Renal tubular function of workers exposed to low levels of cadmium

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ABSTRACT Cadmium induced renal tubular effects were examined in 65 female workers in a factory manufacturing nickel cadmium batteries. Urinary β 2-microglobulin (β 2m), urinary N-acetyl-D-glucosaminidase activity (NAG), and serum creatinine and serum urea concentrations were used to assess the renal effects. Of the four measures, only urinary NAG and urinary β 2m showed a strong positive correlation with blood cadmium concentrations ($r = 0.49$ and 0.43 respectively); NAG showed a weaker correlation with urinary cadmium concentrations ($r = 0.35$). Urinary β 2m has weak correlation with urinary cadmium ($r = 0.04$). Only urinary NAG showed a significant deterioration in renal function among the exposed group. NAG detects the largest proportion of abnormalities among the exposed group. Abnormal urinary β 2m is detected in only 15.4% of the workers, half of whom have blood cadmium above $10 \mu\text{g/l}$. The proportion of abnormalities detected by urinary NAG differs significantly from the proportion of abnormalities detected by urinary β 2m ($p < 0.01$). The age adjusted mean urinary NAG excretion showed a significant rise with urinary cadmium of above $3 \mu\text{g/g}$ creatinine. Urinary β 2m failed to show any significant rise. With blood cadmium concentrations, the age adjusted mean urinary NAG excretion showed a rise from $1 \mu\text{g/l}$ of blood cadmium followed by a plateau between blood cadmium concentrations of $3-10 \mu\text{g/l}$. No significant rise in mean urinary excretion in β 2m was seen until blood cadmium concentrations exceeded $10 \mu\text{g/l}$.

Many studies have described evidence of tubular damage or dysfunction among workers exposed to cadmium. The most typical finding is increased urinary excretion of low molecular weight proteins such as β 2-microglobulin (β 2m).¹⁻⁷ In tubular dysfunction the activity of urinary enzymes is also increased, especially those such as N-acetyl-D-glucosaminidase (NAG)⁸ that have high specific activity in the tubules. Recently NAG has been shown to be a sensitive indicator of renal damage after occupational exposure to heavy metals⁹⁻¹³ and is seen as having considerable potential for screening workers engaged in handling nephrotoxic chemicals.⁸

The aim of the present study was to compare the use of urinary NAG activity with other measures of renal function.

Materials and methods

SUBJECTS

Sixty five female production workers from a nickel cadmium battery factory were examined; they had been working at the factory for between one and 14 years (mean 3.7 years). Only those who fulfilled the following criteria were selected for the study:

- (a) no past history of renal disease, hypertension, or diabetes mellitus;
- (b) no family history of renal disease, hypertension, or diabetes mellitus;
- (c) no evidence of haematuria on clinistix examination;
- (d) systolic blood pressure of less than 140 mm Hg and diastolic blood pressure below 90 mm Hg;
- (e) non-smoker; and
- (f) not pregnant.

Nine sedentary female office staff with no history of exposure to cadmium formed the non-exposed group.

Table 1 General characteristics of study population

	Exposed group (n = 65)	Non-exposed group (n = 9)
Age distribution (years):		
< 20	2 (3.1)	0
20-29	18 (27.7)	1 (11.1)
30-39	26 (40.0)	8 (88.9)
40-49	16 (24.6)	0
≥ 50	3 (4.6)	0
Mean	34.6	32.7
SD	8.8	5.5
Racial distribution:		
Chinese	18 (27.7)	7 (77.8)
Malays	38 (58.5)	2 (22.2)
Indians	9 (13.8)	0

They were selected using the same criteria as the exposed group.

BIOLOGICAL MEASUREMENTS

Blood sample was obtained by venepuncture using cadmium free disposable syringes. An early morning urine sample was collected and adjusted to pH 7 to avoid degradation of β 2m.¹⁴ Samples were collected and stored in cadmium free tubes.

The following analyses were made:

- (i) blood cadmium;
- (ii) urinary cadmium;
- (iii) urinary NAG activity;
- (iv) urinary β 2m concentration;
- (v) urinary creatinine;
- (vi) serum creatinine; and
- (vii) serum urea.

Blood and urinary cadmium concentrations were assayed using a graphite atomic absorption spectrophotometer. External quality control was carried out under the National External Quality Assessment Scheme (NEQAS) in the United Kingdom and the Interlaboratory Comparison Programme of Centre de Toxicologie du Quebec in Canada. Urinary β 2m concentration was assayed using an enzyme immunoassay test kit (Phadezym beta2-micro test); the estimation of urinary NAG activity was performed by the method of Noto *et al.*¹⁵

The urinary measurements were all corrected with the urinary creatinine concentration and expressed per

Table 2 Blood and urinary cadmium concentrations of study population

	Exposed group	Non-exposed group
Blood cadmium (μ g/l):		
Mean	7.57	0.79
SD	5.89	0.44
Range	1.00-25.80	0.20-1.40
Urinary cadmium (μ g/g creatinine):		
Mean	1.73	0.09
SD	3.00	0.08
Range	0.05-20.67	0.02-0.21

gram creatinine; NAG activity was expressed as nmol/h/mg creatinine.⁸

ENVIRONMENTAL MONITORING

The time weighted average concentration of cadmium in the air was estimated during normal work between 0930 and 1630. The concentration on the various production lines ranged from 0.007 to 0.039 mg/m³. This is below the 0.05 mg/m³ recommendation by ACGIH.¹⁶

Results

GENERAL DESCRIPTION OF STUDY POPULATION

Of the exposed group 29% were over 40 whereas most of the non-exposed were in the 30 to 39 age group. Most of the exposed group were Malays whereas the non-exposed were mainly Chinese (table 1).

As expected the blood and urinary cadmium concentrations of the exposed group are much higher than those of the non-exposed group (table 2).

CORRELATION OF BLOOD CADMIUM AND MEASURES OF RENAL FUNCTION

Blood and urinary cadmium concentrations had a correlation coefficient of 0.36 (table 3). Of the four measures of renal function, only NAG and β 2m correlated well with the blood cadmium concentration (0.49 and 0.43 respectively). Urinary cadmium had a poor correlation with urinary β 2m ($r = 0.04$) but was moderately well correlated with urinary NAG ($r = 0.35$). The correlation coefficient between urinary NAG and urinary β 2m was 0.37.

Table 3 Correlation matrix of blood cadmium with measures of renal function

	Blood cadmium	Urinary cadmium	Serum creatinine	Blood urea	NAG	β 2m
Blood cadmium	1.00	0.36	-0.02	0.05	0.49	0.43
Urinary cadmium		1.00	0.01	0.09	0.35	0.04
Serum creatinine			1.00	0.07	-0.04	0.24
Blood urea				1.00	0.18	0.08
CNAG					1.00	0.37
β 2m						1.00

Table 4 Age adjusted measures of renal function of exposed and control groups

	Exposed group		Non-exposed group		p Value
	Mean	Adj mean*	Mean	Adj mean*	
Serum creatinine (mg/dl)	0.92	0.92	0.81	0.81	0.12
Serum urea (mg/dl)	27.06	27.00	23.11	23.59	0.32
Urinary β 2m (μ g/g creatinine)	213.2	212.1	95.2	103.2	0.55
Urinary NAG (nmol/h/mg creatinine)	218.4	218.3	108.6	108.5	0.04

*Adjusted for age using analysis of covariance (ANCOVA).

COMPARISON BETWEEN EXPOSED AND CONTROL GROUPS

When the age adjusted measures of renal function are compared between exposed and non-exposed groups, each was higher in the exposed group (table 4) but NAG activity showed a statistically significant difference between the groups ($p = 0.04$).

PROPORTION OF WORKERS WITH ABNORMAL MEASUREMENTS

Several studies had derived different normals for urinary NAG activity. Most studies in the West, presumably using Caucasian subjects, give an upper normal limit (± 2 SDs) of between 56.0 to 101.5 nmol/h/mg creatinine (table 5). An earlier study in Singapore using a similar method of determination gave an upper normal limit of 147.0 nmol/h/mg creatinine.¹² The present study on the nine sedentary staff gave a slightly lower value of 139.0 nmol/h/mg creatinine.

Urinary NAG activity detected the largest proportion of abnormalities in the exposed group whichever of the last two upper limits was used (table 6). Furthermore, with increasing blood cadmium concentrations, an increasing proportion of workers had abnormal urinary NAG activity. For exposed workers with a blood cadmium below 5 μ g/l, more than 50% had abnormal NAG levels. Only 15.4% of the exposed workers had abnormal urinary β 2m levels, and of these, only one had a normal urinary NAG activity (table 7). Of the exposed workers, 33 had normal urinary β 2m but abnormal urinary NAG activity. This difference in distribution is highly significant (Sign test, 2 tailed $p < 0.001$).¹⁷

Table 6 Proportion of workers with abnormal renal function

	Non-exposed	Exposed group, blood cadmium concentrations (μ g/l)				
		< 3.0	3.0–< 5.0	5.0–< 10.0	≥ 10.0	Total
No of subjects	9	13	15	24	13	65
Serum urea (> 40 mg/dl)	0	0	2 (13.3)	1 (4.2)	0	3 (4.6)
Serum creatinine (> 1.3 mg/dl)	0	0	0	1 (4.2)	0	1 (1.5)
β 2m (> 200 μ g/g cr)	0	2 (15.4)	0	3 (12.5)	5 (38.5)	10 (15.4)
NAG (> 147) ^a	0	5 (38.5)	9 (60.0)	15 (62.5)	10 (76.9)	39 (60.0)
NAG (> 139) ^a	0	6 (46.2)	9 (60.0)	16 (66.6)	11 (84.6)	42 (64.6)

^aNAG activity expressed as nmol/h/mg creatinine.

DOSE RESPONSE RELATION

With increasing urinary cadmium concentrations, serum creatinine, serum urea, and urinary β 2m failed to show any consistent rise (figs 1 and 2). Age adjusted urinary NAG activity showed a statistically significant rise with urinary cadmium concentrations above 3 μ g/g creatinine (fig 3). Even a urinary cadmium concentration between 1 and 3 μ g/g creatinine produced a doubling of the age adjusted urinary NAG activity, although this was just outside the conventional levels of statistical significance ($p = 0.055$).

Table 5 Normal NAG values

Source	Study population	Mean $\pm$ SD	Upper limit ($+ 2$ SDs)
Wellwood <i>et al</i> ¹⁹	8 women	36 $\pm$ 10	56.0
Sherman <i>et al</i> ²⁰	18 subjects	23 $\pm$ 16	55.0
Tucker <i>et al</i> ²¹		57.5 $\pm$ 22	101.5
Ong <i>et al</i> ¹²	30 subjects	87 $\pm$ 30	147.0
Present study	9 women	109 $\pm$ 15	139.0

Similarly the age adjusted mean serum creatinine and serum urea concentrations failed to show a rise with increasing blood cadmium concentrations (fig 4). Even with blood cadmium concentrations greater than 10 μ g/l, no significant rise was noted. Urinary β 2m concentrations increased only when the blood cadmium was greater than 10 μ g/l (fig 5). Urinary NAG, however, showed a doubling with blood cadmium concentrations between 1 and 3 μ g/l (fig 6). This is followed by a plateau until the blood cadmium reaches above 10 μ g/l when the NAG activity was appreciably raised. The urinary cadmium concentration between this blood range was low (1.17 μ g Cd/g creatinine).

Table 7 Distribution of workers with abnormal NAG and β_2 -microglobulin

Description	No	%
Normal NAG, normal β_2 m	22	33.8
Normal NAG, abnormal β_2 m	1	1.5*
Abnormal NAG, normal β_2 m	33	50.8*
Abnormal NAG, abnormal β_2 m	9	13.9

*Significant difference between the proportion of abnormals using the Sign test (2 tailed).

Discussion

This study confirms that occupational exposure to cadmium results in renal tubular dysfunction. Among the four measures of renal function studied, NAG and β_2 m are the most sensitive. Conventional indices of renal function such as total urinary protein, serum urea, and serum creatinine are insensitive indicators of early renal dysfunction (Lauwerys R, Bernard A, at 1st Asia Pacific symposium on environmental and occupational toxicology, Singapore 1987) and become abnormal only when there is major impairment of renal excretory function.

Several indices had been suggested as early indicators of renal dysfunction¹⁸ (and Lauwerys R, Bernard A, Ouled A, at XXIInd International Congress of Occupational Health, Sydney 1987). These include the use of renal enzymes,⁸ which the assay of urinary NAG activity had proved to be useful and practical. There appears to be a great disparity between normal values for urinary NAG activity obtained from different studies.¹²⁻²¹ This may be attributed to differences in methods of NAG determination and possibly to racial differences.

The present study suggests that urinary NAG activity is a more sensitive indicator of early renal tubular impairment in workers exposed to cadmium than β_2 m. NAG appears to be raised at lower blood and urinary cadmium concentrations and is capable of detecting more abnormalities among the exposed

Fig 1 Age adjusted serum urea and serum creatinine by urinary cadmium.

Fig 2 Age adjusted β_2 -microglobulin by urinary cadmium.

workers than β_2 m. Himeno also found NAG to be more sensitive than β_2 m and total protein in his study on workers exposed to mercury.¹⁰ This observation is in contrast to a study by Bernard *et al* in which NAG appeared to be much less frequently raised than β_2 m and retinol binding protein in patients.²² Verschoor *et al* in their recent study among workers exposed to cadmium found a significant relation between urinary NAG activity and urinary cadmium concentrations. They concluded, however, that urinary β_2 m appeared to be more sensitive.

An interesting finding in the present study is the "plateau" effect of urinary NAG activity when blood cadmium concentrations reach 3–10 µg/l. The amount of NAG excreted in this range is high (168.0–186.0 nmol/h/mg creatinine). With acute exposure, the initial high dose of a toxic substance results in an immediate increase in urinary enzyme activity but a second dose fails to provoke an increase. This is possibly due to an extensive loss of tubular cells resulting from the initial dose and because regenerating cells are less susceptible to the toxic agent.^{8,23} The presence of normal urinary NAG activity among

Fig 3 Age adjusted NAG by urinary cadmium.

Fig 4 Age adjusted serum urea and serum creatinine by blood cadmium.

workers with high blood lead concentrations has also been explained by these mechanisms.⁹

In the present study cadmium exposure was low and the initial high and subsequent low urinary enzyme activity seen in animal studies of acute toxic effects would not be expected. The constant NAG activity between 3 and 10 µg/l of cadmium is probably due to a gradual increase in tissue resistance that is keeping pace with the increasing cadmium load. Alternatively, it may indicate that blood cadmium correlates poorly with urinary cadmium at blood concentrations below 10 µg/l and is a poor indicator of the renal cadmium load.

The long term consequence of abnormal measures of renal function (such as β_2 m) is the subject of much debate. Such abnormalities are suggestive of early irreversible renal dysfunction (Lauwerys R, Bernard A, Singapore 1987) and a significant excess of deaths from renal disease has been reported among workers with more than 15 years exposure to cadmium.²⁴ Abnormalities in the renal handling of protein are to be considered as an adverse effect²⁵ and the maximal biological limit of 5 µg Cd/g creatinine may need to be reviewed.²⁶

Fig 5 Age adjusted β_2 -microglobulin by blood cadmium.

Fig 6 Age adjusted NAG by blood cadmium.

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