Letter to the Editor

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Systematic review and meta-analysis of within-subject and between-subject biological variation estimates of serum zinc, copper and selenium

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To the Editor,

Zinc (Zn), copper (Cu) and selenium (Se) are essential trace elements (TrEl) which are involved in various metabolic pathways. Importantly, more than 300 enzymes from all six categories of enzyme systems need Zn for their activity. Cu, similarly to Zn, is an essential component of several enzymes involved in the ATP synthesis in mitochondria. It is also required for the formation of connective tissues and in iron metabolism, for oxidizing ferrous iron to ferric iron [1]. Se is an essential component of the anti-oxidative system and has a crucial role in thyroid functions. Deficiencies of these TrEls have been reported in various clinical situation including growth retardation, immune dysfunctions, neurological disorders etc. [1], and adequate supplies of TrEls are critical in maintaining normal physiological functions and preventing diseases [1]. As the name suggests, the physiological concentrations of TrEls are very low. Correct measurements and understanding the variations affecting the test results are essential for safe and valid clinical interpretation

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of TrEls. In comparison to atomic absorption spectrophotometry (AAS), multiple TrEls can be measured accurately in a single run by new generation inductively coupled plasma mass spectrometry (ICP-MS) [2].

Biological variation (BV) data, including the within-subject (CV_I) and between-subject (CV_G) BV, can be used for (i) the assessment of the index of individuality (II), (ii) the evaluation of the significance in changes in serial test results by the reference change value (RCV), (iii) the calculation of the number of samples required to estimate the homeostatic set point, (iv) the estimation of the total variation around the homeostatic set point for personalized reference intervals [3] and (v) defining analytical performance specifications [4]. These applications, however, require that the BV data is reliable and relevant to the populations and settings to which they will be applied. The aims of this study were to systematically appraise the published BV data of Zn, Cu and Se using the BV Data Critical Appraisal Checklist (BIVAC) criteria [5] and to perform meta-analyses of BV data from BIVAC compliant studies to deliver global BV estimates for Zn, Cu and Se.

A systematic bibliographic research was performed in PubMed and Web of Science for BV studies on Zn, Cu and Se using the terms 'trace elements', 'Zinc', 'Copper', 'Selenium', 'biological variability' and 'biological variation' (cut-date April 2021). In total, 8 papers reporting on BV of TrEls were identified and included in the study (Supplemental Table 1), 4 of which were published before 2000. In this review, the studies are identified by the article number they are given in the EFLM BV database [6]. All papers were evaluated by two independent reviewers for their compliance with the BIVAC criteria (14 quality items [QI]). In the BIVAC, a grade A indicates full compliance with all 14 QIs, a grade B and C indicate that the lowest QI score was a B or a C, respectively. If a study fails to comply with one of the essential BIVAC elements related to the study population, samples and the measurand (QIs 2–4), it is awarded a D-grade. Additionally a subscript system was applied to demonstrate the score of the paper in detail; for example, if a paper was graded as 'C' due to the QI 8 (outlier analysis), 10 (variance homogeneity) and 13 (number of included results), this is shown as '$C_{8,10,13}$' (Table 1), the 8, 10, 13 is where the QIs are deficient.

The method used to extract BV data from the BIVAC compliant studies has been described in detail previously [7]. The inclusion criteria for the meta-analysis were adopted from the EFLM BV database [6]; including studies with BIVAC grades A, B or C where the (1) the study had been performed in healthy subjects 18–75 years old, (2) included ≥3 subjects (3) with ≥3 samples per subject (4) and the sampling intervals ranged from 2 samples per week to 1 sample per month. In addition, BV data obtained from subjects on specific diets were excluded. In the meta-analysis, global estimates were calculated by the weighted median approach, where a factor of 4, 2 and 1 were given to A, B and C graded papers, respectively, and multiplied with the inverse range of the CI of the BV estimates [7]. In the last step, the CI of the meta-analysis estimate for each TrEl was calculated using a percentile boot strap technique [8].

Based on the BIVAC criteria, only 1 study received an A grade [9]. The authors followed the European Biological Variation Study (EuBIVAS) protocol [10], collected weekly samples from 68 health subjects (36 females and 32 males) for 10 weeks and measured serum Zn, Cu and Se using ICP-MS [9]. The 7 remaining papers were graded as C (Table 1). Marked heterogeneity was observed in the study design including study duration (from 3 days to 5 months), sampling intervals (daily, weekly, monthly), study population (women, men) and age groups (between 20 and 89 years).

The meta-analyses for Zn were based on the data of 6 papers (1 A and 5 C studies; reporting results for 8 subgroups) (Table 1) and delivered CV_I and CV_G estimates of 8.9 (6.3–11.0) and 8.3% (4.0–23.3), respectively (Table 2). The two excluded studies reported daily samplings in subjects on a constant diet (paper 52a, c, e) and analysed capillary plasma from elderly subjects (paper 57), reporting a CV_A estimate of 0 in one subgroup (paper 52a). Despite a high degree of heterogeneity in the studies, the reported CV_I estimates ranged for Zn from 6.1 (paper 52b) to 11.0% (paper 97), whereas the CV_G varied from 3.9% (paper 52b) to 23.3% (paper 493). The studies reporting the most extreme results used AAS to measure Zn and were assigned a BIVAC grade C, but the population and the number of samples included in the study were different (Table 1).

The meta-analysis estimates for Cu were based on data of 7 (1 A and 6 C grade) papers (Tables 1 and 2). Similar to Zn, the BV data of the study cohorts with daily sampling and subjects on a constant diet (paper 52a, c, e) were excluded. One study analyzed Cu using a colorimetric method (paper 525), but reported similar CV_I and CV_G estimates as the other studies.

Only 3 papers (1 A and 2 C grade) reported BV of Se (Table 1). The meta-analysis based CV_I and CV_G estimates for Se were 7.8 (2.5–12.0) and 11.4% (6.9–12.9), respectively. One publication (paper 542) reported a low CV_I estimate (2.5% [0.0–4.3]), therefore the CI of the CV_I was wide (2.5–12.0). The CI for the meta-analysis estimates is based on a bootstrap method which when including less than 10 estimates, gives an approximation of the range of the estimates, as the CI.

In conclusion, this study provides evidence-based and updated estimates of CV_I and CV_G values for Zn, Cu and Se. Although the ICP-MS method (paper 546) fulfills the APS based on these estimates, this is not the case for most of the AAS methods applied in the other BV publications. Our systematic review identified a low number of high-quality

Table 1: Overview of studies reporting biological variation data for zinc (Zn), copper (Cu) and selenium (Se) with their associated Biological Variation Data Critical Appraisal Checklist (BIVAC) grade, study details and whether the study fulfilled the meta-analysis (Ma) criteria.

First author (paper number)	Gender	State of well-being	Number of subjects (F/M)	Sample matrix	Analytical method	Sampling intervals and study duration	Trace elements	BIVAC grade	Fulfills criteria for Ma
González-Revaldería (61) 1990	Mixed	Healthy	15 (10/5)	Serum	AAS	One sample/week for four weeks	Zn, Cu	$C_{8,10,13}$	Yes
Gallagher (52a) 1989	Female	Healthy	5	Plasma	AAS	One sample/day for five days	Zn, Cu	$C_{5,7,8,10,12,13}$	No
Gallagher (52b) 1989	Female	Healthy	5	Plasma	AAS	One sample/week for five weeks	Zn, Cu	$C_{5,7,8,10,12,13}$	Yes
Gallagher (52c) 1989	Female	Healthy, constant diet	5	Plasma	AAS	One sample/week for five weeks	Zn, Cu	$C_{5,7,8,10,12,13}$	No
Gallagher (52d) 1989	Female	Healthy	5	Plasma	AAS	One sample/month for five months	Zn, Cu	$C_{5,7,8,10,12,13}$	Yes
Gallagher (52e) 1989	Female	Healthy, constant diet	5	Plasma	AAS	One sample/month for five months	Zn, Cu	$C_{5,7,8,10,12,13}$	No
Giles (57a) 1994	Mixed	Healthy, >60 years old	36 (28/8)	Capillary plasma	AAS	One sample/day for three days	Zn,	$C_{8,10,13}$	No
Giles (57b) 1994	Mixed	Healthy, >60 years old	36 (28/8)	Capillary plasma	AAS	One sample/week for two weeks	Zn,	$C_{8,10,13}$	No
Giles (57c) 1994	Mixed	Healthy, >60 years old	36 (28/8)	Capillary plasma	AAS	One sample/day for three days and one sample/week for two weeks	Zn,	$C_{8,10,13}$	No
Lux (97a) 1995	Mixed	Healthy	12 (4/8)	Plasma	AAS	One sample/week for 12 weeks and one sample/month for three months	Zn, Cu, Se	$C_{7,8,10,13}$	Yes
Lux (97b) 1995	Mixed	Healthy	12 (4/8)	Heparinized whole blood	AAS	One sample/week for 12 weeks and one sample/month for three months	Se	$C_{7,8,10,13}$	Yes
Yücel (493) 2019	Mixed	Healthy	20 (13/7)	Plasma	AAS	One sample/week for 10 weeks	Zn, Cu	C_8	Yes
Bal (542) 2021	Mixed	Healthy	15 (9/5)	Plasma	AAS	One sample/week for 10 weeks	Zn, Cu, Se	C_8	Yes
Coskun (546a) 2021	Female	Healthy	36	Plasma	ICP-MS	One sample/week for 10 weeks	Zn, Cu, Se	A	Yes
Coskun (546b) 2021	Male	Healthy	32	Plasma	ICP-MS	One sample/week for 10 weeks	Zn, Cu, Se	A	Yes
Braga (525a) 2013	Mixed	Healthy	19 (9/10)	Serum	Colorimetric	One sample/two weeks for two months	Cu	$C_{7,10,13}$	Yes
Braga (525b) 2013	Female	Healthy	9	Serum	Colorimetric	One sample/two weeks for two months	Cu	$C_{7,10,13}$	Yes
Braga (525c) 2013	Male	Healthy	10	Serum	Colorimetric	One sample/two weeks for two months	Cu	$C_{7,10,13}$	Yes

F, female; M, male; AAS, atomic absorption spectrophotometry; ICP-MS, inductively coupled plasma mass spectrometry.

Table 2: Meta-analysis derived within-subject (CV_I) and between-subject (CV_G) BV estimates with 95% confidence intervals (CI), index of individuality (II), analytical performance specifications (APS) for imprecision (CV_{APS}) and bias (B_{APS}) for zinc, copper and selenium.

Measurands	Meta-analysis-based estimates							
	N_i	n_i	Mean ± SD	CV_I (CI), %	CV_G (CI), %	II	CV_{APS}%	B_{APS}%
Zinc, µmol/L	6	8	13.4 ± 0.9	8.9 (6.3–11.0)	8.3 (4.0–23.3)	1.07	4.5	3.1
Copper, µmol/L	7	11	14.2 ± 2.0[a]	7.5 (4.7–8.0)	13.1 (4.3–19.6)	0.57	3.7	3.8
Selenium, µmol/L	3	5	1.2 ± 0.3	7.8 (2.5–12.0)	11.4 (6.9–12.9)	0.68	3.9	3.4

N_i, number of studies; n_i, number of subgroups included in meta-analysis. [a]Paper 493 reported very low mean value (0.0013 µmol/L) and was excluded from the mean and SD calculations.

BV studies for Zn, Cu and Se and additional studies using ICP-MS should be encouraged.

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