Urinary N-acetyl- β -D-glucosaminidase activity in workers exposed to inorganic lead

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Abstract

Urinary N-acetyl- β -D-glucosaminidase (NAG) had been shown to be a useful early marker of renal injury. In workers exposed to lead it seems to be the only early marker but the dose response and dose effect relations are weak. Furthermore, the significance and underlying mechanism of increased urinary NAG activity is far from clear. By studying the isoenzyme profiles of urinary NAG, the significance and underlying mechanism may be further clarified. The heat labile (NAG-A) and heat stable (NAG-B) isoenzyme profiles of 128 workers exposed to lead from a lead stabiliser factory were analysed. NAG activity was expressed as total NAG, NAG-A, and NAG-B activity as well as ratios (NAG-B/total NAG and NAG-B/NAG-A). Exposure indices included the recent concentration of blood lead (BPb), a cumulative blood lead index (TBPb), and the recent change in concentration of blood lead (CBPb). The NAG indices correlated best with CBPb. Nearly 50% of the variation in NAG-B activity could be explained by the combination of all three exposure indices but only the CBPb was highly significant. When these exposure indices were entered separately into the regression equation, CBPb accounted for 36.3% of the variation in NAG-B activity, 5.7% was accounted for by TBPb and 2.7% by BPb. There was also no dose-effect or dose-response relation between the NAG variables and BPb or TBPb groups. With CBPb, there were dose-effect and dose-response relations. With CBPb, there was an increase in NAG variables in the group with more than 25% increase in blood lead over the past six months. The increase in NAG activity in this study is likely to be due to a recent increase in concentration of blood lead and hence presumably a recent rise in renal burden of inorganic lead. This suggests that the increase in urinary NAG activity is a form of acute response to a sharp increase in renal burden of lead, rather than to a cumulative dose. Heat stable NAG is part of the lysosomal membrane and is present in the urine when there is breakdown of lysosomes. Our data therefore contradict suggestions that the increase in urinary NAG activity is due to exocytosis.

The urinary activity of N-acetyl-D-glucosaminidase (NAG) was shown to be increased in the early stages of renal injury, even before the onset of abnormalities in excretory function.¹ It has been used as a marker of proximal tubular dysfunction among workers exposed to cadmium² and inorganic lead.³⁻⁵ Seemingly, the cells lining the proximal tubules are most sensitive to lead. The early stage is characterised by the appearance of intranuclear bodies in the proximal tubules.⁶ There is, however, no specific proteinuria. The challenge is to identify a reliable early biological indicator of the kidney damage caused by lead.⁷

Urinary NAG seems to be the only marker indicating the presence of early lead nephropathy.⁸ Meyer *et al* reported an increase in urinary NAG activity among 29 workers exposed to lead.³ Verschoor *et al* also reported a small increase in urinary NAG activity among workers exposed to lead.⁴ NAG seemed to be even more sensitive than retinol binding protein (RBP). Coratelli *et al* found a significantly higher NAG activity compared with matched controls.⁵ In most of these studies, however, there was no strong dose-effect or dose-response relation when blood lead was used as a marker of internal dose.^{3,4} Furthermore, the biological significance and the underlying mechanism leading to increased urinary NAG activity in the urine is unclear.^{8,9}

In human kidneys, there are two main NAG isoenzymes.^{10,11} Isoenzyme A is part of the soluble intralysosomal compartment normally secreted in urine by exocytosis.^{11,12} Isoenzyme B is linked to the lysosomal membrane and is excreted in the urine during tubular damage. The two isoenzymes differ in their heat sensitivity. Isoenzyme A is heat labile whereas isoenzyme B is heat stable.¹³ It had been shown that they can be easily separated by heating the urine samples.^{13,14} By studying the isoenzyme profile, the significance and mechanism leading to increased urinary NAG activity may be further clarified.⁹

The aim of this paper is to study the urinary NAG isoenzyme profile among workers with chronic exposure to inorganic lead.

Materials and methods

STUDY SUBJECTS

The exposed population consisted of 128 workers from a factory manufacturing lead stabiliser. The workers are involved in smelting of lead ingots and the packing of litharge

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(lead oxide) and lead stearate stabilisers. The workers are usually rotated through these various sections and had considerable exposure throughout their working life. The non-exposed group consisted of 152 factory workers with no significant history of exposure to lead or cadmium. The blood lead concentrations ranged from 3.2 to 16.4 $\mu\text{g}/100\text{ ml}$. For a subsample of the non-exposed group, the blood cadmium concentrations ranged from 0.3 to 0.9 $\mu\text{g}/\text{l}$.

The subjects were selected by the following criteria: (1) no history of diabetes, hypertension, or renal diseases; (2) no evidence of haematuria, pyuria, or glycosuria on urinary clinistix examination; (3) systolic blood pressure of less than 140 mm Hg and a diastolic pressure of less than 90 mm Hg; and (4) no use of antibiotics or analgesic for the two weeks before examination.

BIOLOGICAL MEASUREMENTS

A blood sample was obtained by venepuncture with lead free disposable syringes and stored in lead free bottles. A fresh urine sample was collected at the factory between 0900

and 1000. The urine collected was immediately buffered and kept in an ice box for transportation.

Blood lead was determined using an atomic absorption spectrophotometer with a graphite furnace. External quality control was carried out under the National External Quality Assessment Scheme (NEQAS) in the United Kingdom. Table 1 shows the accuracy in determination of blood lead since 1985.

The historical concentrations of blood lead of the exposed workers were also obtained. Concentrations of blood lead had been measured every six months since 1982. There were a total of 1040 examinations of blood lead. Of these, 70 (6.7%) were done before the external quality assurance scheme (before 1985). The average number of examinations per worker was 3.6 (range 1–20). As an index of the body burden of lead, a cumulative blood lead index (TBPb) was computed for each worker by calculating the area under the curve of BPb against time with the formula:

$$0.5 \sum (t_{i+1} - t_i)(y_i + y_{i+1}),$$

where y_i is the measurement at time t_i and y_{i+1} is the next measurement at time t_{i+1} (fig 1).

Not all workers had a pre-employment estimation of blood lead. To control for the variation in the time between first exposure and first estimation of blood lead, the first reading of blood lead was extrapolated backwards to 15 years of age and a concentration of blood lead of 15 $\mu\text{g}/100\text{ ml}$. The concentration of 15 $\mu\text{g}/100\text{ ml}$ was chosen because this was the mean blood lead value of those workers with pre-exposure blood lead estimation.

The percentage change in blood lead (CBPb) over the six months before the analyses of NAG was used as a third index of exposure. This index was used as a measure of recent change in exposure.

Urinary creatinine was measured for all subjects and serum creatinine was determined in 50 of the exposed workers. Urinary NAG activity was measured according to the MCP-NAG method of Noto *et al*¹⁵ and expressed as nmol/min/g creatinine. NAG has a molecular weight of 140 000 and is not normally filtered at the glomerulus in the absence of glomerular damage. The NAG activity in the urine can also be expressed as a function of creatinine clearance (cr cl) as assayed by serum/urine creatinine ratio (nmol/min/ml cr cl).¹⁶

Figure 1 Computation of cumulative blood lead index (TBPb).

Figure 2 Environmental concentration of lead in the factory 1985–91.

Table 1 Accuracy of estimation of blood lead

Year	Range $\mu\text{g}/100\text{ ml}$	Mean difference between local and reference laboratory $\mu\text{g}/100\text{ ml}$ (%)
1985	31.3–72.3	2.94 (6.13)
1986	23.4–71.7	1.41 (2.88)
1987	25.3–69.6	1.94 (3.99)
1988	27.1–73.1	1.68 (3.16)
1989	23.8–64.9	2.88 (6.49)
1990	18.9–68.4	2.27 (5.15)
1991	16.2–64.4	1.82 (4.68)
1992	18.4–60.3	1.17 (2.98)
Overall	16.2–73.1	2.00 (4.44)

Table 2 Description of study population

	Non-exposed	Exposed
Subjects (n)	152	128
Mean age (y (SD))	27.1 (7.10)	28.4 (7.28)
Race n (%):		
Chinese	77 (50.7)	19 (14.8)
Malays	57 (37.5)	67 (52.3)
Indians	18 (11.8)	42 (32.8)
Duration of service (y (SD))	—	2.9 (3.39)
Current concentration of blood lead (μg/100 ml):		
Mean* (range)	8.7 (3.2–16.4)	29.6 (3.9–66.6)
Median	8.7	33.8
Cumulative blood lead index (μg-y/100ml):		
Mean* (range)	—	225.0 (77.0–688.8)
Median	—	208.3
Recent change in concentration of blood lead (%):		
Mean (SD)	—	5.8 (24.39)
Median (range)	—	1.8 (–44.3 to +100)

*Geometric means.

Table 3 Pearson's correlation coefficients between various variables of exposure and effect

	Log (TBPb)	Log (BPb)	CBPb
Log (total NAG) (nmol/min/g cr)	0.025 (128)	–0.008 (128)	0.561 (105)
Log (Total NAG) (nmol/min/ml cr cl)	0.123 (50)	0.164 (50)	0.646 (35)
Log (NAG-B) (nmol/min/g cr)	–0.086 (92)	–0.061 (92)	0.503 (79)
Log (NAG-B) (nmol/min/ml cr cl)	–0.165 (45)	–0.088 (45)	0.602 (32)
Log (NAG-A) (nmol/min/g cr)	0.215 (92)	0.192 (92)	0.312 (79)
Log (NAG-A-G) (nmol/min/ml cr cl)	0.170 (45)	0.308 (45)	0.567 (32)
NAG-B/Total	–0.246 (92)	–0.189 (92)	0.398 (79)
NAG-B/NAG-A	–0.174 (92)	–0.174 (92)	0.440 (79)

( ) Number of subjects; cr = creatinine, cr cl = creatinine clearance.

Figure 3 Proportion of subjects with abnormal NAG indices by recent change in concentration of blood lead.

The separation of the heat stable NAG-B and heat labile NAG-A isoenzymes was carried out by heating the urine sample in a water bath for 30 minutes at 55°C.¹⁴ The amount of NAG-A was calculated by subtracting NAG-B from the total NAG. The ratios of NAG-B/total NAG and NAG-B/NAG-A were also used as indices of NAG activities.

All the urinary NAG variables (except for those expressed as a function of creatinine clearance) were standardised for variability in urine flow using the urinary creatinine concentration and expressed per g creatinine.¹

ENVIRONMENTAL MONITORING

Regular environmental measurements of lead in air were conducted from 1985 (fig 2). There were at least two measurements each year. Except for the smelting area, the environmental concentration of lead was generally below the threshold of 0.15 mg/m³ recommended by the American Conference of Governmental Industrial Hygienists (ACGIH).¹⁷

STATISTICAL ANALYSES

The distributions of urinary NAG, BPb, and TBPb were highly skewed. Logarithmic transformation was used to normalise the distribution. Multiple linear regression analysis was used to compare the relative importance of BPb, TBPb, and CBPb as predictors of increased activities of urinary NAG isoenzymes after adjustment for race. As BPb, TBPb, and CBPb are not highly correlated (correlation coefficients for BPb v TBPb = 0.43, TBPb v CBPb = –0.12, and BPb v CBPb = 0.05), they were entered into the same regression equation simultaneously. Age was not entered into the equation as it is highly correlated with TBPb ($r = 0.82$).

To assess the dose-effect relation by various categories of exposure indices, the mean NAG values were compared between groups after adjustment for age and race by analysis of covariance (ANCOVA). The 95% confidence intervals (95% CIs) for the adjusted means were constructed. To assess the dose-response relation, the normal values for each variable were set at the 90th percentile of the non-exposed group and the trend χ^2 test, or exact probability test when numbers were small, were used.

Results

DESCRIPTION OF STUDY POPULATION

The mean age of the exposed and non-exposed groups were comparable, but their racial distribution was different (table 2). The difference in racial distribution needed to be considered in the subsequent multivariate analyses. The mean duration of exposure for the exposed workers was 2.9 (range <1–12.5) years. The median current concentration of blood lead was 33.8 (range 3.9–66.6) μg/100 ml. The median TBPb was 208.3 (range 77.0–688.8) μg-year/100 ml. The CBPb ranged from –44.3% to +100%.

Table 4 Relative importance of current blood lead (BPb), cumulative blood lead (TBPb) and percentage change in blood lead (CBPb) as predictors of various NAG indices after adjustment for race

Variable	Standardised partial regression coefficients			
	BPb	TBPb	CBPb	r ²
Total NAG (nmol/min/g cr)	-0.11	0.04	0.55***	0.345
Total NAG (nmol/min/ml cr cl)	-0.16	-0.06	0.64***	0.452
NAG-B (nmol/min/g cr)	-0.25**	-0.13	0.56***	0.381
NAG-B (nmol/min/ml cr cl)	-0.27	-0.18	0.58***	0.497
NAG-A (nmol/min/g cr)	-0.16*	0.01	0.31***	0.185
NAG-A (nmol/min/ml cr cl)	-0.02	-0.02	0.58***	0.324
Ratio, NAG-B total	-0.12	-0.18	0.43***	0.214
Ratio, NAG-B/NAG-A	-0.11	-0.17	0.46***	0.249

*p < 0.05; **p < 0.01; ***p < 0.0001.
cr = creatinine; cr cl = creatinine clearance.

INDICES OF EXPOSURE AND EFFECTS

Of the three measurements, CBPb had the strongest relation with each of the NAG variables (table 3). Furthermore, expressing the NAG activity as nmol/min/ml cr cl had higher correlation coefficients compared with NAG corrected to urinary creatinine. When NAG isoenzyme activity was expressed as a ratio, the ratio NAG-B/NAG-A had the highest correlation coefficient.

The CBPb was also the most important predictor for each of the NAG variables after adjustment for race (table 4). For each NAG variable, the CBPb was highly significant. The multiple correlation coefficient (r²) was also higher when NAG was expressed as a function of cr cl. When these exposure

indices were entered separately into the regression equation, CBPb accounted for 36.3% of the variation in NAG-B activity, TBPb 5.7%, and BPb 2.7%.

DOSE-EFFECT AND DOSE-RESPONSE RELATIONS
There was no significant difference in NAG activity between exposed and non-exposed groups. The NAG-B activity was slightly higher among exposed subjects (table 5). There was also no dose-effect or dose-response relation between BPb or TBPb and the various mean NAG activities.

With the CBPb groups, the mean NAG activity was higher when the CBPb was >25% (table 5). The 95% CIs of the adjusted means were, however, quite wide. The proportion of subjects with abnormal NAG concentrations was also significantly higher in this group (fig 3).

Most of the subjects with CBPb of more than 25% had a low to moderate concentration of blood lead in the previous six months (table 6). The range was between 7.40 and 40 µg/100 ml. There seems to be no correlation between the NAG activity and the previous concentrations of blood lead.

Discussion

In the study by Meyer *et al*³ on 29 exposed workers urinary NAG activity was increased

Table 5 Age and race adjusted mean urinary NAG activity between exposed and non-exposed groups and percentage change in blood lead

Variable	Non-exposed	Exposed	Percentage change in blood lead			
			<-5%	-5 to +5%	5 to 25%	>25%
Total NAG† (nmol/min/g cr):						
No	149	128	31	30	26	18
Adjusted*	1.82 (0.3-89)	1.90 (0.3-98)	1.61 (0.3-79)	1.66 (0.3-86)	1.60 (0.3-80)	4.17 (1.92-6.42)
Heat-stable NAG (B)† (nmol/min/g cr):						
No	84	92	29	22	19	9
Adjusted*	0.41 (0.2-58)	0.49 (0.2-65)	0.36 (0.2-52)	0.41 (0.2-68)	0.41 (0.2-74)	1.83 (0.4-36)
Ratio of NAG-B/NAG-A:						
No	84	92	29	22	19	9
Adjusted	0.35 (0.25-0.44)	0.46 (0.37-0.55)	0.31 (0.17-0.44)	0.31 (0.16-0.46)	0.35 (0.18-0.52)	0.67 (0.48-0.86)

*Adjusted for age and race by analysis of covariance (ANCOVA).

†Geometric means.

95% CI of adjusted means in parenthesis.

Table 6 List of subjects with more than a 25% change in their recent concentrations of blood lead

Subject	TBPb (µg y/100ml)	Previous BPb (µg/100ml)	Current BPb (µg y/100ml)	CBPb (%)	Total NAG (nmol/min/g cr)	NAG-B (nmol/min/g cr)
1	278.5	7.4	13.4	81.1	7.18	—
2	104.5	13.7	21.9	59.9	8.72	—
3	172.7	16.7	23.4	40.1	5.47	2.54
4	77.0	15.5	26.7	72.3	11.22	—
5	189.0	18.9	27.0	42.9	4.86	2.15
6	164.1	23.4	29.6	26.5	0.80	—
7	133.7	23.9	30.2	26.4	1.47	—
8	89.9	15.8	31.6	100.0	16.07	—
9	143.0	19.8	32.1	62.1	4.11	2.20
10	260.7	20.4	32.4	58.8	5.92	2.76
11	463.0	25.0	33.0	32.0	3.75	1.39
12	182.8	26.0	34.9	34.2	5.37	2.28
13	481.5	28.4	36.4	28.2	2.57	—
14	178.3	27.8	39.0	40.3	8.70	5.04
15	664.1	32.4	44.7	38.0	6.73	—
16	170.2	34.5	46.6	35.1	1.69	0.68
17	225.9	37.9	47.4	25.1	1.40	0.53
18	254.4	40.0	52.6	31.5	2.13	—

cr = creatinine.

but there was no relation between current concentration of blood lead and NAG activity. In fact, the five workers with a blood lead concentration greater than 70 µg/100 ml had normal NAG values. The addition of lead to urine at reasonable biological concentrations failed to inhibit the activity of NAG. They concluded that urinary NAG activity is increased among workers with concentrations of blood lead below 60 µg/100 ml, which is believed to be the threshold for a nephrotoxic effect.⁶ There was, however, no explanation for the normal NAG activity at concentrations of blood lead of more than 70 µg/100 ml.

In the report by Verschoor *et al.*,⁴ there was a small increase in urinary NAG activity with increasing concentrations of blood lead, but analysis matched for age, smoking habits, socioeconomic state, and duration of employment did not show a significant difference between exposed and non-exposed workers. Similarly, Gerhardtsson *et al.* did not find any difference in urinary NAG activity or other sensitive markers of renal dysfunction between exposed and non-exposed groups.¹⁸ There was no correlation between urinary NAG activity and current concentrations of blood lead, cumulative blood lead (calculated as a sum of annual concentrations of blood lead) or concentrations of lead in the tibia and calcaneum. Coratelli *et al.* also reported no correlation between urinary NAG activity and "biological indicators of lead poisoning"⁵ although there was correlation between the duration of exposure and NAG activity.

The lack of relations between NAG activity and various indices of lead exposure may be due to several factors. Firstly, the various exposure indices may not reflect the renal burden of lead. Furthermore, the early effects of inorganic lead may not be at the proximal tubular cells where NAG is in high concentration. Alternatively the increase in urinary NAG may not be due to the chronic accumulation of lead in the tubules. In our present study, there was also a weak relation between the various indices of NAG activity and the current or cumulative concentrations of blood lead. The relation with recent change in concentration of blood lead is much stronger. This suggests that increase in urinary NAG activity is an acute response to a sharp increase in renal lead. This may explain the findings of poor correlations between current or cumulative blood lead indices and NAG activity and the sharp increase in urinary NAG activity when workers were reexposed after a one month interruption of exposure.^{3-5 18}

The stronger relations between the recent change in blood lead and the indices of NAG-B activity rather than NAG-A activity is important because the heat labile isoenzyme A is believed to be found in urine through normal exocytosis and the heat stable isoenzyme B, which is bound to the lysosomal membranes, is only found in the urine when there is breakdown of the lysosomal membrane.^{11 12}

In our study the contribution of heat

stable NAG to total NAG was 22.5% in the control group. This is very much higher than the values of 5.4% to 9.5% reported by others.^{12 19} Inefficient denaturation of NAG-A in our study or partial denaturation of NAG-B in other studies may explain the difference. It is, however, equally possible that our population was exposed to some unidentified nephrotoxic agents that increased NAG-B excretion. Interestingly, there was a small but significant difference in concentrations of urinary NAG between Chinese, Malays, and Indians. Compared with our controls, there was only a small increase in the contribution of NAG-B to total NAG (table 5) caused mainly by an increase in a small group (n = 9) with a more than 25% increase in blood lead over the last six months. Total NAG excretion was increased in the same group. Because of the small number in this group, the role of lysosome breakdown in lead induced NAG excretion remains open. Our data, however, contradict suggestions on the role of exocytosis.⁸

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