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Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad)

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Abstract

Cadmium, an environmental pollutant, can have adverse effects on the human body. The kidney is the critical organ. In order to improve the understanding of the dose-response relationship between cadmium exposure and health effects, and especially renal dysfunction, a study on a general population group in China was performed. This study was therefore concerned with cadmium exposure biomarkers, such as the concentrations in blood (BCd) and urine (UCd), and effect biomarkers of renal dysfunction, such as β_2-microglobulin (β_2m), retinol binding protein (RBP) and albumin (ALB). To improve the evaluation of exposure levels in relation to the adverse health effects of cadmium exposure in the general population, a quality control program was conducted to determine analytical quality in the determination of cadmium in blood and urine and for β_2m, creatinine, ALB and RBP. The measurements showed that analytical quality was adequate. The exposure and effect biomarkers were studied in the population groups living in three areas, namely a control area and two Cd polluted areas. In the highly exposed area, most of the BCd values were higher than 5 μg/l and most of the UCd values were higher than 5 μg/g creatinine. β_2-microglobulin, retinol binding protein, and albumin in urine were all significantly higher in the population living in the heavily polluted area than in that in the control area. Based on data from all three areas, a marked dose-response relationship between UCd or BCd and the prevalence of renal dysfunction was demonstrated. The number of abnormalities in kidney was related to the level of cadmium exposure. Only one index of renal tubular dysfunction was affected in subjects exposed to low levels of cadmium, but more than two indices of renal function were affected in those exposed to high levels.

Introduction

Cadmium (Cd) is an element belonging to group IIB in the periodic table. It is found in the earth's crust and is widely spread by human activities. Volcanic activities and erosion also give rise to increased concentrations of cadmium in air, land, and water. Major sources of contamination are the industrial production and consumption of cadmium and other non-ferrous

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metals and the disposal of wastes containing cadmium (WHO 1992). Improvements in the work environment have decreased its concentration in workroom air. Occupational threshold limit values in most countries are currently lower than those previously in force. During the 20th century, cadmium production has increased, resulting in increased concentrations in the general environment and particularly in air and water. Because of its adverse toxicological and environmental effects some countries have recently banned many uses of cadmium. At present, in spite of efforts to decrease emissions into the environment, cadmium concentrations in agricultural soils in Scandinavia are increasing by about 0.2% per year. In many countries, cadmium concentrations in crops have increased due to acidification caused in part by acid rain. This process results in an increased dietary cadmium intake in humans. An important toxicological property of cadmium is its exceptionally long biological half-life of 10-30 years in humans (Nordberg 1992; WHO 1992). Absorption of cadmium in humans in the gastrointestinal tract is around 5%, and depends on dietary composition and on the total dose (Nordberg *et al.* 1985; Andersen *et al.* 1992). A number of factors, e.g., low iron stores may increase absorption by a factor of up to 4 compared to humans with normal iron stores. Other factors that give rise to an increased uptake of Cd are low intakes of protein, vitamin D, calcium, and trace elements such as iron, zinc and copper. Intake of fibers can decrease the absorption of cadmium. Once absorbed, cadmium accumulates throughout the lifetime, and renal dysfunction may develop if a critical concentration is reached in renal tissue. With low-level exposure to cadmium, 30–50% of the body burden of cadmium is found in the kidney. Other adverse health effects seen following exposure to cadmium include bone effects, e.g., osteoporosis and more severe forms such as the osteomalacia found in itai-itai patients. With low-level exposure, cadmium is accumulated in the kidney, which is the critical organ in long-term exposure to cadmium. Cadmium has been classified as a human carcinogen (IARC 1993). It should be emphasized however, that carcinogenicity is only expected to occur when cadmium fumes are inhaled. More recent studies, such as Cadmibel and PheeCad (Buchet *et al.* 1990; Staessen *et al.* 1999, 2000; Hotz *et al.* 1999) show that tubular damage may develop at lower exposure levels than previously believed. Environmental epidemiological studies e.g., Cadmibel, PheeCad and several studies from Japan (Suwazano *et al.* 2000) have reported a relationship between Cd exposure in the general population and renal dysfunction. The present study was undertaken in China, where environmental pollution from smelting and mining includes emissions of cadmium. More than 30 places with different sources of cadmium contamination have been identified in China, but only limited information about cadmium exposure in China and its health effects has been reported (Cai *et al.* 1998; Jin *et al.* 1999). In order to improve the understanding of the dose-response relationship between cadmium exposure and possible health effects, e.g. renal dysfunction, a study on the general population was designed and performed in China.

This study was performed in collaboration between Umeå University (UU), Umeå, Sweden, Université Catholique de Louvain, Brussels, Belgium, Institute of Environmental Health, Zhejiang Academy of Medical Science (ZAMS), Hangzhou, China, Institute of Environmental Health and Engineering, Chinese Academy of Preventive Medicine (CAPM), Beijing, PR China, and Department of Preventive Medicine, School of Public Health, Shanghai Medical University, (SMU) Shanghai, China.

The study focused on the concentration of cadmium in blood (BCd), the urinary cadmium concentration (UCd) and estimation of the total lifetime cadmium uptake as indicators of cadmium exposure and internal dose. Biomarkers of renal dysfunction were β_2-microglobulin (β_2m), retinol binding protein (RBP) and albumin (ALB). To increase the likelihood of establishing a dose-response relationship and to evaluate exposure levels in relation to the adverse health effects of cadmium exposure in the general population, a quality control program was conducted to determine the analytical quality in the determination of cadmium in blood and urine and for β_2m, creatinine, ALB and RBP.

Materials and methods

Areas

Based on studies in 1995 of cadmium concentrations in rice from villages located at various distances from non-ferrous metal smelter near the city of Wenzhou (Nordberg *et al.* 1997) it was decided to include the following areas in the present study: Nanbaixiang (‘moderate’ near Wenzhou, Cd in rice 0.51 mg/kg), Jiaoweibao (‘heavy’ near Wenzhou, mean Cd concentration in rice 3.7 mg/kg) and a control area, not

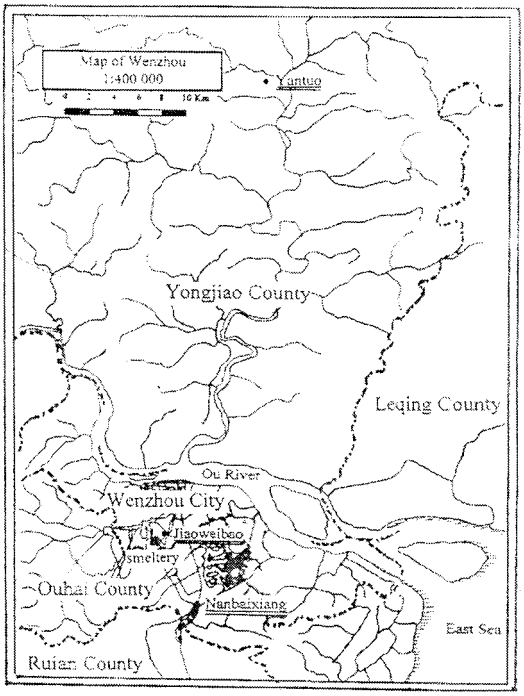

Figure 1. Map of cadmium contaminated areas (Jiaoweibao and Nanbaixiang) and control area (Yantuo).

previously studied regarding Cd in rice Yantuo (40 km from Wenzhou) (see Figure 1).

In the contaminated areas, cadmium levels in rice were higher than the State hygienic standard (0.2 mg/kg).

The Wenzhou smelter is located in Zhejiang province near the city of Wenzhou. Lead and zinc are the main products of this smelter, which came into operation in 1961. It is estimated that about 100,000 tons of industrial wastewater per year are discharged into the Tang River in front of the factory. In addition, waste tailings were stored at the smelter without treatment and were left uncovered, so that they were washed away by rain and polluted the river. The concentration of metals in the wastewater exceeded those laid down in the National Hygienic Standards for drinking water in China (in mg/l: Cu < 1.0 for Pb < 0.1, for Cd < 0.01 and for Zn < 1.0). Residents of the polluted areas used the polluted riverwater to irrigate their fields from 1961–1995. Rice is the main food of the residents in these areas. Many rice fields were converted to other uses in the 1990s (roads, commercial activities) but some were used by new tenants for rice production. In 1996, the residents of the highly polluted area (Jiaweibao) were advised to stop producing rice in their fields and to eat commercial rice from non-polluted areas.

Demographic and nutritional characteristics

Study population

The total number of participants was 790, made up of 253, 243 and 294 persons in the control, moderately and heavily polluted areas, respectively. Based on information available in registries kept by the local authorities, the characteristics of the populations of these areas were described in terms of parameters such as age, sex distribution and birth rate. Historical data from nutrition surveys performed in the period after 1960 were collected and present nutritional status was assessed by means of a targeted interview of 10 families in each area.

Participants were selected based on this information to ensure that living conditions, social and economic conditions and lifestyles were similar in the three areas. Only persons born in the respective areas and who had lived there and consumed locally grown rice throughout their entire lifetime (except the last two years in the highly polluted area) were included in the present study. While observing these inclusion criteria as many as possible of the persons who had participated in a previous study (Jin *et al.* 1999) in the polluted areas were included. An additional requirement was that the person selected was present in his/her home at the time of investigation. During the years immediately preceding the present study, many inhabitants, particularly men took up work outside these areas and thus were not present in their home. Among those present 83.5% agreed to participate. Participants were required to answer a detailed questionnaire administered by trained and supervised interviewers, and to provide blood and urine for biological measurements. The questionnaire was used to obtain information on occupational and environmental exposure to cadmium and use of tobacco. Height and weight were recorded for each subject in order to calculate the body mass index (BMI).

The choice of control area was based on age, nutrition, sex and socioeconomic factors, and the absence of any source of Cd exposure. In order to exclude possible lead exposure that might interfere with the health effects of Cd exposure, Pb in blood was determined, 10 samples from each area being investigated in a pilot study.

Soil

In each of the 3 areas soil samples were taken in 1997 according to the standard method used in China for soil-quality determination for lead and cadmium: KI-MIBK extraction, flame atomic absorption spectrophotometry (BG/T17140-1997, Criteria of China). Top soil (0–10 cm depth) and deep soil (10–20 cm) were taken as separate fractions. After mixing, one kg of each fraction, the sample was collected in a clean container. 10 samples of top and deep soil, i.e., a total of 20 samples, were taken in the rice fields of each of the 3 areas so that a total of 60 soil samples were obtained. The topsoil samples were analyzed for Cd and Pb and the deep samples for Cd only. Analyses were performed by ZAMS by mixing 10 ml $HNO_3/HClO_4$ (3:1) and 2 ml HF at room temperature. For comparison, 3 soil samples were analyzed by an accredited laboratory (SGAB, Sweden). The method used employs under pressure solution in HNO_3 and H_2O_2 and is based on US Environmental Protection Agency methods no 200.7 and 200.8.

High concentrations of lead and cadmium were found by the Chinese laboratory in top soil from the highly polluted area (Pb 448 ± 140 mg/kg, Cd 0.87 ± 0.16 mg/kg) and from the moderately contaminated area (Pb 38.6 ± 3.58 mg/kg, Cd 0.055 ± 0.010 mg/kg). Top soil from the control area gave values of Pb of 32.4 ± 3.8; and of Cd of 0.04 ± 0.01. In deep soil, Cd concentrations were 0.04 ± 0.01 mg/kg in the control area, 0.82 ± 0.20 mg/kg in the highly contaminated area and 0.05 ± 0.01 mg/kg in the moderately contaminated area.

Considerably higher values were found in the top soil by the Swedish accredited laboratory namely Pb: 456–507 mg/kg and Cd:18 −26 mg/kg in the highly polluted area, and Pb: 43.5 mg/kg and Cd 0.27–0.58 mg/kg in the moderately polluted area. In soil from the control area, the concentrations were more similar to the values obtained in the Chinese laboratory, Pb: 34.2 mg/kg and Cd: 0.090 mg/kg. Differences in dissolution methods may possibly explain the differences between laboratories.

Rice

Rice samples were collected in 1997 from 50 households in both the 'moderately exposed' and the control areas and from 35 households of new tenants in the 'highly exposed' area by the method previously described (Nordberg *et al.* 1997). Rice from 5 adjacent households was mixed to obtain a pooled sample, so that a total of 27 pooled rice samples were collected. The cadmium concentrations in rice are given in Table 1. Comparison of the values obtained in China with those of control analyses performed in Sweden showed good agreement ($r = 0.97$ and slope $= 0.67$, $n = 4$).

Table 1. Cadmium concentration in rice (mg/kg) collected in 1997.Number of samples analyzed (n), collected as described in text, Mean values, Standard deviation (SD) and Statistical significance (P) in relation to control area (see text).

Areas	n	Mean	± SD	P
Highly polluted area	7	2.4	1.89	0.017
Moderately polluted area	10	0.48	0.123	0.000
Control	10	0.05	0.014	

Estimation of total cadmium uptake

Cadmium uptake was estimated as described by Cai *et al.* (1998). Rice is the main food in the three areas studied and is considered to be the major source of cadmium intake. Smoking is an important source of cadmium exposure, but no tobacco is produced in these areas and only commercial cigarettes are smoked. The average cadmium concentration of eight different brands of commercial cigarettes, which are generally used by local residents, was determined and found to be 1.50 mg/kg in 1995 (Jin *et al.* 1999). For a person who smoked 10 g of tobacco per day, i.e., 20 cigarettes per day, for 25 years, the uptake of Cd from smoking was calculated to be about 4.05 mg, which is much lower than the cadmium uptake from food.

Since smoking does not have much influence on the value of total cadmium uptake in the present study, it was not considered when calculating total cadmium uptake. The lifetime cumulative uptake of cadmium was calculated based on rice consumption, an age-related weighting factor also being taken into account (0–9 years, 0.413; 10–19 years, 0.885; 20–59 years, 1.000; 60+ years, 0.823). The fractional uptake of cadmium from food was assumed to be 0.05. Thus cumulative uptake of cadmium = daily cadmium intake (daily rice consumption * average rice Cd concentration)* weighting factor * years * 0.05. According to this formula, the average cadmium uptakes were 22.4 (11.3–36.6), 105.8 (85.7–117.1) and 545.1 (256.8–582.5) mg in the control, moderately and highly polluted areas, respectively.

Ethical considerations

Local ethics committees of The Medical Faculty, Umeå University (UU) and of Shanghai Medical University (SMU) gave permission to perform the study. It was also carried out with the permission of the local authority and was in conformity with local guidelines for human investigations. All participants in this study were informed about the content and the objectives of the study, and gave their informed consent to participate.

Statistical methods

Data were entered in a database on a microcomputer using Epi-info (version 6.04b) and analyzed by means of the X^2 test and the X^2 trend test provided in the Statcalc program of the Epi-info package. The cut off values (abnormal values) for the criterion variables were defined as the 95% upper limit values, which were calculated from the control group. For comparison between more than two groups, one-way analysis of variance (ANOVA) was used. For calculations e.g. regression, correlation and curve estimation of all analytical evaluations, data were transferred to the SPSS program that was used. Distributions of the biological measurements were normalized by logarithmic transformation. The excel program was also used. A cut-off for statistical significance was set at $p < 0.05$.

Pilot study

Blood and urine samples. Blood samples (venous whole blood in metal-free tubes) and urine samples were obtained from 10 randomly selected persons, 5 men and 5 women, in each area. Blood samples were analyzed for Pb and Cd by ZAMS or SMU and aliquots of 3 of the samples were analyzed in Umeå, as QC analyses. Collection of samples followed a strict protocol (Basun *et al.* 1994; Cornelis *et al.* 1995).

Pb and Cd in blood. Cd values were higher in the exposed areas than in the control area. Pb in blood samples for controls ($n = 11$) was 287 μg Pb/kg, SD 194, for moderately Cd-exposed subjects was 196 μg Pb/kg, SD 124 and for highly Cd-exposed subjects was 96.5 μg Pb/kg with SD of 39.1. These results show that lead exposure was low compared to cadmium exposure in the polluted areas.

Quality control (QC)

Cadmium in urine. A quality control program was conducted to determine analytical quality for Cd in urine. Samples containing Cd were distributed to participating laboratories to which the Cd concentration was unknown and the results compared. Analytical methods were adapted to optimize analytical performance with the available equipment.

Collection and storage of urine samples. Urine samples were collected from the farmers and kept on ice transported to the laboratory. The pH was measured and found to be higher than 6. Each sample was subdivided by pouring (not by pipetting) into smaller tubes adapted for the specific analysis to be performed.

Urine samples for Cd analyses were prepared by adding a few drops of acid. Samples for determination of protein and β_2-microglobulin were kept on ice, and stored frozen at $-18\,^{\circ}$C after addition of NaOH (pH 7–8) until analyzed. For albumin analyses, another sample was prepared. RBP was analyzed at UCL in Belgium and stored unbuffered at $-18\,^{\circ}$C.

Analyses of urinary indicators of kidney dysfunction and methods for quality control. For RBP-determinations UCL used LIA (latex immuno assay),in which polyclonal antibodies against the protein of interest are absorbed on latex particles, thereby agglutinating the antigen measured.

Ten samples were used for QC for RBP, the QC laboratory for UCL was CDC, Atlanta, USA. UCL used the Immulite method of DPC and BMII of Behring for additional QC of β2-microglobulin and albumin, respectively.

B_2M was assayed by ZAMS using the RIA method. Kits were purchased from the China Institute of Atomic Energy, China. Aliquots of QC samples were sent to UCL, Brussels, where QC was performed by means of the Latex method (Bernard *et al.* 1987). Urinary albumin was measured by the ELISA method (Neuman & Cohen 1989) by ZAMS. QC samples were analyzed by UCL. Creatinine was analyzed by ZAMS and SMU by a spectrophotometric (751 nm) method (Hare 1950), with good agreement between SMU and ZAMS.

Determination of cadmium in blood and urine

Equipment available at the participating laboratories: CAPM and SMU Hitachi UNICAN GFA/AAS

(AAS 180–70). SGAB in Sweden: inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-quadrupole mass spectrometry (ICP-QMS). Umeå: Perkin-Elmer Z-3030 AAS with a HGA atomizer or Perkin-Elmer 4100 ZL with a THGA. Both systems used the Zeeman effect for background correction. At ZAMS, GFA/AAS, Hitachi, UNICAN was used.

In the main study, cadmium was determined in blood and urine using the standard addition method. Blood samples were mixed with 0.1% HNO_3 and a modifier (0.1% Pd and 0.06% $Mg(NO_3)_2$) was added in the HGA. A certified reference sample was inserted between each ten samples.

Urine samples were digested with concentrated nitric acid and boiled for 2 h. A combined modifier containing Pd (6 μg) and NH_4NO_3 (0.5 mg) was used (Snell *et al.* 1997). A certified reference sample was inserted between each ten samples.

Developing skills in AAS

In the pilot phase, the precision of measurements of cadmium concentrations in digested urine expressed as μg/l was fairly poor among the participating Chinese laboratories, while systematic errors were made in analyses of reference materials. This was probably due to the fact that all the laboratories used wall atomisation, peak height evaluation and standardization by means of a calibration graph, constructed from a randomly selected urine sample low in cadmium. It is well known (Welz 1992) that the mentioned procedures were used when AAS was introduced but has been found to suffer from non-spectral interference effects. These can usually be corrected by using matrix matched standards. However, since urine samples vary greatly in their chemical composition, accurate results for wall atomization and peak height evaluation can only be obtained by using the method of standard additions. This method was thus used for determinations in the main study and reported in the 'results' section of the present paper. It should be mentioned that the laboratories concerned could not convert to the Stabilized Temperature Platform Furnace (STPF) concept because this was not compatible with the available instrumentation. The results of the second intercomparison exercise in 1998, as reported by the two participating laboratories, showed a marked improvement, and were of sufficient accuracy and precision.

Evaluation of U-Cd method used in China during the pilot phase

In separate experiments, performed during a visit by Chinese chemists to Umeå, the performance of the analytical method previously used in China for the determination of Cd was investigated. Wall atomization and peak height evaluation was compared with state of the art methods, namely platform atomization and peak area evaluation. The results are shown in Table 2. Five samples from reconstituted reference material, Biorad with a recommended Cd concentration of 7.2 μg/l, were diluted 20 times and slopes of calibration graphs and Cd concentrations were determined using platform and wall atomization combined with peak area and peak height evaluation. For calibration, the method of standard additions was used for each procedure.

As can been seen from the first line of Table 2, repeated independent analysis of the reference material by the peak area method provides results of good accuracy and precision, indicating that both the methodology and the instrumentation are satisfactory.

The last three lines of Table 2 show mean values of different urines obtained from individuals participating in the study. Six to seven samples from different subjects were analyzed and dilution factors of 10, 5 and 2 were used for the determinations and the construction of the calibration graphs. As can be seen, the slope of the calibration graphs is practically unchanged for different dilutions of urine samples when using platform atomization combined with peak area evaluation. The standard deviation of the slopes from the different urine samples, shown in the last three lines, is reasonably small, so that variations in the chemical composition and matrix concentrations, within the limits of the test performed, do not change the sensitivity of the method when platform atomization in combination with peak area evaluation is used. Hence, a single calibration solution can, in this case, be used for the different types of urine samples. For all other modes of operation, large changes in the slopes between different urine samples are observed, both within each dilution group and between the groups. It should be noted that a change in the slope translates directly into a corresponding change in the accuracy of the analytical results, if external calibration is used. Variations in the slopes can be explained by the fact that the composition of individual urine samples is unique. As previously mentioned, the concentrations

Table 2. Results of measurements of Cd in urine using a Perkin Elmer 3030 AAS instrument, and comparison of slopes for wall and platform atomization using peak height and peak area signal evaluation. The ± values show one standard deviation and n is the number of samples analyzed. For 20 times dilution, the same sample was used, whereas each of the last three lines shows mean concentration values and slopes with SD of slopes for urines obtained from different individuals. All values were obtained by the method of standard addition. Considerable differences in mean slopes can be seen for different dilutions of urine samples, except when platform atomisation with peak area is used.

Platform atomisation					
Peak area			Peak height		
Dilution	Conc μg/l	Slope As/(μg l^{-1})	Conc μg/l	Slope A/ug/l	*n*
20	7.4 ± 0.58	0.071 ± 0.003	8.0 ± 0.17	0.074 ± 0.002	5
10	7.7	0.071 ± 0.002	8.2	0.093 ± 0.007	7
5	4.9	0.071 ± 0.005	5.2	0.084 ± 0.004	6
2	1.4	0.071 ± 0.005	1.4	0.086 ± 0.013	7
Wall atomisation					
Peak area			Peak height		
Dilution	Conc μg/l	Slope s/μg/l	Conc μg/l	Slope A /μg/l	*n*
20	7.1 ± 1.67	0.029 ± 0.002	7.4 ± 0.24	0.117 ± 0.003	5
10	7.9	0.019 ± 0.005	6.7	0.083 ± 0.022	7
5	4.4	0.018 ± 0.002	4.5	0.075 ± 0.005	6
2	1.5	0.020 ± 0.006	1.5	0.067 ± 0.016	7

in the last three lines of Table 2 are mean values for different individuals.

Main study: Results

Results of quality control of UCd and BCd and indicators of renal dysfunction

In the light of the pilot study and the evaluation of the methods previously used, it was realized that sufficient precision and accuracy in determining Cd concentrations in urine in the Chinese laboratories could only be achieved by the use of the standard addition method. This method was therefore used in the final analyses of UCd and BCd (main study).

For BCd the correlation coefficient was 0.83 between analyses performed by the independent Laboratory in Sweden (UU) and the Chinese laboratory (SMU). The slope was 0.8. Urinary cadmium concentrations were also measured by (wall) graphite-furnace atomic absorption spectrophotometry (GF-AAS) with peak area evaluation (ZAMS). A reference urine sample (Seronorm trace element urine, Nycomed, Oslo, Norway) was inserted in each run of ten samples. Every tenth sample was sent to the reference laboratory in Sweden (UU) using the methods described by Snell *et al.* 1997. The correlation coefficient value was 0.851 and the slope was 0.914. A second quality control run was performed by dividing 10 samples into four parts, two of which were sent either to the laboratory in China or to that in Sweden. Each laboratory therefore measured two subdivided samples from the same sample. The value of the correlation coefficient was 0.9997 and the slope of the calibration graph was 0.9422. One contaminated sample was excluded.

Urine pH was measured before any addition was made. It was found that, for 95% of the samples pH was above 6. For β_2m, the regression coefficient was 0.81–0.900 and the slope 0.63–0.970 in two runs. Albumin analyses gave a regression coefficient of 0.9 (polynomial regression) and a slope of 1.01. For RBP, the regression coefficient was 0.91 and the slope 1.05.

Main Study: Cd in blood and Urine

The concentrations of cadmium in blood, geometric mean, and in urine in different areas and for both sexes are shown in Table 3. Urinary cadmium concentrations

Table 3. Geometric mean values (G) of blood cadmium ○ and urinary cadmium ○ in different areas and both sexes.

Indicator/Area	Total		Women		Men	
	n	G	n	G	n	G
BCd (μg/l)						
Control area	244	1.41	146	1.30	98	1.58
Moderately polluted area	238	3.66*	158	3.55*	80	3.88*
Highly polluted area	288	9.05*$^{\Delta}$	170	9.90*$^{\Delta}$	118	7.96*$^{\Delta}$
UCd (μg/g creatinine)						
Control area	253	1.83	155	1.79	98	1.58
Moderately polluted area	243	3.55*	162	4.45*	81	2.27*
Highly polluted area	294	11.18*$^{\Delta}$	171	12.86*$^{\Delta}$	123	9.20*$^{\Delta}$

*Compared with control area: $P < 0.05$.
$^{\Delta}$Compared with moderately exposed area: $P < 0.05$.
○Determination by standard addition method with proven accuracy (see text).

Table 4. Geometric means of urinary total β_2m, RBP and ALB in different areas and both sexes.

Indices/Area	Total		Female		Male	
	n	Mean	N	G	N	G
B2M(mg/g creatinine)						
Control area	253	0.165	155	0.153	98	0.184
Moderately exposed area	243	0.160	162	0.156	81	0.169
Highly exposed area	294	0.332*$^{\Delta}$	171	0.324*$^{\Delta}$	123	0.343*
RBP (mg/g creatinine)						
Control area	233	0.059	171	0.088	93	0.060
Moderately exposed area	239	0.075*	159	0.078	80	0.070*
Highly exposed area	288	0.139*$^{\Delta}$	140	0.139*$^{\Delta}$	118	0.140*$^{\Delta}$
ALB(mg/g creatinine)						
Control area	253	3.06	155	3.39	98	2.60
Moderately exposed area	243	4.34	162	5.12	81	3.11
Highly exposed area	294	5.95*	171	6.92*	123	4.82*

*Compared with control area: $P < 0.05$. $^{\Delta}$Compared with moderately exposed area: $P < 0.05$.

and blood cadmium concentrations of residents in the high and moderately exposed areas were significantly higher for both sexes than those of residents in the control area. In addition, values for the high exposure group were higher than those for the moderately exposed group. The detection limit of Cd in urine and in blood was the same 0.05 μg/l and no subjects had values below the detection limit.

The distribution of blood cadmium and urinary cadmium in different areas and both sexes is shown in Figure 2 and 3, respectively. Figure 2 shows clearly that most of the BCd values were less than 2 μg/l in the control area for both men and women. In the moderately exposed area, most values of BCd were lower than 5 μg/l. In contrast, most of the BCd values were higher than 5 μg/l in the highly exposed area. A similar result was found for the distribution of cadmium in urine, as shown in Figure 3, Most of the UCd values were higher than 5 μg/g creatinine in the highly exposed group.

Compared with the control area, a significantly higher concentration of cadmium in blood is seen in the polluted areas both for the total population studied and for the two sexes. Smoking is more prevalent among men than among women, and this may explain the higher blood values found in men as compared with women in the control and moderately exposed areas. A similar difference is not seen for urinary Cd,

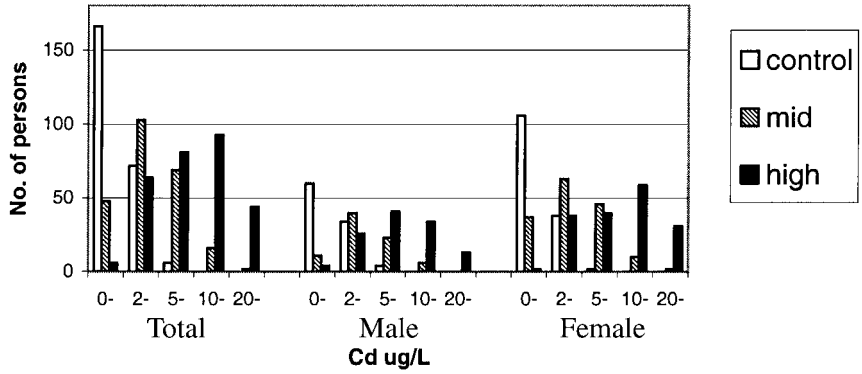

Figure 2. Distribution of cadmiun concentration in blood for men and women on a numerical basis an for the total number of subjects for each area studied.

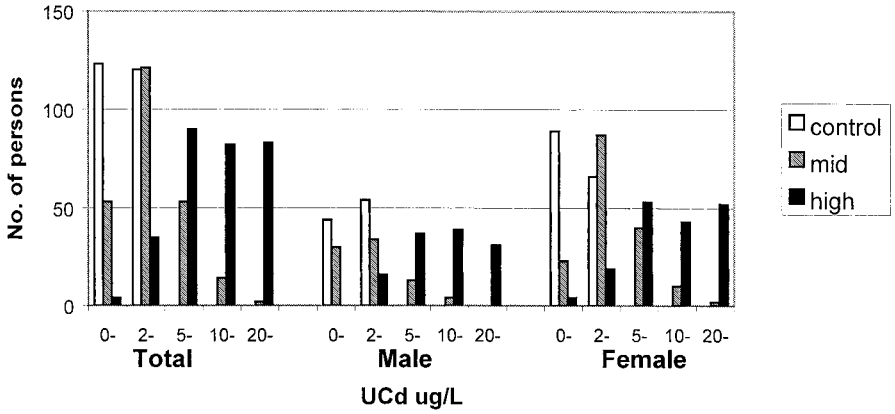

Figure 3. Distribution of cadmium in urine for men and women on a numerical basis and for the total number of subjects for each area studied.

since Cd from smoking probably has less influence on urine values than on blood values. In the highly exposed area, the influence of smoking is not seen since Cd exposure from dietary intake is much greater.

Main Study: Renal dysfunction indicators and relationship to dose indicators

The levels of urinary β_2 microglobulin, RBP, and albumin in the different areas and both sexes expressed as geometric means, are shown in Table 4. The values for urinary β_2 microglobulin and RBP are statistically significantly higher for subjects in the highly exposed area compared to subjects from the control area and in some instances also when compared to the moderately exposed area. This difference is marked for both the total group and for the two sexes. It will be seen that the albumin values in the highly cadmium exposed area are higher than those in the control area. For both women and men in the highly exposed area, the increase, as compared with the control group, was statistically significant.

A multiple regression analysis was performed, and the correlation coefficients between the indicators of cadmium exposure and those of renal dysfunction are shown in Table 5. The correlation coefficients for blood cadmium, urinary cadmium and total uptake were higher than 0.5 and those between indicators of internal dose (BCd, UCd) and effect indicators were also statistically significant.

The relationship between blood cadmium and the prevalence of increased urinary protein excretion is shown in Table 6. The β_2 microglobulin cut-off was 300 μg/g creatinine and for RBP was 300 μg/g creatinine. ALBuria was calculated, based on a cut off level of 15 mg ALB/g creatinine. There were clear and statistically highly significant dose-response relationships for all the indicators of renal effect namely β2m, RBP and ALB. ALBuria began to increase significantly when the blood cadmium concentration

Table 5. Results of multiple regression analysis: Correlation coefficients* between indicators of cadmium exposure and indexes of renal effect.

	BCd	UCd	Cd uptake	Albumin
BCd	1.000			
UCd	0.600	1.000		
Cd uptake	0.528	0.496	1.000	
B2M	0.199	0.207	0.183	
RBP	0.230	0.235	0.216	
Albumin	0.133	0.186	0.170	1.000

* P-values of all correlation coefficients are less than 0.001.

Table 6. Prevalence of increased protein excretion in urine for different levels of blood cadmium (BCd).

BCd (μg/l)	B2M +/−	%	RBP +/−	%	ALB +/−	%
<2	28/192	12.73	14/194	6.73	31/189	14.09
2–	27/212	11.30	14/214	6.14	29/210	12.13
5–	20/136	12.82	13/140	8.50	28/128	17.95
10–	39/70	35.78	32/76	29.03	25/84	22.94
20–	26/20	56.52	20/24	45.45	13/33	28.26
χ^2	83.18		89.35		12.44	
P	0.0000		0.0000		0.0000	
Linear trend χ^2	73.048		79.961		10.795	
P	0.0000		0.0000		0.0000	

Table 7. Prevalence of increased protein excretion in urine for different levels of urinary cadmium (UCd).

UCd*	B2M +/−	%	RBP +/−	%	ALB +/−	%
<2	20/146	12.05	13/144	8.28	18/148	10.84
2–	28/243	10.33	14/243	5.54	28/243	10.33
5–	26/137	15.95	19/141	11.88	25/138	15.34
10–	34/95	26.36	21/106	16.54	28/101	21.71
20–	36/25	59.02	30/30	50.00	28/33	45.90
χ^2	89.96		91.75		53.31	
P	0.0000		0.0000		0.0000	
Linear Trend χ^2	83.384		77.876		50.171	
P	0.0000		0.0000		0.0000	

*UCd (μg/g creatinine).

Table 8. Prevalence of increased excretion of proteins and enzymes in urine for different levels of cadmium uptake (Cd uptake).

Cd uptake (mg)	B2M +/−	%	RBP +/−	%	ALB +/−	%
<2	39/218	12.19	20/208	6.67	33/287	10.31
100–	18/190	8.65	12/192	5.91	32/176	15.38
500–	87/175	42.23	65/193	25.19	62/200	23.61
χ^2	60.06		54.44		19.13	
P	0.0000		0.0000		0.0000	
Linear trend χ^2	54.220		51.892		18.496	
P	0.0000		0.0000		0.0000	

exceeded 20 μg/l, as shown by a significant trend for each response.

Table 7 shows the relationship between urinary cadmium and the response expressed as the prevalence of proteinuria, and the pattern is similar to that seen in Table 6. There was a significant linear trend for all three indexes of response. The group with urinary cadmium of 2–5 μg/g creatinine did not show this response.

The relationship between total cadmium uptake and the prevalence of ALBuria is shown in Table 8, where a definite significant linear trend was also seen. When total cadmium uptake was more than 500 mg, the prevalence of ALBuria started to increase significantly.

The hierarchical classification of glomerular and tubular damage with urinary cadmium, blood cadmium and Cd uptake is shown in Table 9. There was a significant difference between the total renal damage, glomerular and tubular damage following increasing exposure to cadmium, as indicated by urinary cadmium, blood cadmium and Cd uptake

Discussion

The present study clearly shows that considerable exposure to cadmium and accumulation of cadmium in the body occur in population groups residing in contaminated areas of China. The levels of BCd and UCd in both sexes in the two polluted areas were significantly higher than those in the control area. In the highly polluted area, the majority of UCd and BCd values

exceeded 5 μg/g creatinine and 5 μg/l, respectively, demonstrating high exposure and accumulation in the body in this area, where the participants had been exposed to cadmium via the diet for approximately 35 years.

Only a few previous studies have reported adequate biomonitoring data for cadmium in human populations, since many studies carried out in the past lacked adequate quality control (Clarkson *et al.* 1988; Nordberg & Nordberg 1988; Alessio *et al.* 1994).

In biological monitoring, the quality of the overall process is dependent on the analytical procedures, sampling, sample storage, protection from contamination, and the representativeness of the samples. All analyses should include the use of adequate equipment and laboratory procedures, certified reference material, CRM and control reference material and/or interlaboratory tests. Performance over time should be documented (control chart). Knowledge of the field and awareness of the pitfalls of biological monitoring are important in obtaining correct results, together with the ability to prevent external contamination by using a clean room, pure reagents and clean benches. These prerequisites for adequate trace element determinations have been described by Cornelis *et al.* 1995 and Basun *et al.* 1994. In the present study, a training program was conducted so as to ensure that these requirements were satisfied.

Reference values for blood-cadmium levels (B-Cd) are available for only a limited number of geographical areas and for particular population strata (by sex, age and smoking habits). In conformity with the TRACY guidelines, between 1976 and 1992 only four out of 800 publications were used to provide provisional reference values (Herber *et al.* 1997). Smoking and dietary Cd intake are important determinants of Cd levels in 'control' areas. In the control area in this study BCd and UCd were lower than in 'non-contaminated' areas of Japan (Watanabe *et al.* 2000 a), but BCd was higher and UCd lower than in another low Cd-intake area (Shanxi province) in China (Watanabe *et al.* 2000b).

Cadmium in whole blood is a useful indicator of human exposure. BCd is derived from several body compartments, and when there is ongoing exposure, is related mainly to recent exposure (Nordberg & Nordberg 1988). However, after cessation of Cd exposure, blood levels reflect the body burden of Cd. The kinetics of BCd is described by a two-compartment model with a fast phase (Jarup *et al.* 1983) with a half-time of 75–130 days and a slow phase with a half-time of 7.4–16 years. BCd can thus reflect cadmium body burden, particularly in long term low level exposure and after cessation of exposure. Many studies have supported this view (Jarup *et al.* 1983; Gambini & Leurini 1992; Mc Diarmid *et al.* 1997). Cadmium in urine is bound mainly to metallothionein, and mainly reflects past exposure, body burden and renal accumulation. In the present study, blood cadmium and urinary cadmium were used together as indicators of the internal dose of cadmium in the exposure assessment of the general population groups studied. The good correlations found in the present study (Table 5) between BCd, UCd, and Cd Uptake, and between these dose indicators and indicators of renal dysfunction (β_2m, RBP, ALB) demonstrate the usefulness of these indicators.

Chronic cadmium exposure gives rise to renal tubular dysfunction, with increased excretion of low molecular weight proteins, such as β_2m. Urinary β_2m is a well established early and sensitive biomarker of renal dysfunction caused by cadmium (Bernard 1988; WHO 1992). However, the instability of the protein in urine can be a disadvantage of the method when urinary pH is below 5.5 (Evrin & Wibell 1972). Urine specimens should be made alkaline immediately after collection in order to prevent degradation. In the present study, the pH before addition of alkaline was already above 6 and no degradation was expected.

Albumin has a high molecular weight and an increased level of albumin in urine may reflect glomerular dysfunction. In quantitative terms, albumin is the major urinary protein derived from plasma. Its concentration in normal urine is on average at least 5 times higher than that of other high molecular weight proteins. Since albumin is easy to quantify in urine, increased urinary albumin is widely used as an index of glomerular damage (Jarup *et al.* 1983).

Environmentally Cd exposed farmers have been shown to have increased albumin levels in urine (Nogawa 1984; Aoshima & Kasuya 1991). The interpretation of increased levels of urinary high molecular weight proteins in cadmium-exposed humans has been debated. It has been suggested that such levels may result mainly from the increased permeability of the glomerular membrane caused by cadmium. In this study, urinary albumin concentrations in the highly polluted area were higher than those in the control area, suggesting that high cadmium exposure may result in damage to the glomerular barrier and enhanced glomerular permeability. Compared with the 5% prevalence of renal dysfunction in the control area, a prevalence of about 38.4% of renal tubular dysfunc-

Table 9. Hierarchical classification of glomerular and tubular damage with urinary cadmium, blood cadmium and Cd uptake.

Glomerular damage*	−			+			Total		Glomerular damage		Tubular damage	
Tubular damage**	0	1	2	0	1	2	χ^2	P	χ^2	P	χ^2	P
UCd(μg/g Creatinine)							172.84	0.0000	53.31	0.00	119.53	0.000
<2	137	9	2	10	2	6						
2–	221	17	5	23	2	3						
5–	116	9	13	20	2	3						
10–	78	11	12	17	2	9						
20–	18	4	11	7	4	17						
BCd (μg/L)							124.24	0.000	12.44	0.014	111.80	0.000
<2	171	14	4	23	2	6						
2–	189	14	7	22	4	3						
5–	113	8	7	23	2	3						
10–	64	8	12	6	3	16						
20–	17	5	11	3	1	9						
Cd uptake (mg)							97.25	0.000	19.13	0.0000	78.12	0.000
<100	255	26	6	27	0	6						
100–	165	6	5	24	5	3						
500–	150	18	32	26	7	29						

*Glomerular damage: +With glomerular damage; −Without glomerular damage.
**Tubular damage: 0: Without tubular damage; 1: Only one parameter of tubular damage with higher value; 2. Two parameters of tubular damage with higher values.

tion and 10.6% of glomerular damages were found among residents of the highly polluted area in this study. This demonstrates that long-term cadmium exposure gives rise mainly to renal tubular dysfunction in the general population but only to a limited extent to glomerular damage. It is important to take adequate preventive measures to protect the general population from environmental cadmium exposure, and particularly important to prohibit the discharge of wastewater without prior treatment. Fortunately, the local authority has realized the importance of environmental protection, and after 1995 most of the fields of the polluted area were used for other purposes than to growing rice.

The dose-response relationship between cadmium exposure, urinary cadmium and renal effects in populations exposed to cadmium is well documented (Friberg *et al.* 1986; Nogawa & Ishizaki 1979; Lauwerys & De Wals 1981; Ishizaki *et al.* 1989; Kido & Nogawa 1983; Kjellstrom 1986; Nordberg *et al.* 1985; Yamaka *et al.* 1998). However, the relationship between blood cadmium and renal dysfunction has not previously been well described. The present study shows a good dose-response relationship between blood cadmium and the prevalence of renal dysfunction for the population concerned. A possible explanation for this situation is that the relationship between BCd and the response depend on the duration and intensity of the exposure to cadmium of the particular population studied. Prolonged high-level exposure over a period of approximately 35 years via the diet explains the good relationship found in the present study. A somewhat different relationship can be expected in populations under other conditions. The fact that the farmers in the most highly exposed area stopped eating contaminated rice 2 years before the present study may further have improved the usefulness of BCd as an indicator of body burden, since the short half-life compartment of BCd would to a large extent have disappeared. The BCd in the present study will thus represent mainly the long half-life compartment indicative of the body and renal burden of Cd (cf Nordberg and Nordberg 1988; Jarup *et al.* 1983).

Urinary cadmium as an indicator of the internal dose of cadmium has mainly been used in studies of the dose-response relationship. Many studies have shown that the prevalence of renal dysfunction such as β_2-microglobulinuria increases significantly when urinary cadmium exceeds 5 ug/g creatinine. The present results are in agreement with these observations. An intake of 2 g of cadmium has been reported (Nogawa *et al.* 1992) to result in β_2-microglobulinuria and met-

allothioneinuria in both sexes. In the present study, a total uptake exceeding 100 mg calculated on the assumption of 5% fractional uptake of cadmium gave rise to tubular dysfunction. This corresponds to a cumulative intake of 2,000 mg and supports the previous observations of an increased prevalence of renal dysfunction at such an intake. The critical concentration of cadmium in urine and kidney cortex is still a matter of debate. Further studies are therefore needed.

The hierarchical classification showed that total renal damage, glomerular and tubular damage were significantly related to the level of cadmium exposure, and accumulation (BCd, UCd, and Cd uptake). Only one index of renal tubular dysfunction was affected in subjects exposed to low levels of cadmium, but changes in two tubular indicators occurred more frequently among those with high UCd or BCd levels. Thus more indices were involved as exposure to cadmium was increased.

This project has been of great value to China in improving the performance of biological monitoring and increasing knowledge of quality control and showing how preventive action can be taken to improve the quality of life by investigating the effects of exposure. The educational value of this study is great, since the techniques used are also applicable to agents other than cadmium. The project also shows that epidemiological studies can be performed as joint projects between countries and laboratories of different backgrounds and with different facilities for quality control in biological monitoring, thereby improving the exchange of knowledge between countries.

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