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Creatinine adjustment of biological monitoring results

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Background Biological monitoring (BM) aids exposure assessment but where this is based on incomplete collections of single urine voiding measurement of creatinine is often used to adjust analyte concentrations for the effects of fluid balance.

Aims To provide reference data on creatinine concentrations in urine samples from a population of UK workers.

Methods Urine samples sent to the Health and Safety Laboratory were analysed for creatinine by an automated kinetic Jaffe technique using alkaline picric acid and the results stored in a database. Statistical analysis of the data used linear mixed effects models on the natural log-transformed data.

Results Between 1996 and 2007, the laboratory analysed 49 506 urine samples from 20 433 UK adult workers. In the 42 817 samples where gender was known, 93% were from men and 7% were from women. The overall mean and median creatinine concentrations were both 12 mmol/l corresponding to 1.36 g/l. The mean (13 mmol/l) and median (12 mmol/l) creatinine concentrations for men were higher than those (9 and 10 mmol/l, respectively) for women.

Conclusions Gender differences in creatinine concentrations and the range of 0.3–3.0 g/l (2.653 and 26.53 mmol/l) traditionally used for confirming acceptability of urine samples mean that 2.5% of samples from male and 9% from female workers were flagged as 'low creatinine' and required a repeat sample. In addition, care should be taken interpreting any apparent gender differences in BM results to ensure that they are due to exposure and not an artefact of creatinine adjustment.

Key words Biological monitoring; creatinine; occupational exposure; urine.

Introduction

Biological monitoring (BM), based on the analysis of hazardous substances or their metabolites in biological fluids, is a useful means of assessing systemic exposure through inhalation, ingestion and dermal absorption. Historically in the UK, BM was most used for statutory monitoring of exposure to lead but its practical utility has more recently found applications in a wide range of other chemical exposures, and there are now BM guidance values for over a hundred different substances [1–3].

In the UK, the Health and Safety Executive (HSE) developed its framework for BM in the mid 1990s. To increase the acceptance of BM, HSE has a preference for non-invasive sampling where possible [4]. In practice, this means collection of urine samples, but unlike clinical practice, where 24 h total urine collections are routine, the only practical workplace approach is that of collecting incomplete single voiding before, during or at the end of work. In such circumstances, the concentrations of analytes may be affected by urine concentration or dilution depending on the fluid balance. The most common approach to compensate for this involves measurement of the creatinine concentration in the sample and expression of the concentration of the analyte as a ratio of the creatinine concentration. This approach is not advocated for urine samples with very low (<0.3 g/l) or high (>3 g/l) concentrations of creatinine [2,3]. Whether it is appropriate to use creatinine to adjust analyte concentrations depends on whether the analyte and creatinine are excreted in the same way.

It has been known for many years that the production of creatinine in an individual reflects muscle mass [5]. Creatinine is the metabolite of creatine, an important energy store for muscles in the form of the creatine phosphate bond. The production of creatinine is relatively constant within an individual, increasing from childhood into adulthood with a slow decline after 65 years of age. The excretion of creatinine is almost entirely by glomerular filtration in the kidneys, therefore, measurement of creatinine in both blood plasma and urine has been used clinically for many

years to estimate glomerular filtration rate [6–8]. However, there is some minor renal tubular secretion, and possible tubular rediffusion, of creatinine that may cause deviations of creatinine excretion rates from expected at the extremes of diuresis. Routine analytical methods for creatinine have historically relied on the colorimetric Jaffe method [9,10], which, with accurate control of temperature and timings of readings found in autoanalysers, can give fairly precise and accurate estimates of creatinine concentration. Relatively recent colorimetric/spectrophotometric-specific enzymic methodologies have reduced the potential for interferences in the original Jaffe method [11].

In many cases, the mechanism of renal excretion of industrial chemicals is not known and the published BM guidance values are based on published studies with results expressed as either simple concentrations or creatinine-adjusted values. Comparison of BM results in one format to a guidance value, in the other relies on assumptions about median creatinine concentrations. For simplicity of calculation, a nominal creatinine concentration of 1 g/l is sometimes assumed but this is probably an underestimate of median creatinine concentrations. Reference values for creatinine excretion in clinical or general populations may include the young as well as adults and be inappropriate for populations of workers [12]. The work reported here aims to derive reference values for creatinine in urine samples from the UK working population, to use the dataset to develop a mixed effects analysis to identify important determinants of creatinine and to test the validity of the assumption of a nominal creatinine concentration of 1 g/l.

Methods

The Health and Safety Laboratory (HSL) analyses urine samples from workers in a wide range of workplaces with exposures to hazardous substances who have given informed consent for samples to be sent for analysis by occupational health practitioners and HSE Medical Inspectors. Since 1996, all BM data have been stored in a database together with basic worker details and some contextual information on exposure and we searched this database for data on creatinine concentrations in urine.

Urinary creatinine has always been measured at HSL by an automated kinetic Jaffe technique, where creatinine and alkaline picric acid produce a red/orange complex [13]. In essence, the assay uses a 1:10 sample:total volume ratio, after an initial 1 + 24 dilution of all urine samples. The reagent is alkaline picric acid (pH 13). Colour change at 500 nm is measured as the absorbance difference between 50 s and 135 s after mixing of sample and reagent. Standardization uses a stock creatinine hydrochloride dissolved in 0.1 M HCl. All reagent/sample additions, timing for absorbance measurements and maintenance of reaction mixture at 37°C are carried out on standard clinical chemistry automation. Most of the data collected were analysed on a COBAS MIRA S PLUS instrument. Long-term precision of the assay is 3–4% based on data from internal quality control results since 1995, for example, the overall coefficient of variation from the quality control material in use since March 2009 is 3.68%. The laboratory has participated in a number of external quality assurance schemes for creatinine over the period of this study. In the last 10 rounds of an external quality assurance scheme (RIQAS; Randox Laboratories, Crumlin, UK), the assay showed percentage biases of −0.33% (CI −2.20% to +1.36%) against all-method results (n = 213–304); −5.38% (CI −7.54% to −3.22%) against isotope dilution mass-spectrometry methods (n = 7–22 labs) and −4.48% (CI −6.37 to −2.57%) for enzymic methods (n = 5–8 labs). The lower limit of detection of the assay is ∼0.04 mmol/l.

The statistical analysis of urinary creatinine data used linear mixed effects models on the natural log-transformed data. Fixed effects were used to model differences due to age, smoking, gender and the time of day of the sample. Random person (worker) effects model other systematic differences between individuals, which are apparent with repeated measurements per individual. The model can be represented in the following form:

$$\ln\left(Y_{i,j}\right) = \mu + \delta_i + \alpha \times \text{age}_i + \beta_1 I_i(\text{smoker}) + \beta_2 I_i(\text{female}) + \beta_3 I_{i,j}(\text{PM}) + \varepsilon_{i,j},$$

Table 1. Summary of urine samples analysed for creatinine by the HSL 1996–2007

	All	Female	Male	Gender unknown
Number of samples	49506	3207	39610	6689
Number of individuals	20433	1558	15111	3764
Number of sites	1536	360	1413	173

Table 2. Age distribution of individuals at the times sampling

Age	16–22	22–28	28–34	34–40	40–46	46–52	52–58	58–64	64–70
Number (%)	1121 (2.3)	3310 (6.7)	4968 (10)	6105 (12)	5711 (12)	4395 (8.9)	3562 (7.2)	1527 (3.1)	152 (0.31)

where $Y_{i,j}$ is the jth urine creatinine measurement on the ith person, μ is the log-transformed baseline, α is the trend with age, and β_1, β_2 and β_3 are the differences (fixed effects) for smoking, gender and time of day, respectively. Capital 'I's represent indicator variables corresponding to whether the worker smoked, their gender and the timing of the sample. On a log scale, the random effects (δ_i) are assumed to be normally distributed with mean zero and standard deviation σ_{bp}, while the within-person variations ($\varepsilon_{i,j}$) were assumed to be normally distributed with mean zero and standard deviation σ_{wp}. A value of half the limit of detection was substituted in place of the small number (<0.2%) of urinary creatinine results less than the limits of detection. Where time of day was not recorded, post-shift values were considered to be PM and pre-shift values were considered to be AM.

Table 3. Summary statistics for urinary creatinine levels (mmol/l)

	All	Female	Male
1st percentile	1.5	1.1	1.8
5th percentile	3.2	2.0	3.7
10th percentile	4.6	2.9	5.2
25th percentile	8	5.2	8.5
50th percentile	12	8.8	12
75th percentile	16	13	16
90th percentile	20	18	21
95th percentile	23	21	24
99th percentile	30	28	31
Mean	12	9.8	13
Geometric mean	11	7.9	11
SD	6.3	6.1	6.2
Geometric SD	1.9	2.1	1.8
% of samples below limit of detection	0.14	0.03	0.01

Results

The dataset consisted of 49 506 spot samples on 20 433 UK adult workers, taken between 1996 and 2007. Further details of the dataset, including a breakdown by gender and age are given in Tables 1 and 2. In the 42 817 samples where gender was known, 93% were from men and 7% were from women. While the majority (66%) of individuals had provided just a single sample, the dataset nevertheless contains a substantial number of repeat measurements, with ~700 individuals having provided between ≥10 samples and 11 providing ≥100 samples. Approximately 13% of samples came from known smokers and 29% from known non-smokers. The smoking habits of the majority of workers were unknown.

Summary statistics for creatinine including various percentiles and the percentage of samples less than the limit of detection are given in Table 3. The mean urinary creatinine level was 12 mmol/l, though it was significantly lower for females (10 mmol/l) compared to males (13 mmol/l). Fewer than 0.2% of samples were less than the limit of detection.

The main findings from the mixed effects analysis are that creatinine levels decrease with age at a rate of 0.76% (95% CI 0.68–0.84%) per year (Figure 1), that men have substantially higher levels of urinary creatinine than women (~45% higher; Figure 2) and that creatinine levels are typically slightly lower in the afternoon than the

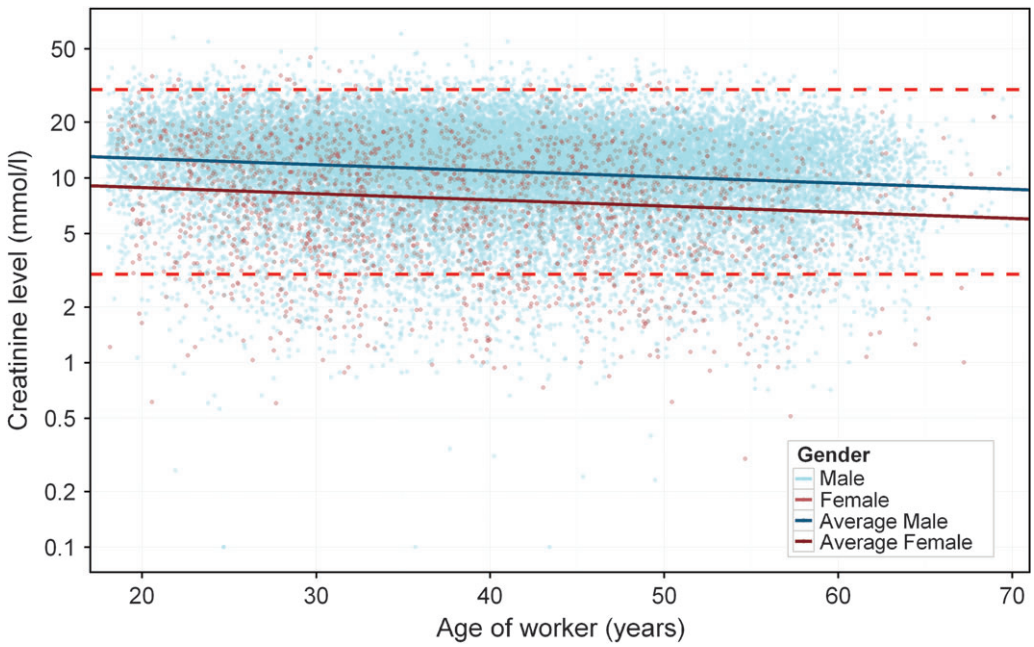

Figure 1. Relationship between male and female urinary creatinine concentrations and age.

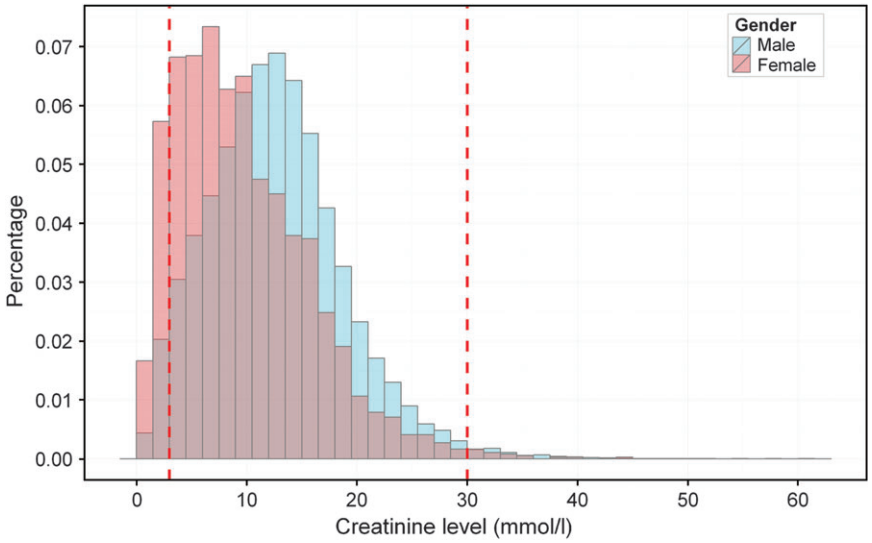

Figure 2. Distribution of male and female urinary creatinine concentrations.

morning (15%). These first two findings are consistent with creatinine levels being strongly related to an individual's muscle mass.

Smoking was not found to be a significant factor in determining creatinine levels. Inter and intra-individual variation in urinary creatinine were both relatively modest, at least in comparison with the variation seen in many biomarkers used to assess occupational exposure, with SDs (log scale) of 0.35 and 0.49, respectively.

Discussion

This study found that the mean (13 mmol/l) and median (12 mmol/l) creatinine concentrations for men were higher than the mean (9 mmol/l) and median (10 mmol/l) for women. The assumption of a nominal creatinine concentration of 1 g/l (8.8 mmol/l) sometimes used in conversions is really only applicable to women. The mean and median creatinine concentrations of 12 mmol/l for all 49 506 samples from adults in this study correspond to 1.36 g/l which may be more appropriate for such calculations for workplace populations.

The study showed that the range of creatinine concentrations of 0.3–3.0 g/l (2.653–26.53 mol/l) traditionally used for confirming acceptability of the sample [2,3], correspond to the 2.5th and 97.4th percentiles and the 8.7th and 98.4th percentiles, respectively, of the male and female creatinine distributions in this study. In practice, this means that although only 2.5% of samples from male workers will be flagged as 'low creatinine' and a repeat sample requested, 9% of samples from women would result in a request for a repeat sample. If the acceptable range was reduced to 0.5–2.5 g/l (4.4–22 mmol/l [14], it would result in ~15% of samples from women being outside the range at the low end and almost 10% of samples from men being outside at the high end. HSL uses the acceptable range of 0.3–3 g/l but reports results in SI units and rounds the values to give a range of 3–30 mmol/l and this results in 5% of all samples being flagged as low creatinine and 1% as 'high creatinine'.

BM guidance values are derived from published workplace studies that are likely to be based on predominantly male subjects. The gender differences in creatinine concentrations mean that care should be taken to check that any apparently higher creatinine-adjusted BM results in females are indeed due to higher exposure and not simply an artefact produced by adjustment of lower creatinine concentrations.

Key points

- The mean and median creatinine concentrations of 12 mmol/l for all 49 506 samples from adults in this study correspond to 1.36 g/l and this value should be used instead of 1 g/l for conversion calculations.
- The range of 0.3 and 3.0 g/l (2.653 and 26.53 mol/l) traditionally used for confirming acceptability of the sample corresponded to the 2.5th and 97.4th percentiles and the 8.7th and 98.4th percentiles, respectively, of the male and female creatinine distributions in this study. In practice this means that 2.5% of samples from men and 9% of samples from women result in a repeat sample request.
- Health and Safety Laboratory uses the acceptable range of 0.3–3 g/l and rounds the values in SI units to give a range of 3–30 mmol/l and this results in 5% of all samples from workers being flagged as low creatinine and 1% flagged as high creatinine.

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Conflicts of interest

None declared.

References

1. HSE. EH40/2005 Workplace exposure limits. Table 2 Biological Monitoring Guidance Values. http://www.hse.gov.uk/coshh/table2.pdf (8 December 2010, date last accessed).
2. DFG Deutsche Forschungsgemeinschaft. *List of MAK and BAT Values 2010. Commission of the Investigation of Health Hazards of Chemical Compounds in the Work Area*. Weinheim, Germany: Wiley-VCH Verlag GmbH, 2010. Report No. 46.
3. ACGIH. *TLVs and BEIs Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*. Cincinnati, OH: ACGIH, 2010.
4. HSE. *Biological Monitoring in the Workplace: A Guide to its Practical Application to Chemical Exposure*. *HSG 167*. Suffolk: HSE Books, 1997 http://www.hse.gov.uk/pubns/books/hsg167.htm (8 December 2010, date last accessed).
5. Moore F *et al*. *The Body Cell Mass and its Supporting Environment*. Philadelphia, PA: W.B. Saunders, 1963.
6. Camara AA, Arn KD, Reimer A *et al*. The twenty-four hourly endogenous creatinine clearance as a clinical measure of the functional state of the kidneys. *J Lab Clin Med* 1951;**37:**743–763.
7. Miller BF, Leaf A, Mamby AR, Miller Z. Validity of the endogenous creatinine clearance as a measure of glomerular filtration rate in the diseased human kidney. *J Clin Invest* 1952;**31:**309–313.
8. Burkhardt H, Bojarsky G, Gretz N *et al*. Creatinine clearance, cockcroft-gault formula and cystatin C: estimators of true glomerular filtration rate in the elderly? *Gerontology* 2002;**48:**140–146.
9. Taussky HH. A procedure increasing the specificity of the Jaffe reaction for the determination of creatine and creatinine in urine and plasma. *Clin Chim Acta* 1956;**1:** 210–224.
10. Hervey GR. Determination of creatinine by the Jaffe reaction. *Nature* 1953;**171:**1125.
11. Moss GA, Bondar RJ, Buzzelli DM. Kinetic enzymatic method for determining serum creatinine. *Clin Chem* 1975;**21:**1422–1426.
12. *ICRP Publication 89: Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values. Annals of the IRCP Vol. 32 issues 3–4*. Valentin J, ed. Pergamon, 2003.
13. Vasiliades J. Reaction of alkaline picrate with creatinine. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. *Clin Chem* 1976;**22:**1664–1671.
14. DFG. Creatinine as a reference parameter. In: Greim H, Lehnert G, eds. *Biological Exposure Values for Occupational Toxicants and Carcinogens. Vol. 3*. Weinheim, Germany: Wiley-VCH, 1999; 35–44.