Environment International 134 (2020) 105218

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Urine selenium concentration is a useful biomarker for assessing population level selenium status

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ARTICLE INFO

Handling Editor: Hefa Cheng

Keywords:
Biomarkers
Biomonitoring
Micronutrient survey
Selenium status
Sub Saharan Africa
Urine

ABSTRACT

Plasma selenium (Se) concentration is an established population level biomarker of Se status, especially in Se-deficient populations. Previously observed correlations between dietary Se intake and urinary Se excretion suggest that urine Se concentration is also a potentially viable biomarker of Se status. However, there are only limited data on urine Se concentration among Se-deficient populations. Here, we test if urine is a viable biomarker for assessing Se status among a large sample of women and children in Malawi, most of whom are likely to be Se-deficient based on plasma Se status. Casual (spot) urine samples (n = 1406) were collected from a nationally representative sample of women of reproductive age (WRA, n = 741) and school aged children (SAC, n = 665) across Malawi as part of the 2015/16 Demographic and Health Survey. Selenium concentration in urine was determined using inductively coupled plasma mass spectrometry (ICP-MS). Urinary dilution corrections for specific gravity, osmolality, and creatinine were applied to adjust for hydration status. Plasma Se status had been measured for the same survey participants. There was between-cluster variation in urine Se concentration that corresponded with variation in plasma Se concentration, but not between households within a cluster, or between individuals within a household. Corrected urine Se concentrations explained more of the between-cluster variation in plasma Se concentration than uncorrected data. These results provide new evidence that urine may be used in the surveillance of Se status at the population level in some groups. This could be a cost-effective option if urine samples are already being collected for other assessments, such as for iodine status analysis as in the Malawi and other national Demographic and Health Surveys.

1. Introduction

Selenium (Se) is an essential trace element in humans where it is an integral component of ~25 selenoproteins. It has roles in immune function, responses to oxidative stress, and cognitive development (Burk and Hill, 2009; Fairweather-Tait et al., 2011). Biomarkers used to assess Se status include blood (whole, plasma or serum), toenails, hair, and urine. Each of these biomarkers has its strengths and limitations

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https://doi.org/10.1016/j.envint.2019.105218
Received 8 July 2019; Received in revised form 12 September 2019; Accepted 23 September 2019
Available online 09 November 2019

which are influenced by contextual (e.g. population Se-status) as well as logistical and technical (e.g. access to instrumentation) factors (Hays et al., 2014).

Blood Se concentration (whole, plasma or serum) is regarded as the most informative biomarker of Se status at both the individual and population level (Fairweather-Tait et al., 2011; Longnecker et al., 1996; Yang et al., 1989). Typically, approximately 50% of the body's selenium is contained in the blood system, and blood Se concentration is relatively responsive to short-to-medium term Se intake or exposure. Plasma Se concentrations of 87 ng mL^{-1} and 65 ng mL^{-1} are used as thresholds of the optimal activities of the selenoproteins glutathione peroxidase 3 (GPx3) and iodothyronine deiodinase (IDI), respectively, in adults (Thomson, 2004). Challenges of using blood Se concentration as a biomarker of Se status include the invasive nature of sampling, including personal sensitivities regarding use of blood samples (e.g. for HIV testing) and cultural beliefs (e.g. 'blood sucking' and witchcraft in some countries). Less-invasive biomarkers of Se status, such as toenail and hair Se concentration, have been used (Fairweather-Tait et al., 2011; Hays et al., 2014; Longnecker et al., 1996). However, due to potential contamination from dirt and hair cleaning products, their use has been limited.

Urine Se concentration can also been used to assess Se status (Fairweather-Tait et al., 2011; Hays et al., 2014). Urine is the main route of Se excretion, primarily in the form of selenosugar (Combs, 2015). Due to intra-individual variation in hydration-driven urinary flow rate, 24-h urine collection is regarded as the most appropriate method for measuring urine elemental concentrations (Nermell et al., 2008). However, the need for multiple collections limits the use of urine concentration to smaller populations due to logistical challenges from individuals (Yeh et al., 2015). In studies that have analyzed elemental concentrations of a single-void ('spot' or 'casual') urine as a biomarker of status, challenges have been reported due to variation in hydration state, fluid intake, physical activity, temperature, protein malnutrition and genetic factors (Cockell, 2015; Combs, 2015; Nermell et al., 2008). To account for the effect of hydration status (urine dilution) on intra- and inter- individual variation in urine elemental concentrations, creatinine, osmolality and specific gravity corrections can be applied (Hays et al., 2014; Middleton et al., 2016a, 2018). For a dilution correction to perform robustly, it must meet a number of prerequisites, including: (i) be an accurate physicochemical measure of dilution/concentration; (ii) be applied in a manner representative of underlying physiological changes in analyte excretion in relation to fluctuations in urinary flow rate. Each of these corrections is likely to have advantages or disadvantages over the others in different contexts. For example, creatinine correction can be affected by protein energy malnutrition, which reduces creatinine excretion (O'Brien et al., 2017; Yeh et al., 2015), whilst osmolality is affected by age, sex, race/ethnic and body mass index (Miller et al., 2004; O'Brien et al., 2017), although less-so than creatinine. Specific gravity is lower cost, easy to conduct in the field, and less influenced by age, gender and body size (Suwazono et al., 2005). However, specific gravity can be affected by medical conditions that increase urinary glucose and protein (Moriguchi et al., 2005). Corrections are not routinely applied during urinary iodine population surveillance (Watts et al., 2019).

The aim of this study was to test if Se concentration in casual urine is a potentially useful biomarker for population-level Se status and to examine the effect of correction for urinary dilution using three routinely applied methods: specific gravity, osmolality and creatinine. Our study focused on a design-unbiased national sample of non-pregnant women of reproductive age (WRA) and school-aged children (SAC) in Malawi. These groups have previously been shown to be Se-deficient based on their plasma Se concentration (Phiri et al., 2019).

2. Methods

Our study was conducted as part of the 2015–16 Malawi Demographic and Health Survey (MDHS) and National Micronutrient Survey (NMS), for which blood plasma Se concentration has been reported previously (Phiri et al., 2019). The MDHS and NMS are periodic surveys through which blood and urine specimens are collected from a representative sample of the Malawian population and tested for various health and nutrition indicators. During the 2015–16 NMS, casual urine samples were collected from WRA (age 15–49) and SAC (age 6–14, female and male) for primary analysis of urinary iodine. These data therefore allow urine and plasma from the same individuals to be compared via a unique anonymized identifier. The sampling design also provides a powerful comparison at individual, household and Enumeration Area (EA), i.e. 'cluster' level. Individuals within household and households within a cluster are more likely to have similar dietary patterns, and share the same environmental exposure, which may influence urine Se concentration.

2.1. Sampling

Study design and methods are described in detail elsewhere (NSO, 2017; NSO/CHSU/CDC, 2017; Phiri et al., 2019). Briefly, the 2015–16 NMS represented a subsample of the wider cross-sectional study of the 2015–16 MDHS with a two-stage cluster sampling design. The study was conducted in all 28 districts of the Republic of Malawi targeting both urban and rural populations. For the MDHS, 850 clusters with inclusion-probability proportional to population size. National Micronutrient Survey clusters (n = 105) were then randomly selected from MDHS clusters (35 in each of the North, Central, and South regions) with representation from urban and rural areas. In each of the selected households, eligible participants (defined as having spent the night prior to the survey in that household) were invited to participate from all the demographic groups as follows: pre-school children (PSC, aged 6–59 months), school aged children (SAC, aged 6–14 years), non-pregnant women of reproductive age group (WRA, aged 15–45 years) and men. Urine was collected only from WRA and SAC. Urine was not collected from PSC and men due to difficulties in getting urine and the small sample size, respectively. Urine was collected as a casual sample at the time of interview, or within 24 h if that was not possible. No other information on the urine samples (e.g. flow rate, time since last void) was collected. Exclusion criteria were applied to participants based on the requirements of the NMS and not for the purposes of this study. Ethical approval and a material transfer agreement was granted by the Malawi National Health Sciences Research Committee (NHSRC), reference number NHSRC 15/5/1436. Individual informed consent was also secured by the NMS field team from every adult participant before sample collection, and from caregivers before collecting from SACs.

2.2. Sample collection

At each collection site, a temporary laboratory was established to minimize contamination and facilitate accurate record keeping for traceability of samples. Strict quality control measures were followed using well-trained nurses, clinicians and laboratory technicians under the supervision of the Centers for Disease Control & Disease Prevention (CDC), Georgia, USA. Casual urine samples were obtained in 125 mL polypropylene urine cups with screw caps (model 1131202, Heinz Herenz Medizinalbedarf GmbH, Hamburg, Germany). In total, 1406 urine samples were collected from WRA (n = 741) and SAC (n = 665). The samples were frozen within an hour of collection and kept at −20 °C for maximum of 8 h before being transferred to a central laboratory at the Community Health Sciences Unit (CHSU) where the samples were stored at −80 °C. Samples were labelled with barcodes to enable tracking. Aliquots of urine were dispensed into 2 mL iodine free cryo-vial tubes, and transferred (frozen in cool boxes at the point of dispatch) to the UK for elemental analyses and hydration adjustment. After analysis, remaining samples were disposed of in accordance with the Material Transfer Agreement (MTA).

2.3. Analysis of Se concentration in urine

Total elemental concentrations of a routine suite of approximately 30 elements were determined in thawed urine samples following a ×10 dilution in 1% v/v HNO_3, 0.5% v/v HCl using an Agilent 7500cx series inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies, Santa Clara, USA) under the conditions described in Middleton et al. (2016a,b). Selenium was measured in hydrogen (H_2) reaction mode to reduce polyatomic interferences associated with ^{78}Se, such as $^{38}Ar^{40}Ar^{+}$. Internal standardization was achieved through the simultaneous introduction of tellurium (Te) via a T-piece. The limit of detection for Se was 0.3 μg L^{-1} based on 3× standard deviation of 137 analytical blanks. Certified reference materials (CRMs) were analyzed with urine samples: NIES No. 18 Human Urine (National Institute for Environmental Studies, Tsukuba, Japan) (total Se certified value: 59 ± 5 μg L^{-1}; recovery: 115%; precision: 6%; n = 57) and Seronorm Trace Elements Urine L-1 (LGC Group, Teddington, UK) (total Se certified value: 13.9 μg L^{-1}, recovery: 98%; precision: 3%; n = 20). Plasma Se concentration data measured using ICP-MS are described in Phiri et al. (2019).

2.4. Correction for hydration status

Correction of urine Se concentration data was undertaken using three approaches, osmolality, specific gravity, and creatinine, to explore how these corrections affected relationships between urine and plasma Se concentration.

Urinary osmolality (osm) was measured using an Osmomat 030 cryoscopic osmometer (Gonotec, Berlin, Germany) as described by Middleton et al. (2016a). This method involves comparing the freezing point of urine and that of pure water to determine osmolality expressed in milliosmoles (mOsm) per kg of water (Levine and Fahy, 1945)

For any given sample, with measured osmolality (o_{meas}), the measured Se concentration (Se_{uncor}) was corrected for osmolality to give a corrected value (Se_{osm}) as follows:

$$Se_{osm} = Se_{uncor} \times \frac{\bar{O}}{O_{meas}} \quad (1)$$

where $\bar{O}$ is the mean osmolality of all samples tested in the study, O_{meas}refers to osmolality measured.

Specific gravity (*sg*) was measured using a handheld temperature-corrected refractometer (PAL-10S, Atago, Japan), on 1 to 2 drops of urine. For any given sample, with measured specific gravity (sg_{Meas}), Se_{Mean} was adjusted to give a corrected value Se_{sg} as follows:

$$Se_{sg} = Se_{uncor} \times \frac{(sg_{Mean} - 1)}{(sg_{Meas} - 1)} \quad (2)$$

where sg_{Mean} is the mean of specific gravity of all samples tested in the study, sg_{Meas} refers to specific gravity measured.

Urinary creatinine (cre_{Meas}) was determined by enzymatic (creatinase) reaction using a Randox Rx Imola, RX series instrument (Randox Laboratories Limited, Crumlin, Co. Antrim, UK) and the following correction applied:

$$Se_{cre} = \frac{Se_{uncor}}{cre_{Meas}} \quad (3)$$

2.5. Data analysis

The objective of the analysis was to examine the relationship between plasma Se concentration and the corresponding urine Se concentration data with and without corrections. The key analytical steps are set out in detail below. In summary, we undertook exploratory analysis to examine the data and to select any necessary transformations. We then used a linear mixed model (LMM), which reflects the hierarchical sampling design (individuals selected within households within clusters), to examine the relationship between plasma and urine Se concentration, and the way in which the variation in plasma Se concentration not explained by a linear function of urine Se concentration is distributed between the individual, household and cluster levels of the sampling. We then estimated the cluster mean values of plasma and urine Se concentration so that the relationship between these variables could be visualized at this generalized, population scale. We used ordinary kriging (OK) to map plasma and urine Se concentrations so that these could be compared visually. Data for SAC were not gender disaggregated.

2.6. Exploratory analyses

Corrections of urine Se concentration were performed to minimize effects of hydration-driven dilution variation of samples among the study population. Both Se_{uncor} and corrected urine Se concentration (Se_{osm}, Se_{sg}, Se_{cre}) was compared to plasma Se concentration the commonly-used biomarker for Se status.

The objective of the exploratory analyses was to make an initial assessment of the appropriate scale at which to compare plasma and urine Se concentrations. Inspection of scatter diagrams, and the output of ordinary least squares regressions of plasma Se and urine Se concentrations, corrected and uncorrected, indicated that both variables were best analyzed on natural log (log_e) scales to make the assumption of linear effects and normally distributed residuals plausible. Ordinary least-squares estimates from these data are suitable only for exploratory purposes because they do not reflect the dependencies among the observations that arise from the sampling design. We also computed the mean value of the residuals from the exploratory models for each cluster and examined the empirical variogram of these values to explore evidence for spatial dependence of the variation of the between-cluster random effect. It is noteworthy that this exploratory empirical variogram is expected to underestimate the variation of the between-cluster random effect. However, this provided initial evidence of spatial dependence of the residuals.

2.7. Linear mixed model (LMM)

For the final analysis we used a LMM, which took the form:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Z}_C\eta + \mathbf{Z}_H\boldsymbol{\alpha} + \boldsymbol{\varepsilon} \quad (4)$$

where $\mathbf{y}$ is a vector of $\mathbf{n}$ observations of log_e-transformed plasma Se concentration, $\mathbf{X}$ is a design matrix with n rows and a column of 1 s (i.e. the value 1 in each cell of that column of the matrix) as it corresponds to a constant fixed effect, either the overall mean (for the null model) or the intercept of the regression on urine Se concentration. For models where urine Se concentration was included in the fixed effects the values of log_e-transformed urine Se concentration were included in a second column of $\mathbf{X}$. The term $\boldsymbol{\tau}$ is a vector containing the fixed effects parameters, respectively an intercept and a regression coefficient for the urine Se concentration. The term $\boldsymbol{\eta}$ is a random component, with a random value for the mean difference between the fitted and observed transformed plasma Se concentration under the model in each cluster of the original sample design. Each entry in this random vector is associated with the corresponding observation by the design matrix $\mathbf{Z}_C$ which indicates the cluster to which each observation belongs. Similarly, the term $\boldsymbol{\alpha}$ is a random effect which represents the deviation between the mean error of the fixed model within each household and the mean for the cluster to which that household belongs. The term $\mathbf{Z}_H$ is the design matrix which associates each observation with its corresponding household. Finally, $\boldsymbol{\varepsilon}$ is a random effect, the random deviation of the observation for each individual from its corresponding household mean.

In the LMM, Eq. (4), the two random effects, $\boldsymbol{\varepsilon}$ and $\boldsymbol{\alpha}$, are independent Gaussian random variables, each identically and

independently distributed with mean zero. The variances of these random effects are of interest, namely the variance of $\boldsymbol{\varepsilon}$ (the between-individual within household variance) and the variance of $\boldsymbol{\alpha}$ (the between-household within cluster variance). The random effect $\boldsymbol{\eta}$, the between-cluster random component, is modelled as a spatially-correlated random variable so the observations have a zero mean and a covariance matrix which takes the form:

$$\mathbf{V} = \sigma_C^2\mathbf{R}, \tag{5}$$

where $\sigma_C{}^2$ is the between-cluster variance and the correlation matrix $\mathbf{R}$ has entries

$$\mathbf{R}[i, j] = \rho(|\mathbf{x}_i - \mathbf{x}_j|; \kappa, \phi), \tag{6}$$

where $|\mathbf{x}_i - \mathbf{x}_j|$ denotes the distance in space between the locations, $\mathbf{x}_i$ and $\mathbf{x}_j$, of the i^{th} and j^{th} cluster respectively and $\rho(u; \kappa, \phi)$ is a Matérn correlation function (Matérn, 1986) where u is the lag distance between two locations in space and κ and ϕ are parameters which determine, respectively, the spatial smoothness of the variation of $\boldsymbol{\eta}$ over short distances and the rate at which the autocorrelation of the variable decays with distance in space.

Stein (1999) provides details of the Matérn correlation function. Briefly, the parameter κ controls the smoothness of the spatial random variable whereas ϕ controls, with κ, the spatial scale over which the variable shows spatial dependence (Stein, 1999). The larger the value of κ the smoother is the spatial variation of the random variable. The overall LMM therefore has five parameters for the random variation, the two parameters of the Matérn correlation function and the variances of the three random effects. These were estimated by residual maximum likelihood (REML) (Diggle and Ribeiro, 2007) using code written for the R platform (R Core Team, 2017). The use of REML minimizes the effects of uncertainty in the fixed effects parameters (in $\boldsymbol{\tau}$) on the variance parameter estimates. Once the variance parameters are estimated, they can be used to estimate the fixed effects parameters by weighted least squares (Lark and Cullis, 2004). One can also compute the standard error of these estimates. We followed Diggle and Ribeiro (2007) in estimating the κ parameter by a profile likelihood approach, considering some discrete values of κ (0.1, 0.5, 1 and 2), fixing κ at each of these values in turn and finding the corresponding REML estimates of the remaining parameters (Diggle and Ribeiro, 2007). The model for which the likelihood was largest was then selected. To characterize the scale of spatial dependence of a fitted model we computed the effective range. This is the lag distance at which the Matérn correlation takes a small value (0.05) and can be obtained by multiplying the parameter ϕ by a constant which depends on the value of κ as in Table 1.

We first fitted the model specifying a constant mean as the only fixed effect. We call this the null model. Our interest is in how including each of the predictors (urine Se concentration) reduced the variance of each random effect in the model, since the reduction of the random variance of some term indicates that the predictor provides information on the variation of plasma Se concentration observed at that level of the sampling design (between cluster, between household within cluster, or between individual within household). One way to quantify this is to compute an approximate adjusted R^2 statistic for each random component of the model. For example, if $\sigma_{C,N}{}^2$ is the estimated between-cluster variance for the null model (fixed effect a constant mean) and $\sigma_{C,U1}{}^2$ is the corresponding variance for the model with some corrected or uncorrected urine Se concentration U as a predictor variable, then the corresponding approximate adjusted R^2 is:

$$R^2_{C,U} = 1 - \sigma^2_{C,U}/\sigma^2_{C,N}. \tag{7}$$

We call this an approximate R^2 statistic because, with the variances estimated separately by REML rather than ordinary least squares, the values are not bounded between zero and 1. However, one can see that if including urine Se concentration accounted for all the variation in plasma Se between households then the expected value of $\sigma_{C,U}{}^2$ would be zero and $R_{C,U}{}^2$ would equal one. Similarly, if including urine Se concentration accounted for no variation in plasma Se concentration between households then the expected values of $\sigma_{C,U}{}^2$ and $\sigma_{C,N}{}^2$ would be equal and $R_{C,U}{}^2$ would equal zero. To aid the interpretation of our results we computed approximate adjusted R^2 statistics for between-individual (within household) variation, $R_{I,U}{}^2$, for between-household (within cluster) variation, $R_{H,U}{}^2$, and for between-cluster variation, $R_{c,U}{}^2$.

Table 1
Effective range expressed as a multiple of the ϕ parameter for different values of the κ parameter of the Matérn correlation function.

κ	Effective range
0.1	$1.4 \times \phi$
0.5	$3 \times \phi$
1.0	$4 \times \phi$
2.0	$5.5 \times \phi$

The κ and ϕ correlation parameters are described in the text following their appearance in Eq. (6).

2.8. Mapping urine Se concentration versus plasma Se concentration for WRA

Urine Se concentration as a potential biomarker for Se status for WRA was explored through mapping, and based on comparisons with plasma Se concentration whose mapping was described in Phiri et al. (2019). Briefly, corrected and uncorrected urine Se concentration data were used. An ordinary kriging (OK) estimate of individual urine Se concentration was computed at nodes of a 500 by 500 m square grid scale by exponentiation which gives a median-unbiased central value of the prediction distribution on the original scale (Pawlowsky-Glahn and Olea, 2004). The prediction grid data were mapped using ArcGIS (v10.3, ESRI, Redlands, CA, USA).

The urine Se concentration cluster means ($\log_e$ scale) were estimated for uncorrected data and for the three corrections (Se_{osm}, Se_{sg}, Se_{cre}). This was done with a weighted least-squares estimator using the variance components from the LLMs where each individual within a household with two WRA gets less weighting in the cluster mean than household with only one WRA. The cluster means for urine Se concentration were then plotted against the corresponding values for plasma Se concentration.

3. Results

3.1. Urine selenium concentration

The mean and median Se_{uncor} for WRA were 25.7 μg L^{-1} and 16.2 μg L^{-1}, respectively (standard deviation, SD ± 34.7 μg L^{-1}; range 0.8 to 398 μg L^{-1}; Table 2). The mean and median Se_{uncor} for SAC were 27.1 μg L^{-1} and 15.0 μg L^{-1}, respectively (SD ± 45.1 μg L^{-1}; range 0.8 to 562.8 μg L^{-1}). For WRA, the mean corrected urine Se concentrations (Se_{osm}, Se_{sg}, Se_{cre}) were smaller than Se_{uncor} (Table 2). For SAC, the mean Se_{uncor} concentration was larger than the corrected values for Se_{osm} and Se_{sg} except Se_{cre} (Table 2); this might arise as a consequence of protein energy malnutrition (PEM) being more prevalent among SAC. All Se_{uncor} data were above the detection limit of 0.3 μg L^{-1}.

3.2. Spatial comparison of urine and plasma Se concentrations

Table 3 shows the components of variance of plasma Se concentration ($\log_e$ scale) for WRA in a model for variation about the

Table 2
Urine Se concentration (all units are μg mL^{-1}, except creatinine, μg Se g $creatinine^{-1}$) for WRA and SAC.

Characteristics	Urinary dilution correction	Mean	Median	SD	Min	Max
WRA (n = 741)	Uncorrected	25.7	16.2	34.7	0.8	398
	Osmolarity	23.6	16.0	29.6	2.0	339
	Specific gravity	22.8	16.3	25.9	2.0	281
	Creatinine	20.4	14.3	25.7	3.3	300
SAC (n = 665)	Uncorrected	27.1	15.0	45.1	0.8	563
	Osmolarity	26.0	17.1	37.3	1.6	473
	Specific gravity	25.1	16.9	35.0	2.6	414
	Creatinine	31.9	20.9	52.7	4.3	651

n = sample number in the demographic group; SD = standard deviation; min = minimum value; max = maximum value, WRA = Women of reproductive age (aged 15–49 years); SAC = School aged children (aged 6–14 years).

overall mean value of the population. These results show that the largest component of variance is at the between-cluster level. The variance at between-household, within-cluster, level is an order of magnitude smaller, and is somewhat smaller than the variance between individuals within households. For SAC (Table 3) the between and within-household variance components are also an order of magnitude smaller than the between-cluster variance, but the between-household variance is the larger of the two.

Table 3 also shows the corresponding variance components for plasma Se concentration when Se_{osm}, Se_{sg}, Se_{cre} is included as a predictor. In so far as urine Se concentration succeeds as a predictor of variation of plasma Se concentration at each level of the sampling, the corresponding variance component will be reduced relative to its value in the "null" model (with no predictor) on the top row. The approximate adjusted R^2 values shown in the table show the reduction in each variance component as a proportion of the value in the null model and therefore measure the extent to which a particular predictor succeeds in accounting for variation in plasma Se concentration at the corresponding level.

It is notable that the corrected urine Se concentration values have markedly larger adjusted R^2 than does the Se_{uncor} urine Se concentration at the between-cluster and between-household level for both WRA and SAC. This shows the success of the corrections in reducing the variations in urinary Se concentrations that are likely to be attributable to variations in hydration status. It emphasizes that a suitable correction to concentrations in casual urine data is needed if these are to be used as a biomarker. We see that the R^2 are larger for Se_{cre} and Se_{sg} (approximately 0.6) for WRA at cluster level than for Se_{osm}. This is also the case at household level (approximate R^2 of 0.44 and 0.50 Se_{cre} and Se_{sg}, respectively).

The approximate R^2 values are smaller for SAC than for WRA (Table 3), showing that casual urinary Se is less useful as a biomarker for this demographic group. A further notable difference between the results for WRA and SAC is that the effective range (the largest distance over which there is spatial dependence) of the between-cluster variation is markedly reduced by including urine Se concentration as a predictor for WRA but not for SAC. The effective range of urine Se concentration at cluster level for WRA was 38–49 km, depending on the correction, compared to 118 km for the null model (Table 3). For SAC, the effective range was 64–87 km compared to 74 km for the null model (Table 3). These results indicate that whilst much of the long range spatially correlated variation in plasma Se concentration is predicted by urine Se concentration in WRA, and that this is relatively insensitive to correction factors, in SAC, less of long range spatially correlated variation in plasma Se concentration is predicted by urine Se concentration and it is more dependent on the correction factor.

Table 3 also gives the estimated intercept and slope for the linear function that predicts plasma Se concentration from urine Se concentration, with the associated standard errors. For both WRA and SAC, the slopes for corrected urine Se concentration are an order of magnitude larger than their standard errors, showing strong evidence for the linear relationship. The difference is smaller in both cases for Se_{uncor}, although still large enough to support the linear model. Thus, urine Se concentration is a significant predictor of Se status at a population level.

3.3. Cluster mean correlation between urine Se concentration and plasma Se concentration

There are correlations between urine Se concentration and plasma Se concentration at between-household and between-cluster levels (Table 3). One way to visualize these relationships is to plot the estimated mean urine and plasma Se concentrations of the different clusters (Fig. 1). For WRA, the relationship between cluster mean Se_{osm}, Se_{sg} or Se_{cre} and plasma Se concentration is stronger than for Se_{uncor}, as indicated by the approximate R^2 values (Table 3). A similar pattern was observed in SAC, where there is a stronger correlation between Se_{sg} and

Table 3
Variance parameters for random effects for plasma selenium concentration in WRA (aged 15–49 years) and SAC (aged 6–14 years) in Malawi.

Predictor of plasma Se concentration	κ	Variance: between-individuals within households	Variance: between-households within clusters	Variance: between clusters	$R_{I,U}^2$	$R_{H,U}^2$	$R_{C,U}^2$	ϕ for between-cluster random effects	Effective range	Intercept: WLS estimate and standard error		Slope: WLS estimate	
WRA													
Null model	0.5	0.03	0.02	0.15				39.4	118				
Urine-Osm	2	0.03	0.01	0.07	−0.11	0.39	0.52	7.22	39.7	3.85	0.06	0.17	0.02
Urine-SG	2	0.03	0.01	0.06	−0.11	0.50	0.59	6.97	38.3	3.71	0.06	0.22	0.02
Urine-Cre	1	0.03	0.01	0.06	−0.04	0.44	0.61	12.3	49.4	3.61	0.07	0.27	0.02
Urine-Unc	2	0.03	0.01	0.11	−0.11	0.22	0.29	8.28	45.5	4.16	0.06	0.07	0.01
SAC													
Null model	0.5	0.02	0.04	0.16				24.7	74.1				
Urine-Osm	0.5	0.02	0.03	0.09	−0.05	0.24	0.39	21.9	65.6	3.78	0.07	0.15	0.02
Urine-SG	0.5	0.02	0.03	0.08	−0.11	0.32	0.51	21.5	64.5	3.61	0.07	0.20	0.017
Urine-Cre	0.5	0.02	0.03	0.09	−0.16	0.32	0.43	28.9	86.7	3.55	0.09	0.21	0.021
Urine-Unc	0.5	0.02	0.03	0.12	−0.05	0.08	0.19	23.7	70.7	4.06	0.07	0.06	0.01

Approximate adjusted R^2 values (Equation [4]) are based on nested variance components: $R_{I,U}^2$ = variation between-individuals within household; $R_{H,U}^2$ = variation between-households within clusters; $R_{c,U}^2$ = variation between-clusters. GLS: weighted least squares estimates. The Matérn correlation parameters κ and ϕ are described in the text where they appear in Eq. (6). Note, if κ is larger, the random variation is spatially smoother than for a smaller κ. The κ parameters in Table 3 were selected on the basis of their maximum profile likelihood, following the procedure described by Diggle and Ribeiro (2007). The κ parameters selected indicate that the random variation in urine Se concentration is spatially smoother in WRA than in SAC.

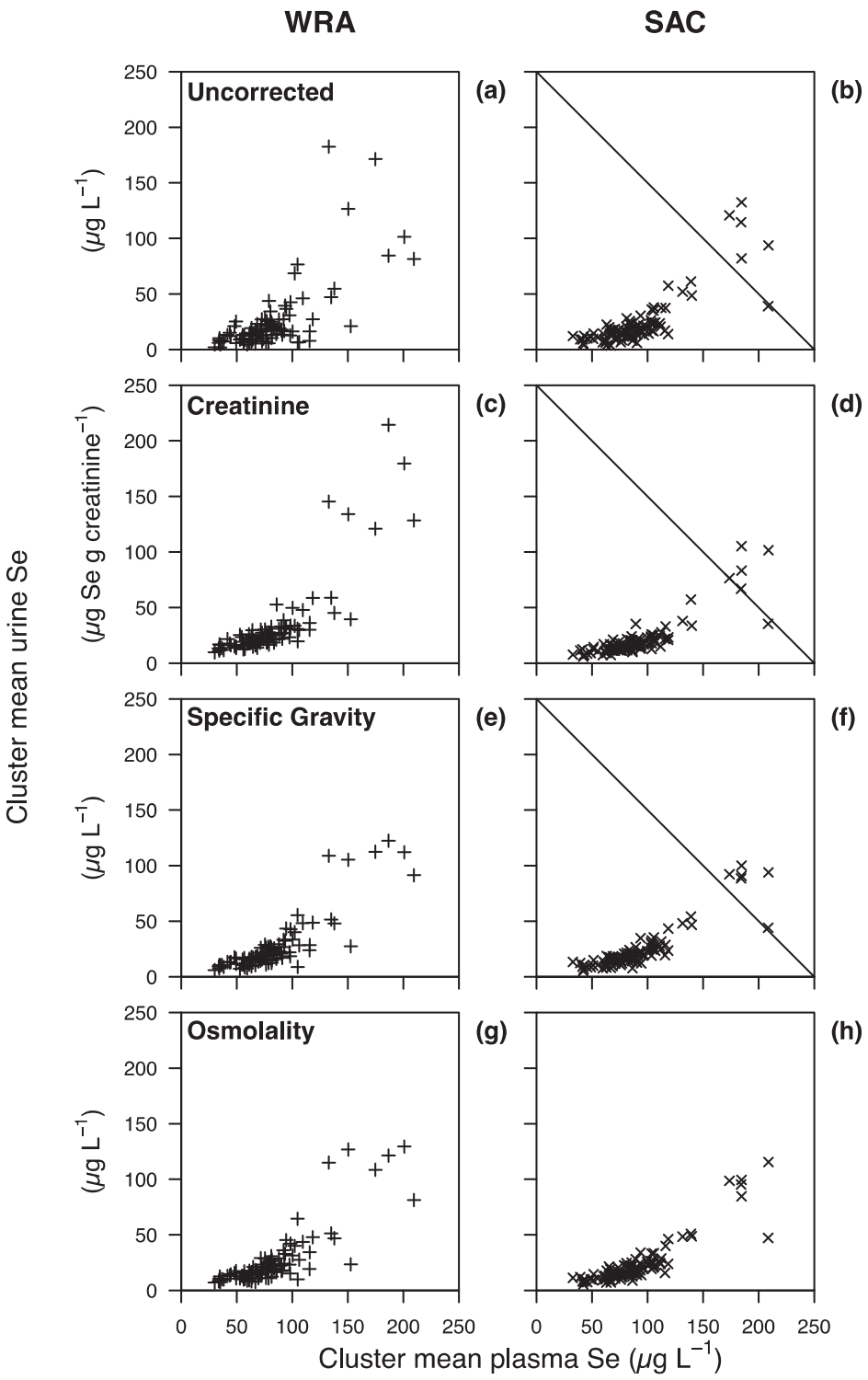

Fig. 1. Estimated cluster mean concentrations of plasma selenium concentration and (a) Se_{Uncor}, (b) Se_{Osm}, (c) Se_{SG} and (d) Se_{Cre} for women of reproductive age (WRA) and school-aged children (SAC). Note that these are unbiased back-transformations of the estimates on the $\log_e$ scale.

plasma Se concentration than for Se_{osm}, Se_{cre} and Se_{uncor} (Table 3).

3.4. Mapping of casual urine Se concentration versus plasma Se concentration for WRA

The median-unbiased back transformed values of the OK predictions of urine Se concentration (WRA, all corrections) are presented as maps, along with the corresponding map of plasma Se concentration reported by Phiri et al. (2019), (Fig. 2). These show the spatial pattern of the predicted concentrations of the different biomarker at national scale. The broad spatial pattern of Se concentration in urine and in blood plasma (Phiri et al., 2019) are similar. Concentrations of Se in both urine and plasma are greatest in the southern part of Malawi along the Shire River, and in areas closer to Lake Malawi (Fig. 2). However, not all the variations seen in plasma Se concentration are reflected in the urinary measurements. This may reflect the observation that the spatially correlated random variation remains substantial in the mixed models for plasma Se concentration with urine Se concentration as a

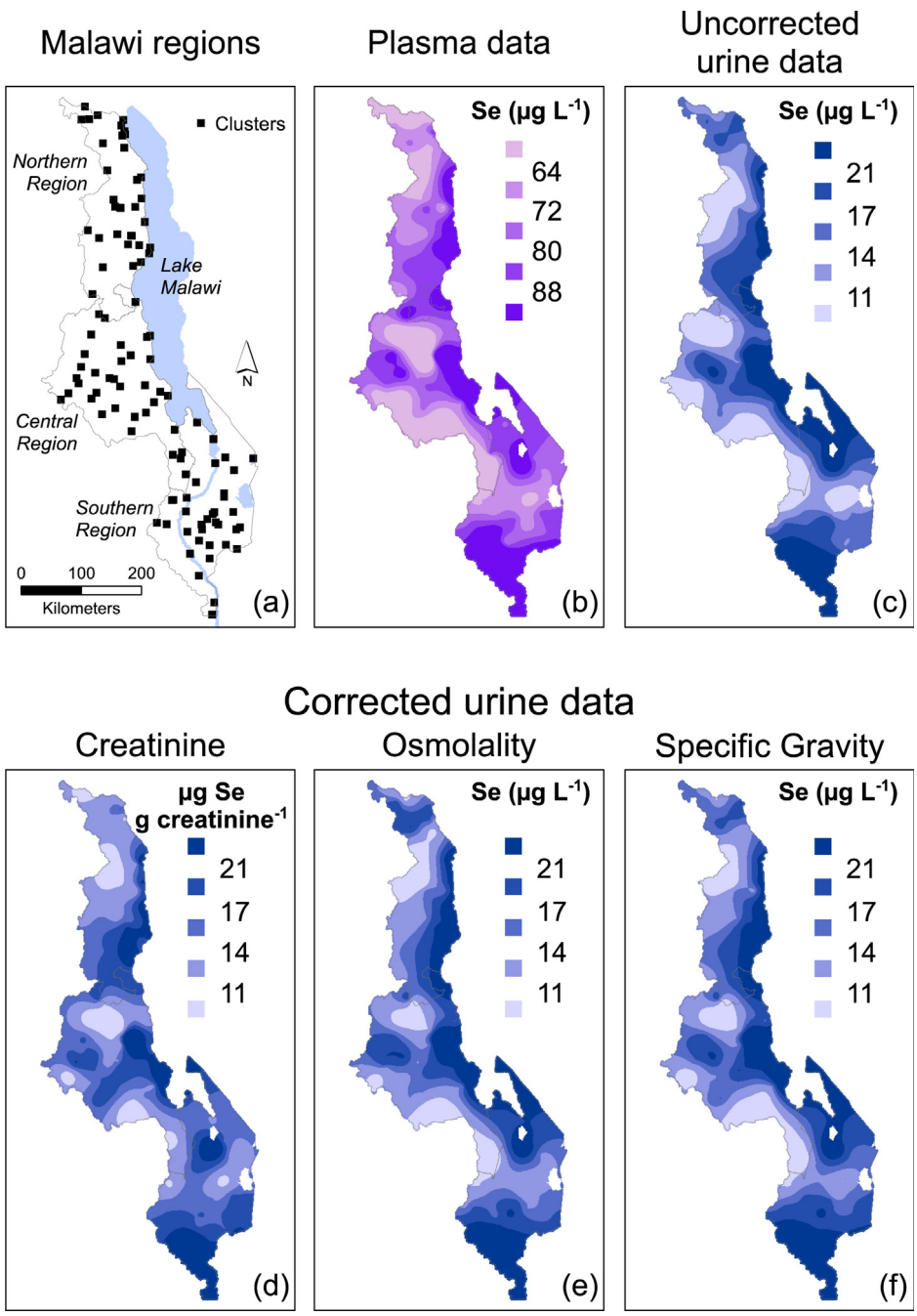

Fig. 2. Predicted Se concentration across Malawi (a), in plasma (b; adapted from Phiri et al., 2019), and urine, Se_{Uncor}, (c) Se_{Osm}, (d) Se_{SG} (e) and Se_{Cre} (f) for WRA.

fixed effect.

4. Discussion

Urine Se concentration predicted some of the variation seen in blood plasma Se concentration at broad regional scales (i.e. between clusters). This shows that at population level, urine Se concentration could be used as a biomarker for assessing Se-status, as predicted previously from a small-scale cross-sectional study of WRA in two areas of Malawi of contrasting dietary Se intakes due to soil type and resulting food composition (Hurst et al., 2013).

There were no significant population-level differences in uncorrected urine Se concentration between WRA and SAC. At a cluster-level, correction of urine Se concentration for hydration status improved the prediction of plasma Se concentration for both demographic groups. In WRA, all corrections predicted plasma Se concentration better than uncorrected urine Se concentration, whilst in SAC the Se_{sg} predicted plasma Se concentration better than $Se_{uncor.}$ The use of specific gravity correction has also shown to work well for other urinary surveillance, including cadmium and steroid hormone concentrations (Miller et al., 2004; Suwazono et al., 2005). Unexpectedly, Se_{sg} appeared to perform better than Se_{osm} within this study. Osmolality is typically considered a more robust measure of urinary dilution than specific gravity. It is already known that creatinine correction should be used with caution in settings where protein energy malnutrition (PEM) is likely, such as in many parts of sub-Saharan Africa (Allen et al., 2006; Bain et al., 2013; Cockell, 2015), which is further supported by the findings for SAC in this study.

At cluster level, urine Se concentration predicted plasma Se concentration better in WRA than in SAC. To our knowledge, there is no

evidence why this is the case, however, it has previously been reported that urinary Se retention is greater in adult males than adult females (Combs, 2015). Thus, urine Se concentrations may be greater, and more strongly correlated with Se intake, in female-only cohorts. Many other factors, genetic and environmental, are likely to influence Se retention and excretion in urine; these factors along with body size, age, and gender will also affect the performance of urinary adjustments for hydration status. Further exploration of these factors is outside the scope of the current study.

The approximate R^2 value of 0.50 for Se_{sg} concentrations at household level for WRA suggests that the biomarker may have some value for indicating differences between households, but with more uncertainty than at between-cluster scale. In contrast, the R^2 values between individuals, for both WRA and SAC, are all very small, showing that differences between individuals in urine Se concentration have no predictive value for showing between-individual differences in Se status compared to plasma Se concentration. This is consistent with observations on urinary iodine data, which is widely used for population assessments but is not appropriate for assessing individual-level status (WHO, 2007). It may partially be due to the effect of intra- and inter-individual differences in hydration, as observed in studies in multiples biomarkers (Koch et al., 2014; Middleton et al., 2018; O'Brien et al., 2017), which were not entirely addressed by the corrections of the raw urine Se concentrations in the present study.

The spatial patterns in urine Se concentration for WRA, and the effective ranges of spatial correlation of up to ~50 km, is consistent with previous observations for plasma Se concentration in WRA in Malawi (Phiri et al., 2019). The spatial dependency of urine Se concentration can be hypothesized to be influenced in the same ways as plasma Se concentration, for example, by soil type and dietary patterns (Chilimba et al., 2011; Hurst et al., 2013; Joy et al., 2015a,b). As seen previously for plasma Se concentration in WRA, urine Se concentration is much greater in clusters in the south of Malawi and near to Lake Malawi, where vertisol soil types are more prevalent, leading to greater grain Se concentration and dietary Se intakes. These results are therefore consistent with a previous small cross-sectional study in Malawi showing associations between dietary Se intake, plasma Se concentration, and casual urine Se concentration (Hurst et al., 2013), and with national-scale geostatistical modelling shown that Se deficiency risks in Malawi are influenced by soil type, dietary patterns and proximity to the lake (Phiri et al., 2019).

Taken together, the results provide evidence for the opportunity of using casual urine as a population level biomarker of Se status in sub-Saharan Africa. It may be most useful where nutrition surveillance systems such as Demographic and Health Surveys and National Micronutrient Surveys are already in place and where urine samples are already being collected. In such contexts, the Se status of the population, and regional spatial variations that may be important both for understanding the causes of deficiency and for prioritizing interventions, can be analyzed at marginal cost if there is access to sensitive instrumentation and good quality control during sample collection and reporting.

The approaches for correcting urine Se concentrations in the present study were those routinely used in the literature. Specific gravity correction for hydration status is a relatively low-cost method for hydration corrections with a simple to operate refractometer and no on-going consumables, capable of field or laboratory measurements. It might therefore be considered a good first option during surveillance in similar contexts. Modifications of these correction equations have been explored recently (Middleton et al., 2016a) and have shown to be promising to improve current practice. However, such modifications were outside the scope of the present study, and further studies to assess the use of different corrections for measuring Se status using casual urine are recommended, especially in different geographic contexts to those of most existing datasets, such as this study population represents.

In cases where micronutrient surveillance collects blood, our study does not provide evidence that urine Se concentration has any technical advantages over plasma Se concentration in assessing the Se status of the population, but it may provide a cost effective and less invasive method for standalone studies requiring population Se-status data.

5. Conclusions

To our knowledge this the largest reported assessment of Se status using urine Se concentration among a nationally representative groups in a low Se-status context, and also the first study which shows evidence of spatial variation in urine Se concentration in sub-Saharan Africa. These results suggest that casual urine Se concentration is a viable alternative biomarker to plasma Se concentration for assessing population level Se status. Urine Se concentration can identify regional spatial variations in Se status, which is likely to reflect differences in Se characteristics of these food systems, e.g. driven by soil type and access to different food groups. This evidence supports the potential to review polices around national monitoring and surveillance systems of micronutrient deficiencies, also referred to as hidden hunger, where urine samples are already collected for analysis of iodine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Funding for this study was provided by the University of Nottingham (UoN) UK, the British Geological Survey (BGS) UK, and the Royal Society-Department for International Development (DFID) UK, under project AQ140000, "*Strengthening African capacity in soil geochemistry for agriculture and health*". We thank The Government of Malawi (Ministry of Health; Ministry of Agriculture, Irrigation and Water Development; National Statistics Office), and the Centers for Disease Control & Prevention (CDC), notably Katie Tripp, Anne Williams, and Carine Mapango, for enabling this work to be conducted, together with all of the participating field teams and volunteers.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105218.

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