposure assessment. *Arch Environ Health* 1997;**52**:104–12.
6 Kjellstrom T. Mechanism and epidemiology of bone effects of cadmium. *IARC Sci Publ* 1992;**118**:301–310.
7 Roels H, Bernard AM, Cardenas A, *et al*. Markers of early renal changes induced by industrial pollutants. III. Application to workers exposed to cadmium. *Br J Ind Med* 1993;**50**:37–48.
8 Hido T, Honda R, Yamada Y, *et al*. α1-Microglobulin determination in urine for the early detection of renal tubular dysfunctions caused by exposure to cadmium. *Toxicol Lett* 1985;**24**:195–201.
9 Steenbock H, Black A. Vitamin D. *J Biol Chem* 1924;**61**:405.
10 Hess AF, Weinstock M. Vitamin D. *J Biol Chem* 1924;**62**: 301
11 Holick MF. The cutaneous photosynthesis of pre-vitamin D3: a unique photo-endocrine system. *J Invest Dermatol* 1981;**77**:51–8.
12 Windaus A, Schenk F, von Werder F. Isolation of Vitamin D. *Hoppe-Seyler's Zeitschrift fur Physiologische Chemie* 1936;**241**: 100–1.
13 Takeuchi A, Okano T, Kobayashi T. The existence of 25-hydroxyvitamin D3–1 alpha-hydroxylase in the liver of carp and bastard halibut. *Life Sciences* 1991;**48**: 275–82.
14 Fraser DR, Kodicek E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature* 1970;**228**: 764–6.
15 Burgos-Trinidad M, DeLuca HF. Kinetic properties of 25-hydroxyvitamin D- and 1,25-dihydroxyvitamin D-24-hydroxylase from chick kidney. *Biochim Biophys Acta* 1991; **1078**:226–30.
16 Gray RW, Ghazarian JG. Solubilization and reconstitution of kidney 25-hydroxyvitamin D3 1α- and 24-hydroxylases from vitamin D replete pigs. *Biochem J* 1989;**259**:561–8.
17 Mandel ML, Moorthy B, Swartz SJ, *et al*. Monoclonal antibodies to chick renal calcium regulating hemeproteins. Biochemical and immunohistochemical interactions with mitochondrial 25-hydroxyvitamin D3 hydroxylases. *J Clin Lab Immunol* 1990;**31**:1–10.
18 Rubin LP, Yeung B, Vouros P, *et al*. Evidence for human placental synthesis of 24,25-dihydroxyvitamin D3 and 23,25-dihydroxyvitamin D3. *Ped Res* 1993;**34**:98–104.
19 Bikle DD, Halloran BP, Riviere JE. Production of 1,25 dihydroxyvitamin D3 by perfused pig skin. *J Invest Dermatol* 1994;**102**:796–8.
20 Guilhou JJ, Colette C, Monpoint S, *et al*. Vitamin D metabolism in psoriasis before and after phototherapy. *Acta Derm Venereol* 1990;**70**:351–4.
21 Wasserman RH, Brindak ME, Buddle MM, *et al*. Recent studies on the biological actions of vitamin D on intestinal transport and the electrophysiology of peripheral nerve and cardiac muscle [review]. *Prog Clin Biol Res* 1990;**332**:99–126.
22 Fuss M, Pepersack T, Gillet C, *et al*. Calcium and vitamin D metabolism in granulomatous diseases. *Clin Rheumatol* 1992;**11**:28–36.
23 Miyaura C, Suda T. Vitamin D [review]. *Jap J Clin Med* 1993;**51**:893–900. [Japanese]
24 Rubinger D, Krause I, Alkoby-Gibbs M, *et al*. 24,25(OH)2D3 affects the calcemic effects of 1,25(OH)2D3 by mechanisms independent of intestinal calcium absorption. *Bone and Mineral* 1991;**15**:219–26.
25 Matsumoto T, Yamato H, Okazaki R, *et al*. Effect of 24,25-dihydroxyvitamin D3 in osteoclasts. *Proc Soc Exp Biol Med* 1992;**200**:161–4.
26 Yamato H, Okazaki R, Ishii T, *et al*. Effect of 24R,25-dihydroxyvitamin D3 on the formation and function of osteoclastic cells. *Calcif Tissue Int* 1993;**52**:255–60.
27 Grosse B, Bourdeau A, Lieberherr M. Oscillations in inositol 1,4,5-trisphosphate and diacyglycerol induced by vitamin D3 metabolites in confluent mouse osteoblasts. *J Bone Miner Res* 1993;**8**:1059–69.
28 Fullmer CS, Rosen JF. Effect of dietary calcium and lead status on intestinal calcium absorption. *Environ Res* 1990;**51**:91–9.
29 Long GJ, Rosen JF, Pounds JG. Lead impairs the production of osteocalcin by rat osteosarcoma (ROS 17/2.8) cells. *Toxicol Appl Pharmacol* 1990;**106**:270–7.
30 Pounds JG, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone, [review]. *Environ Health Perspect* 1991; **91**:17–32.
31 Fullmer CS, Edelstein S, Wasserman RH. Lead-binding properties of intestinal calcium-binding proteins. *J Biol Chem* 1985;**260**:6816–9.
32 Rosen JF, Chesney RW, Hamstra A, *et al*. Reduction in 1,25-dihydroxyvitamin D in children with increased lead absorption. *N Engl J Med* 1980;**302**:1128–31.
33 Yoshiki S, Yanagisawa T, Kimura M, *et al*. Bone and kidney lesions in experimental cadmium intoxication. *Arch Environ Health* 1975;**30**:559–62.
34 Kazantzis G, Armstrong BG. Renal function in relation to low levels of cadmium exposure in a group of smelter workers. *Environ Health Perspect* 1984;**54**:193–9.
35 Kazantzis G, Lam TH, Sullivan KR. Mortality of cadmium-exposed workers. A five-year update. *Scand J Work Environ Health* 1988;**14**:220–3.
36 Lidor C, Sagiv P, Amdur B, *et al*. Decrease in bone levels of 1,25-dihydroxyvitamin D in women with subcapital fracture of the femur. *Calcif Tissue Int* 1993;**52**: 146–8.

37 Edelstein S, Charman M, Lawson DE, *et al*. Competitive protein-binding assay for 25-hydroxycholecalciferol. *Clin Sci Mol Med* 1974;**46**:231–40.
38 Macart M, Gerbaut L. An improvement of the Coomassie blue dye binding method allowing an equal sensitivity to various proteins: application to cerebrospinal fluid. *Clin Chim Acta* 1982;**122**:93
39 Macart M, Gerbaut L. Evaluation of an improved Coomassie blue dye binding method for urinary protein assay. *Clin Chim Acta* 1984;**141**:77
40 Rosen JF, Chesney RW. Medical progress: circulating calcitriol concentrations in health and disease. *J Pediatr* 1983;**103**:1–17.
41 Brody DJ, Pirkle JL, Kramer RA, *et al*. Blood lead levels in the US population. Phase I of the third National Health and Nutrition Examination Study (NHANES III 1988 to 1991). *JAMA* 1994;**272**:277–83.
42 Staessen J, Lauwerys R. Health effects of environmental exposure to cadmium in a population study. *J Hum Hypertens* 1993;7:195–9.
43 Mahaffey KR, Rosen JF, Chesney RW, *et al*. Association between age, blood lead concentration, and serum 1,25-dihydroxycholecalciferol levels in children. *Am J Clin Nutr* 1982;**35**:1327–31.
44 Ghazarian JG. The renal mitochondrial hydroxylases of the vitamin D3 endocrine complex: how are they regulated at the molecular level? [review]. *J Bone Miner Res* 1990;**5**: 897–903.
45 Long GJ, Pounds JG, Rosen JF. Lead intoxication alters basal and parathyroid hormone-regulated cellular calcium homeostasis in rat osteosarcoma (ROS 17/2.8) cells. *Calcif Tissue Int* 1992;**50**:451–8.
46 Katsuta O, Hiratsuka H, Matsumoto J, *et al*. Cadmium-induced osteomalacic and osteopetrotic lesions in ovariectomized rats. *Toxicol Appl Pharmacol* 1994;**126**:58–68.
47 Gur E, Waner T, Barushka Eizik O, *et al*. Effect of cadmium on bone repair in young rats. *J Toxicol Environ Health* 1995; **45**:249–60.
48 Nolan CV, Shaikh ZA. Lead nephrotoxicity and associated disorders: biochemical mechanisms, [review]. *Toxicology* 1992;**73**:127–46.
49 Walton JR. Intracellular inclusions in the lead-poisoned cultured kidney cell. *J Pathol* 1974;**112**:213.
50 Goyer RA. Transplacental transport of lead, [review]. *Environ Health Perspect* 1990;**89**:101–5.
51 Ehle AL, McKee DC. Neuropsychological effect of lead in occupationally exposed workers: a critical review. *Crit Rev Toxicol* 1990;**20**:237–55.
52 Saha HH. Calcium and vitamin D homeostasis in patients with heavy proteinuria. *Clin Nephrol* 1994;**41**:290–6.
53 Navarro JA, Granadillo VA, Salgado O, *et al*. Bone metal content in patients with chronic renal failure. *Clin Chim Acta* 1992;**211**:133–42.
54 Nogawa K, Tsuritani I, Kido T, *et al*. Serum vitamin D metabolites in cadmium-exposed persons with renal damage. *Int Arch Occup Environ Health* 1990;**62**:189–93.