Reproducibility of essential elements chromium, manganese, iron, zinc and selenium in spot samples, first-morning voids and 24-h collections from healthy adult men

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Abstract

Evaluation of Cr, Mn, Fe, Zn and Se in humans is challenged by the potentially high within-individual variability of these elements in biological specimens, which are poorly characterised. This study aimed to evaluate their within-day, between-day and between-month variability in spot samples, first-morning voids and 24-h collections. A total of 529 spot urine samples (including eighty-eight first-morning voids and 24-h collections) were collected from eleven Chinese adult men on days 0, 1, 2, 3, 4, 30, 60 and 90 and analysed for these five elements using inductively coupled plasma-MS. Intraclass correlation coefficients (ICC) were utilised to characterise the reproducibility, and their sensitivity and specificity were analysed to assess how well a single measurement classified individuals' 3-month average exposures. Serial measurements of Zn in spot samples exhibited fair to good reproducibility (creatinine-adjusted ICC = 0·47) over five consecutive days, which became poor when the samples were gathered months apart (creatinine-adjusted ICC = 0·33). The reproducibility of Cr, Mn, Fe and Se in spot samples was poor over periods ranging from days to months (creatinine-adjusted ICC = 0·01–0·12). Two spot samples were sufficient for classifying 60 % of the men who truly had the highest (top 33 %) 3-month average Zn concentrations; for Cr, Mn, Fe and Se, however, at least three specimens were required to achieve similar sensitivities. In conclusion, urinary Cr, Mn, Fe, Zn and Se concentrations showed a strong within-individual variability, and a single measurement is not enough to efficiently characterise individuals' long-term exposures.

Key words: Essential elements: Urine samples: Reproducibility: Variability: Misclassification

Mn, Fe, Zn and Se are well-known essential elements that act as cofactors for a variety of important proteins and enzymes[1]. Many toxicological and human evidence have revealed that a deficiency of these elements was associated with many chronic diseases (e.g. CVD, diabetes and chronic liver disease)[2–5]. However, excessive exposure to these elements, either voluntarily via supplementation or involuntarily via intake of contaminated food and water, has also been linked to increased risks of diabetes[4], the metabolic syndrome[6], kidney[7] and gut diseases[8], and cancer[9]. Cr is also postulated to be involved in regulating the metabolism of carbohydrates and lipids by enhancing insulin's efficacy[10], though the European Food Safety Authority found no convincing evidence that Cr is an essential element[11].

The collection of spot samples is non-invasive and convenient to implement[12,13]. Therefore, many epidemiological studies, especially large-scale biomonitoring investigations, such as the Canadian Health Measures Survey (CHMS)[14], the German Environmental Specimen Bank (ESB)[15] and the French National Nutrition and Health Survey (ENNS)[16], all have utilised single spot urine sample to determine individuals' exposure status of Cr, Mn, Fe, Zn and Se. However, the concentrations of these elements in spot samples may vary greatly over time due to the variation in external exposure (driven by the changing lifestyle or dietary habits)[17,18] and urinary excretion rate (UER) (driven by changing salt intake and physiological status)[19]. Using creatinine to adjust for urine dilution may also introduce variation that does not reflect urine dilution[20].

Very few studies to date have explored the reproducibility of urinary concentrations of Cr, Mn, Fe, Zn and Se. In a study conducted in the USA, Gargas *et al.*[21] found that Cr concentrations in spot urine samples varied by orders of magnitude in ten

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volunteers. In a Belgium study, Smolders *et al.*[17] reported poor reproducibility in urinary Mn concentrations over a 6-d period in four couples (creatinine-adjusted intraclass correlation coefficient (ICC) = 0·28). In an Italy study, Paglia *et al.*[22] found high seasonal variations in Mn, Zn and Se in first-morning voids (all ICC < 0·50) in seven healthy volunteers. However, no studies have comprehensively assessed the within-day, between-day and between-month variability of Cr, Mn, Fe, Zn and Se concentrations in different sample types (e.g. spot samples, first-morning voids or 24-h collections). The impact of different concentration correction methods (i.e. creatinine adjustment, creatinine as a covariate and UER) on the reproducibility of these elements in urine was also unclear.

To fill the data gap in this study, we characterised the within-day, between-day and between-month variability of serial measurements of Cr, Mn, Fe, Zn and Se in spot samples, first-morning voids and 24-h collections from eleven Chinese adult men, using different correction models. Additionally, we mimicked the exposure classification process in epidemiological studies to calculate the sensitivity and specificity for classifying individuals' high (top 33 %) 3-month average exposures with single or repeated specimens.

Methods

Study population

From 22 October 2012 to 21 January 2013, eleven healthy adult men were recruited to participate in the study designed to explore the variability in urinary heavy metals and haloacetic acids[18,23]. Their basic demographics have been shown in our previous study[18]. To reduce the uncertainty related to demographic characteristic and lifestyle factors that may contribute to the variability in urinary element concentrations, we included only volunteers who were non-smokers and did not have any occupational exposure to metals. Their age and BMI ranged from 21 to 28 years (mean: 23·64 ± 1·96) and 18·5 to 25·1 kg/m² (mean: 21·67 ± 2·13), respectively. On average, participants urinated six times per d (range: 3–11 times), and the volume of spot samples was 195 ml (range: 20–741 ml). Participants had no restriction on their daily dietary intake but were requested to record the types of food consumed throughout the eight sampling days. Three men reported eating fish only once in the eight sampling days during the 3-month sampling period, and none of the participants took dietary supplementation or ate seafood other than fish on the sampling days. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Ethics Committee of Tongji Medical College. All participants signed informed consent forms before participation.

Urine sample collection

During the 3-month sampling period, all spot samples were collected from the eleven adult men on days 0, 1, 2, 3 and 4 (days apart) and on days 30, 60 and 90 (including day 0, months apart). To reduce the effect of changing dietary patterns on the weekend[24], all the samples were collected on weekdays. Detailed

sampling procedure has been described in our prior publications[18,23]. Briefly, participants were asked to collect all their spot samples in element-free plastic containers throughout the sampling days. Research staff recorded the total volume and collection time of each void, decanted the samples into a trace element-free polyethylene cup and then stored at −40°C refrigerator until analysis. A total of 529 spot samples (six of 535 possible samples were missing) were finally gathered, including eighty-eight first-morning voids and 24-h collections.

Spot samples were defined as any individual urine void collected throughout a given day. A first-morning void was the first sample gathered from an individual starting at 05.00 hours. We reconstructed 24 h collections using the volume-weighted average of all voids gathered from an individual at or after 00.00 hours[18,23].

Determination of chromium, manganese, iron, zinc and selenium

Urinary concentrations of Cr, Mn, Fe, Zn and Se were quantified using our established method[25,26]. Briefly, 3·0 ml of urine sample was nitrified with 15 μl HNO₃ 67 % v/v (Optima™ grade; Fisher) at 5°C overnight, of which 1·0 ml was diluted five times using HNO₃ 1·2 % v/v (Optima™ grade; Fisher). The target analytes were then quantified using an Agilent 7700x inductively coupled plasma-MS (ICP-MS; Agilent Technologies). Standard reference materials (SRM) (2670a and 1640a) and spiked pool urine samples were used as quality controls. Detected values of Cr, Mn, Fe, Zn and Se were within the range of SRM 2670a and 1640a. The spiked recoveries of these elements ranged from 90 % to 110 %, and the within-day and between-day variations were <10 %. To control for possible contamination, a reagent blank sample (i.e. 3 ml deionised water) was processed together with every twenty samples. Analyte values in all blank samples were lower than the limits of quantification (LOQ). When the detected concentrations in urine are below LOQ, it was substituted by $LOQ/(2^{1/2})$. Urinary creatinine concentrations were determined using an automated clinical chemistry analyser[27].

Statistical analyses

All analyses were performed using Stata (version 13.1; Stata Corp) and R software (version 3.5.1, https://www.r-project.org/). Descriptive statistics were used to assess the normality of urinary Cr, Mn, Fe, Zn and Se concentrations. Because of their skewed distribution, log-transformed values were applied for subsequent analyses.

Urinary measurements of Cr, Mn, Fe, Zn and Se were expressed in the following four ways: (a) as unadjusted concentrations (μg/l), (b) as creatinine-adjusted concentrations (μg/g creatinine) calculated by dividing the unadjusted concentrations by urinary creatinine[15], (c) by adding creatinine as a covariate[28], and (d) as the UER of each element (μg/h), computed by dividing the total mass by the time since prior void[29]. Akaike information criterion values were used to assess the fit of the above-mentioned models (lower value indicates a better model fit).

A line chart was constructed to visually assess the within-individual variability in Cr, Mn, Fe, Zn and Se concentrations

Table 1. Unadjusted (µg/l), creatinine-adjusted (µg/g creatinine) and urinary excretion rate (µg/h) of chromium, manganese, iron, zinc and selenium concentrations in spot samples, first-morning voids and 24-h collections from eleven men*
(Mean values, medians and interquartile ranges (IQR))

Exposures	Spot samples (n 529)			First-morning voids (n 88)			24-h collections (n 88)		
	Mean	Median	IQR	Mean	Median	IQR	Mean	Median	IQR
Unadjusted									
Cr	4·6	2·2	1·4–3·2	13	2·2	1·4–3·1	5·7	2·3	1·9–3·0
Mn	2·0	1·4	0.98–1·9	1·7	1·4	1·2–1·9	2·0	1·6	1·3–2·1
Fe	39	28	21–41	38	32	23–43	39	33	26–44
Zn	343	264	149–451	597	530	327–764	349	296	227–441
Se	9·3	8·1	4·7–13	14	13	9·1–17	9·0	8·3	6·1–10
Creatinine-adjusted									
Cr	5·9	2·3	1·4–3·4	18	1·3	0.92–2·3	7·1	2·2	1·7–3·1
Mn	2·5	1·4	0.83–2·5	1·2	0.95	0.63–1·4	2·0	1·7	1·2–2·3
Fe	48	29	18–50	27	20	14–36	39	33	25–46
Zn	314	279	190–391	371	365	246–494	326	316	236–386
Se	8·2	8·2	6·9–9·7	8·4	8·4	7·3–9·9	8·3	8·4	7·1–9·5
Urinary excretion rate									
Cr	0·29	0·11	0.068–0·18	0·80	0·057	0.038–0·091	0·38	0·11	0.081–0·15
Mn	0·13	0·072	0.040–0·14	0·057	0·039	0.026–0·073	0·099	0·081	0.061–0·11
Fe	2·5	1·4	0.87–2·6	1·3	0·89	0.60–1·85	1·9	1·6	1·2–2·2
Zn	16	15	9·2–20	17	16	11–22	16	16	11–20
Se	0·43	0·41	0·32–0·53	0·39	0·40	0·33–0·48	0·41	0·39	0·33–0·48

*Percentage of samples > limits of quantification for Cr, Mn, Fe, Zn and Se was 99·6, 99·8, 100, 100 and 100, respectively.

in spot samples during the study period. Mixed random-effect models were utilised to estimate the between-individual, within-individual inter-day and within-individual intra-day variances in spot samples. For the first-morning and 24-h specimens, only the between-individual and within-individual variances were estimated. The variance apportionment of these elements was further separately reported for samples that were collected days apart (on days 0, 1, 2, 3 and 4) and months apart (on days 0, 30, 60 and 90). In addition, a stratified analysis was conducted to compare the variance apportionment for samples collected in the morning (0.00–11.59 hours), afternoon (12.00–18.00 hours) and evening (18.01–23.59 hours)[30]. ICC, the proportion of between-individual variance and the total variance, was used to identify the degree of reproducibility. An ICC value <0·40 is regarded as poor reproducibility, 0·40–0·75 as fair to good reproducibility and ≥0·75 as excellent reproducibility[31].

Mixed regression models were constructed to evaluate how well spot or first-morning specimens predicted the same-day 24-h collections. The predictive ability of the models was evaluated using coefficients of determination (R^2). Marginal R^2 and conditional R^2 were separately computed to characterise the proportion of variance explained by fixed or random effects[32].

To evaluate how well single or repeated randomly selected specimens identified the highest tertile (top 33 %) of 3-month average exposures[33], the arithmetic mean of log-transformed Cr, Mn, Fe, Zn and Se concentrations were computed for each participant using all their spot samples gathered over 3 months; the men who were truly in the highest (top 33 %) exposure group were then identified. Afterwards, ten data sets were created, and each containing a single randomly selected spot sample (or first-morning void) from each participant; the 'predicted' highest (top 33 %) exposure group was also identified. The average sensitivity and specificity across ten separate data sets were reported. The analyses were replicated to evaluate whether collecting repeated specimens (two or three) from each participant improved the classification.

Statistical power

The sample size of this study was estimated using the R 'ICC.Sample. Size-package' based on the method developed by Zou[34]. Assuming that the ICC of creatinine-adjusted Cr, Mn, Fe, Zn and Se were higher than 0·15, according to previously published data[17,22], 461 measurements would be sufficient to achieve a power of 90 % for our reliability analysis.

Results

Table 1 presents the unadjusted (µg/l), creatinine-adjusted (µg/g creatinine) and UER (µg/h) of urinary Cr, Mn, Fe, Zn and Se in three different sample types. Detection rates of Cr, Mn, Fe, Zn and Se in spot samples were 99·6 %, 99·8 %, 100 %, 100 % and 100 %, respectively. Line chart showed that creatinine-adjusted concentrations of Cr, Mn, Fe, Se and Zn changed up to two or three orders of magnitude (online Supplementary Fig. S1). However, no apparent rhythmic pattern was observed.

Table 2 shows the variance apportionment of Cr, Mn, Fe, Zn and Se in spot samples based on unadjusted, creatinine-adjusted, creatinine as a covariate and UER models. The lowest Akaike information criterion value was achieved with creatinine-adjusted models for Zn, creatinine as a covariate model and UER model for Se. However, the between-individual and within-individual variance components of these elements were similar based on different correction models. Given that modelling creatinine as a covariate cannot be implemented for 24-h collections and that the UER model is not practical for large population studies since sampling volume and time are required for

Table 2. Variance apportionment of log-transformed concentrations of chromium, manganese, iron, zinc and selenium in spot samples collected from eleven men (*n* 529)
(Akaike information criteria (AIC); variances (σ^2) and percentages)

Models	Cr σ^2	Cr %	Mn σ^2	Mn %	Fe σ^2	Fe %	Zn σ^2	Zn %	Se σ^2	Se %
Unadjusted (µg/l)										
AIC*	601		600		511		620		561	
Between-individual σ^2†	0·001	1	0·001	1	0·007	4	0·058	24	0·022	12
Within-individual, inter-day σ^2‡	0·068	32	0·033	17	0·018	11	0·032	13	0·026	13
Within-individual, intra-day σ^2§	0·143	67	0·155	82	0·134	85	0·155	63	0·143	75
Creatinine-adjusted (µg/g creatinine)										
AIC*	701		875		765		93		492	
Between-individual σ^2†	0·001	1	0·023	7	0·021	8	0·052	41	0·001	3
Within-individual, inter-day σ^2‡	0·049	21	0·015	5	0·006	2	0·021	17	0·017	50
Within-individual, intra-day σ^2§	0·185	78	0·278	88	0·231	90	0·053	42	0·016	47
Creatinine modelled as a covariate										
AIC*	579		606		510		156		150	
Between-individual σ^2†	0·001	1	0·001	1	0·007	5	0·050	38	0·002	4
Within-individual, inter-day σ^2‡	0·061	31	0·033	17	0·016	10	0·021	16	0·018	34
Within-individual, intra-day σ^2§	0·137	68	0·155	82	0·133	85	0·060	46	0·033	62
Urinary excretion rate (µg/h)										
AIC*	799		966		878		328		85	
Between-individual σ^2†	0·003	1	0·016	4	0·017	5	0·063	38	0·014	17
Within-individual, inter-day σ^2‡	0·043	16	0·006	2	0·001	1	0·013	8	0·015	18
Within-individual, intra-day σ^2§	0·228	83	0·342	94	0·293	94	0·091	54	0·055	65

*AIC values were used to compare the model fits.
† Proportion of between-person variance to total variance.
‡ Proportion of within-person inter-day variance to total variance.
§ Proportion of within-person intra-day variance to total variance.

calculation, only creatinine-adjusted concentrations were used for all consequent analyses.

Table 3 shows the variance apportionment of creatinine-adjusted Cr, Mn, Fe, Zn and Se concentrations in three different sample types. Fair to good reproducibility was obtained for serial measurements of Zn (creatinine-adjusted ICC = 0·41) in spot samples over 3 months, whereas Cr, Mn, Fe and Se showed poor reproducibility (creatinine-adjusted ICC = 0·01–0·08). For Cr, Mn, Fe and Zn, the within-individual intra-day variance was the largest component of the total variance (range: 42–90 %). For Se, however, the within-individual inter-day variance was predominant (50 %). Compared with spot samples, apparent increases in ICC were obtained for Mn and Fe in first-morning voids, and Mn, Fe and Zn in 24-h collections. However, the within-individual variance remained larger than the between-individual variance, except for Zn in 24-h collection. The results were largely unchanged when additionally including 'time interval since the prior urination' as a covariate (*n* 529; data not shown).

Stratified analyses showed that the reproducibility of serial measurements of Zn in spot samples collected days apart was fair to good (creatinine-adjusted ICC = 0·47), which became poor when the samples were gathered months apart (creatinine-adjusted ICC = 0·33). Poor reproducibility ranging from 0·01 to 0·12 was observed for Cr, Mn, Fe and Se, irrespective of whether the spot samples were gathered days apart or months apart. Compared with spot samples, apparently higher ICC (>0·20) was obtained for Mn, Fe and Zn in first-morning and 24-h specimens gathered days apart, and Mn and Fe in first-morning and 24-h specimens gathered months apart (Table 3). The variance apportionment of Cr, Mn, Fe, Zn and Se in the samples collected at different times of the day was also estimated. The

reproducibility of Cr, Mn, Fe and Se was poor irrespective of whether the samples were collected in the morning, afternoon or evening (ICC = 0·01–0·13). Fair to good reproducibility was observed for Zn in the afternoon and evening samples (ICC = 0·55 and 0·48, respectively) (online Supplementary Table S1).

Table 4 shows how well spot or first-morning specimens predicate the same-day 24-h collections. Low to moderate predictive power was achieved for Cr, Mn, Fe, Zn and Se based on spot samples (conditional R^2: 0·27–0·65). Compared with spot samples, using first-morning voids as predictors provided apparent increases in conditional R^2 for Cr (0·73 *v.* 0·27) and slight increases for Zn (0·81 *v.* 0·65) and Se (0·75 *v.* 0·59).

Table 5 shows the results of sensitivity and specificity analyses. When single spot sample or first-morning void was used to identify the highest tertile of 3-month average concentrations, relatively low sensitivities in a range of 0·23–0·50 were observed for Cr, Mn, Fe, Zn and Se. Compared with a single measurement in spot samples, ≥10 % increases in sensitivities were observed for all five elements when two or three spot samples were randomly selected from each men days apart. However, moderate sensitivity was obtained only for Zn (0·67). Similarly, using two or three first-morning voids as predictors showed higher sensitivity for Cr, Mn, Fe, Zn and Se compared with a single first-morning void. Collection of specimens months apart did not offer an apparent improvement in exposure classification over the specimens collected days apart.

Discussion

Among eleven non-occupationally exposed healthy adult men, high within-individual variability in the concentrations of Cr, Mn,

Table 3. Variance of log-transformed creatinine-adjusted chromium, manganese, iron, zinc and selenium concentrations in three sample types*
(Intraclass correlation coefficients (ICC); variances (σ^2) and percentages)

Type of samples	Cr σ^2	%	Mn σ^2	%	Fe σ^2	%	Zn σ^2	%	Se σ^2	%
Samples collected during a 3-month period										
Spot samples (n 529)										
ICC	0.01		0.07		0.08		0.41		0.03	
Between-individual σ^2†	0.001	1	0.022	7	0.021	8	0.052	41	0.001	3
Within-individual, inter-day σ^2‡	0.048	20	0.015	5	0.006	2	0.021	17	0.017	50
Within-individual, intra-day σ^2§	0.185	79	0.278	88	0.231	90	0.053	42	0.016	47
First-morning voids (n 88)										
ICC	0.06		0.37		0.41		0.44		0.07	
Between-individual σ^2†	0.021	6	0.063	37	0.060	41	0.049	44	0.002	7
Within-individual σ^2‡	0.356	94	0.109	63	0.086	59	0.063	56	0.026	93
24-h collections (n 88)										
ICC	0.01		0.27		0.32		0.57		0.05	
Between-individual σ^2†	0.001	1	0.037	27	0.032	32	0.045	57	0.001	5
Within-individual σ^2‡	0.202	99	0.099	73	0.068	68	0.034	43	0.020	95
Samples collected days apart										
Spot sample (n 326)										
ICC	0.01		0.09		0.06		0.47		0.12	
Between-individual σ^2†	0.001	1	0.026	9	0.014	6	0.060	47	0.003	12
Within-individual, inter-day σ^2‡	0.031	20	0.013	4	0.003	1	0.010	8	0.010	40
Within-individual, intra-day σ^2§	0.123	79	0.262	87	0.207	93	0.058	45	0.012	48
First-morning voids (n 55)										
ICC	0.18		0.35		0.32		0.71		0.16	
Between-individual σ^2†	0.023	18	0.051	35	0.046	32	0.084	71	0.004	16
Within-individual σ^2‡	0.105	82	0.095	65	0.100	68	0.035	29	0.021	84
24-h collections (n 55)										
ICC	0.01		0.30		0.29		0.74		0.20	
Between-individual σ^2†	0.001	1	0.042	30	0.022	29	0.058	74	0.003	20
Within-individual σ^2‡	0.075	99	0.096	70	0.053	71	0.020	26	0.012	80
Samples collected months apart										
Spot sample (n 265)										
ICC	0.01		0.09		0.09		0.33		0.01	
Between-individual σ^2†	0.001	1	0.027	9	0.026	9	0.039	33	0.001	1
Within-individual, inter-day σ^2‡	0.072	23	0.006	2	0.003	1	0.035	30	0.017	43
Within-individual, intra-day σ^2§	0.233	76	0.274	89	0.248	89	0.044	37	0.022	56
First-morning voids (n 44)										
ICC	0.01		0.36		0.39		0.33		0.03	
Between-individual σ^2†	0.001	1	0.071	36	0.058	39	0.036	33	0.001	3
Within-individual σ^2‡	0.643	99	0.127	64	0.092	61	0.072	67	0.030	97
24-h collections (n 44)										
ICC	0.01		0.43		0.42		0.39		0.04	
Between-individual σ^2†	0.001	1	0.054	43	0.052	42	0.030	39	0.001	4
Within-individual σ^2‡	0.327	99	0.072	57	0.072	58	0.047	61	0.022	96

*Days apart: days 0, 1, 2, 3, and 4; months apart: days 0, 30, 60, and 90.
† Proportion of between-individual variance to total variance.
‡ Proportion of within-individual inter-day variance to total variance.
§ Proportion of within-individual intra-day variance to total variance.

Fe and Se was exhibited in spot samples over periods ranging from days to months (creatinine-adjusted ICC = 0·01–0·12). The reproducibility of Zn concentrations in spot samples was fair to good over five consecutive days (creatinine-adjusted ICC = 0·47), which became poor when the specimens were gathered months apart (creatinine-adjusted ICC = 0·33). Similar to the trend of ICC, our classification analysis showed that using one, two or even three spot samples to identify the highest tertile of 3-month average concentrations of Cr, Mn, Fe and Se could result in high degree of classification error (i.e. low sensitivities); by contrast, two and three randomly selected spot samples appeared to offer moderate to high sensitivities (≥ 0·6) for Zn.

Cr, Mn, Fe, Zn and Se are proposed to be essential elements that are naturally present in food and water and are also available as dietary supplements[1,35]. Additional intake may benefit individuals who have insufficient intake. However, excessive exposures have demonstrated adverse health effects both in animal and in human studies[1,35]. In this study, we found high within-individual variability in urinary concentration of Cr, Mn, Fe and Se over both short and long periods of time, which is not unexpected, given the changing exposure status due to the variation in the daily dietary consumption[19]. The changing urinary flow rate that is related to salt intake and physiological status may also affect the reproducibility of these elements[19]. The reproducibility of Zn concentrations in spot samples was fair to good over five consecutive days (creatinine-adjusted ICC = 0·47). Within-individual variation in biomarker concentrations is affected by various factors, especially the elimination half-life.

Table 4. Creatinine-adjusted models of 24-h concentrations of chromium, manganese, iron, zinc and selenium using the same-day spot samples or first-morning voids as predictors†
(β-Coefficients, 95 % confidence intervals, intercepts, marginal coefficients of determination (R^2) and conditional R^2)

Types of sample	β	95 % CI	Intercept	Marginal R^2§	Conditional R^2‖
Spot samples (n 529)‡					
Cr	0·48*	0·40, 0·55	0·32	0·22	0·27
Mn	0·21*	0·17, 0·26	0·28	0·12	0·37
Fe	0·19*	0·15, 0·23	1·80	0·10	0·39
Zn	0·38*	0·34, 0·43	2·20	0·31	0·65
Se	0·56*	0·52, 0·61	0·57	0·56	0·59
First-morning voids (n 88)					
Cr	0·63*	0·55, 0·71	0·40	0·72	0·73
Mn	0·38*	0·20, 0·56	0·33	0·18	0·31
Fe	0·28*	0·10, 0·45	1·67	0·12	0·30
Zn	0·63*	0·54, 0·72	1·26	0·69	0·81
Se	0·75*	0·66, 0·84	0·32	0·74	0·75

* $P < 0.001$.
† Spot samples and first-morning voids were analysed on a log-transformed scale.
‡ Spot samples included first-morning voids.
§ Proportion of variance explained by fixed effects.
‖ Amount of variance explained by fixed and random effects.

Table 5. Sensitivity and specificity of one, two or three urine samples as a predictor to identify the highly exposed men (top 33 %) based on their 3-month average measures*

Type of samples	Cr Sensitivity	Cr Specificity	Mn Sensitivity	Mn Specificity	Fe Sensitivity	Fe Specificity	Zn Sensitivity	Zn Specificity	Se Sensitivity	Se Specificity
Spot samples										
One sample	0·27	0·73	0·33	0·75	0·37	0·76	0·43	0·79	0·37	0·76
Two samples† (days apart)	0·40	0·78	0·37	0·76	0·43	0·79	0·60	0·85	0·47	0·80
Two samples† (months apart)	0·27	0·73	0·33	0·75	0·33	0·75	0·60	0·85	0·43	0·79
Three samples† (days apart)	0·37	0·76	0·47	0·80	0·47	0·80	0·67	0·88	0·47	0·80
Three samples† (months apart)	0·40	0·78	0·33	0·75	0·40	0·78	0·73	0·90	0·63	0·86
First-morning voids										
One sample	0·40	0·78	0·23	0·71	0·37	0·76	0·50	0·81	0·37	0·76
Two samples† (days apart)	0·43	0·79	0·40	0·78	0·50	0·81	0·57	0·84	0·37	0·76
Two samples† (months apart)	0·37	0·76	0·37	0·76	0·47	0·80	0·57	0·84	0·40	0·78
Three samples† (days apart)	0·53	0·83	0·47	0·80	0·37	0·76	0·70	0·89	0·50	0·81
Three samples† (months apart)	0·63	0·86	0·43	0·79	0·43	0·79	0·53	0·80	0·30	0·74

*Days apart: days 0, 1, 2, 3, and 4; months apart: days 0, 30, 60, and 90. Calculations use creatinine-adjusted Cr, Mn, Fe, Zn and Se concentrations (µg/g creatinine) on a $\log_{10}$ scale.
† Specimens were collected on different days.

Zn is mainly stored in muscle and bones with a half-life of 244 d[36–38], which is longer than that of Cr, Mn, Fe and Se (range from <30 h to <116 d)[39–41]. Consistent with our findings, previous studies have conformably revealed that the within-individual variance exceeds the between-individual variance for urinary concentration of Cr, Mn, Zn and Se[17,22], though the reported ICC vary slightly across studies, probably because of the differences in study design (e.g. study population, sample types and sampling duration). For instance, Paglia *et al.*[22] reported a poor reproducibility for Zn in first-morning voids across seasons (creatinine-adjusted ICC = 0·37), which was comparable to our first-morning voids that were collected months apart (creatinine-adjusted ICC = 0·33) but was much lower than

our samples that were collected days apart (creatinine-adjusted ICC = 0·71).

Making collections over 24 h is proposed as the 'gold standard' to evaluate an individuals' daily exposures to chemicals that are excreted mainly through urine[42]. In the present study, only low to moderate predictive ability (conditional R^2 0·27–0·65) was obtained when using spot samples as predictors of the same-day 24-h concentrations of Cr, Mn, Fe, Zn and Se. A first-morning void is recommended as a better sample type than spot sample since it is more concentrated and reflects accumulative exposures[33,43]. However, compared with spot samples, using first-morning voids to predict the same-day 24-h average exposures showed an apparent advantage only for Cr

(conditional R^2: 0·73 *v.* 0·27). Moreover, although serial measurements of Mn, Fe and Zn in first-morning voids or 24-h collections showed higher reproducibility than spot samples, their within-individual variance remained larger than the between-individual variance, suggesting that improvements in exposure estimation over weeks or months cannot offset the additional effort needed to gather these two sample types. In support of this speculation, our classification analysis revealed that a single measurement of Cr, Mn, Fe, Zn and Se in first-morning voids only correctly identified ≤50 % of the men who truly had high (top 33 %) 3-month average exposures.

Our findings highlight the importance of characterising analyte-specific patterns of variability to improve exposure estimation in epidemiological studies. In our classification analysis, we revealed a tendency similar to that of the ICC. For instance, two spot samples are sufficient to correctly classify 60 % of the men who had the highest (top 33 %) 3-month average Zn concentrations; for Cr, Mn, Fe and Se, however, at least three specimens were required to achieve a similar sensitivity. In our stratified analyses, we compared the variance apportionment for samples that were collected at different time points and found that the sensitivity and specificity of specimens collected months apart did not offer apparent improvements in exposure classification over the specimens collected days apart. The reproducibility of Cr, Mn, Fe and Se was uniformly poor irrespective of whether the samples were collected in the morning, afternoon or evening, which indicates that changing the collection time of day will not substantially improve the exposure estimation. For Zn, however, specimens collected in the afternoon and evening showed slightly higher reproducibility than that the morning samples, which means changing the collection time of day will optimise exposure assessment when multiple specimens are collected over a period of time.

The geometric mean concentrations of Cr, Mn, Fe, Zn and Se in this study were comparable with those reported among 2004 adult residents of Wuhan, China[25]. However, our geometric mean urinary concentrations of Mn (1·5 μg/l) and Se (7·5 μg/l) were different from those reported for the males from the CHMS of Canada (0·078 μg/l and 57 μg/l for Mn and Se, respectively)[14] and adults from Germany (0·063 μg/l for Mn)[44], implying regional variability in the extent and source of exposure. The main strength of this study was that we comprehensively assessed the within-day, between-day and between-month variability in Cr, Mn, Fe, Zn and Se concentrations in spot, first-morning and 24-h urine specimens. The impact of different concentration correction methods (i.e. creatinine-adjusted, creatinine as a covariate and UER models) on the reproducibility of these elements was also assessed. Our findings support the feasibility of using the creatinine-adjusted model to control for urinary dilution for Cr, Mn, Fe, Zn and Se in healthy adult men. Nevertheless, our study has several limitations. First, our recruited study volunteers were relatively homogeneous (young men aged between 21 and 28 years), living in a restricted district during the study period, which may have resulted in an underestimation of the between-individual variability compared with the general population who have more heterogeneous demographic characteristics (e.g. sex, and age). Second, our study population was

relatively small, which limits our ability to explore the effects of various sources of uncertainties (e.g. exposure uncertainties that were related to diet and lifestyle) on the within-individual and between-individual variances. Third, duplicate aliquots were not carried out to evaluate uncertainty due to analytical factors, which may have also biased our estimation of ICC.

In conclusion, our findings revealed that serial measurements of Cr, Mn, Fe, Zn and Se in spot samples, first-morning voids and 24-h collections uniformly showed strong within-individual variability, and a single measurement is not enough to efficiently characterise individuals' long-term exposures. Collection of repeated specimens is a feasible approach to reduce exposure misclassification. If multiple specimens were collected from each individual over a period of time, changing the collection time of day may optimise exposure assessment for Zn; whereas this may not be necessary for Cr, Mn, Fe and Se.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114519001193

References

1. National Institutes of Health Office of Dietary Supplements (2018) Dietary supplement fact sheets. https://ods.od.nih.gov/factsheets/list-all/ (accessed November 2018).
2. Mohammadifard N, Humphries KH, Gotay C, *et al.* (2017) Trace minerals intake: risks and benefits for cardiovascular health. *Crit Rev Food Sci Nutr* **59**, 1–13.
3. Chu A, Foster M & Samman S (2016) Zinc status and risk of cardiovascular diseases and type 2 diabetes mellitus-a systematic review of prospective cohort studies. *Nutrients* **8**, 707.

4. Shan Z, Chen S, Sun T, *et al.* (2016) U-shaped association between plasma manganese levels and type 2 diabetes. *Environ Health Perspect* **124**, 1876–1881.

5. Himoto T & Masaki T (2018) Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. *Nutrients* **10**, 88.

6. Yuan Z, Xu X, Ye H, *et al.* (2015) High levels of plasma selenium are associated with metabolic syndrome and elevated fasting plasma glucose in a Chinese population: a case-control study. *J Trace Elem Med Biol* **32**, 189–194.

7. Shil K & Pal S (2018) Metabolic adaptability in hexavalent chromium-treated renal tissue: an *in vivo* study. *Clin Kidney J* **11**, 222–229.

8. Fang S, Zhuo Z, Yu X, *et al.* (2018) Oral administration of liquid iron preparation containing excess iron induces intestine and liver injury, impairs intestinal barrier function and alters the gut microbiota in rats. *J Trace Elem Med Biol* **47**, 12–20.

9. Kok FJ, Van Duijn CM, Hofman A, *et al.* (1988) Serum copper and zinc and the risk of death from cancer and cardiovascular disease. *Am J Epidemiol* **128**, 352–359.

10. Vincent JB (2001) The bioinorganic chemistry of chromium(III). *Polyhedron* **20**, 1–26.

11. European Food Safety Authority (2014) Scientific opinion on dietary reference values for chromium1. *EFSA J* **12**, 4185–4185.

12. Esteban M & Castano A (2009) Non-invasive matrices in human biomonitoring: a review. *Environ Int* **35**, 438–449.

13. Sun Q, Bertrand KA, Franke AA, *et al.* (2017) Reproducibility of urinary biomarkers in multiple 24-h urine samples. *Am J Clin Nutr* **105**, 159–168.

14. Health Canada (2013) Second report on human biomonitoring of environmental chemicals in Canada. http://www.healthyenvironmentforkids.ca/sites/healthyenvironmentforkids.ca/files/HumanBiomonitoringReport__EN.pdf (accessed November 2018).

15. Wiesmuller GA, Eckard R, Dobler L, *et al.* (2007) The environmental specimen bank for human tissues as part of the German Environmental Specimen Bank. *Int J Hyg Environ Health* **210**, 299–305.

16. Fréry N, Saoudi A, Garnier R, *et al.* (2011) Exposition de la population française aux substances chimiques de l'environnement. Tome 1 (Exposure of the French population to chemical substances in the environment. Vol. 1). *Toxicol Anal Clin* **29**, 441–482.

17. Smolders R, Koch HM, Moos RK, *et al.* (2014) Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 1: metals. *Toxicol Lett* **231**, 249–260.

18. Wang YX, Feng W, Zeng Q, *et al.* (2016) Variability of metal levels in spot, first morning, and 24-hour urine samples over a 3-month period in healthy adult Chinese men. *Environ Health Perspect* **124**, 468–476.

19. Aylward LL, Hays SM, Smolders R, *et al.* (2014) Sources of variability in biomarker concentrations. *J Toxicol Environ Health B Crit Rev* **17**, 45–61.

20. Barr DB, Wilder LC, Caudill SP, *et al.* (2005) Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect* **113**, 192–200.

21. Gargas ML, Norton RL, Paustenbach DJ, *et al.* (1994) Urinary excretion of chromium by humans following ingestion of chromium picolinate. Implications for biomonitoring. *Drug Metab Dispos* **22**, 522–529.

22. Paglia G, Miedico O, Tarallo M, *et al.* (2016) Evaluation of seasonal variability of toxic and essential elements in urine analyzed by inductively coupled plasma mass spectrometry. *Expo Health* **9**, 79–88.

23. Wang YX, Zeng Q, Wang L, *et al.* (2014) Temporal variability in urinary levels of drinking water disinfection byproducts dichloroacetic acid and trichloroacetic acid among men. *Environ Res* **135**, 126–132.

24. Gibson RS, Gibson IL & Kitching J (1985) A study of inter- and intrasubject variability in seven-day weighed dietary intakes with particular emphasis on trace elements. *Biol Trace Elem Res* **8**, 79–91.

25. Feng W, He X, Chen M, *et al.* (2015) Urinary metals and heart rate variability: a cross-sectional study of urban adults in Wuhan, China. *Environ Health Perspect* **123**, 217–222.

26. Wan ZZ, Chen HG, Lu WQ, *et al.* (2019) Metal/metalloid levels in urine and seminal plasma in relation to computer-aided sperm analysis motion parameters. *Chemosphere* **214**, 791–800.

27. Wang YX, Sun Y, Huang Z, *et al.* (2016) Associations of urinary metal levels with serum hormones, spermatozoa apoptosis and sperm DNA damage in a Chinese population. *Environ Int* **94**, 177–188.

28. Kim K, Steuerwald AJ, Parsons PJ, *et al.* (2011) Biomonitoring for exposure to multiple trace elements via analysis of urine from participants in the Study of Metals and Assisted Reproductive Technologies (SMART). *J Environ Monit* **13**, 2413–2419.

29. Akerstrom M, Sallsten G, Lundh T, *et al.* (2013) Associations between urinary excretion of cadmium and proteins in a non-smoking population: renal toxicity or normal physiology? *Environ Health Perspect* **121**, 187–191.

30. Preau JL Jr., Wong LY, Silva MJ, *et al.* (2010) Variability over 1 week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. *Environ Health Perspect* **118**, 1748–1754.

31. Rosner B (2000) Multisample inference. In *Fundamentals of Biostatistics*, 5th ed., pp. 511–576. Pacific Grove, CA: Duxbury Press.

32. Nakagawa S & Schielzeth H (2013) A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecolo Evol* **4**, 133–142.

33. Bradman A, Kogut K, Eisen EA, *et al.* (2013) Variability of organophosphorous pesticide metabolite levels in spot and 24-hr urine samples collected from young children during 1 week. *Environ Health Perspect* **121**, 118–124.

34. Zou GY (2012) Sample size formulas for estimating intraclass correlation coefficients with precision and assurance. *Stat Med* **31**, 3972–3981.

35. Sengupta P (2013) Environmental and occupational exposure of metals and their role in male reproductive functions. *Drug Chem Toxicol* **36**, 353–368.

36. Solomons NW (1982) Biological availability of zinc in humans. *Am J Clin Nutr* **35**, 1048–1075.

37. De Roost E, Funck E, Spernol A, *et al.* (1972) The decay of ^{65}Zn. *Z Physik* **250**, 395–412.

38. Chasapis CT, Loutsidou AC, Spiliopoulou CA, *et al.* (2012) Zinc and human health: an update. *Arch Toxicol* **86**, 521–534.

39. Paustenbach D, Panko J, Fredrick M, *et al.* (1997) Urinary chromium as a biological marker of environmental exposure: what are the limitations? *Regul Toxicol Pharmacol* **26**, S23–S34.

40. Roels H, Lauwerys R, Genet P, *et al.* (1987) Relationship between external and internal parameters of exposure to manganese in workers from a manganese oxide and salt producing plant. *Am J Ind Med* **11**, 297–305.

41. Friberg L & Elinder CG (1993) Biological monitoring of toxic metals. *Scand J Work Environ Health* **19**, Suppl. 1, 7–13.

42. US Environmental Protection Agency (2014) Code of federal regulations: priority pollutants list. 2014. https://www.gpo.gov/fdsys/pkg/CFR-2014-title40-vol29/xml/CFR-2014-title40-vol29-part423-appA.xml (accessed November 2018).

43. Kissel JC, Curl CL, Kedan G, et al. (2005) Comparison of organophosphorus pesticide metabolite levels in single and multiple daily urine samples collected from preschool children in Washington state. *J Expo Anal Environ Epidemiol* **15**, 164–171.

44. Heitland P & Koster HD (2006) Biomonitoring of 30 trace elements in urine of children and adults by ICP-MS. *Clin Chim Acta* **365**, 310–318.