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Within- and between-subject biological variation data for serum zinc, copper and selenium obtained from 68 apparently healthy Turkish subjects

https://doi.org/10.1515/cclm-2021-0886
Received August 9, 2021; accepted September 27, 2021; published online October 22, 2021

Abstract

Objectives: Trace elements (TrEL) are nutritionally essential components in maintaining health and preventing diseases. There is a lack of reliable biological variation (BV) data for TrELs, required for the diagnosis and monitoring of TrEL disturbances. In this study, we aimed to provide updated within- and between-subject BV estimates for zinc (Zn), copper (Cu) and selenium (Se).

Methods: Weekly serum samples were drawn from 68 healthy subjects (36 females and 32 males) for 10 weeks and stored at −80 °C prior to analysis. Serum Zn, Cu and Se levels were measured using inductively-coupled plasma mass spectrometry (ICP-MS). Outlier and variance homogeneity analyses were performed followed by CV-ANOVA (Røraas method) to determine BV and analytical variation estimates with 95% CI and the associated reference change values (RCV) for all subjects, males and females.

Results: Significant differences in mean concentrations between males and females were observed, with absolute and relative (%) differences for Zn at 0.5 µmol/L (3.5%), Cu 2.0 µmol/L (14.1%) and Se 0.06 µmol/L (6.0%). The within-subject BV (CV_I [95% CI]) estimates were 8.8% (8.2–9.3), 7.8% (7.3–8.3) and 7.7% (7.2–8.2) for Zn, Cu and Se, respectively. Within-subject biological variation (CV_I) estimates derived for male and female subgroups were similar for all three TrELs. Marked individuality was observed for Cu and Se.

Conclusions: The data of this study provides updated BV estimates for serum Zn, Cu and Se derived from a stringent

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protocol and state of the art methodologies. Furthermore, Cu and Se display marked individuality, highlighting that population based reference limits should not be used in the monitoring of patients.

Keywords: biological variation (BV); copper (Cu); selenium (Se); trace elements (TrEL); zinc (Zn).

Introduction

Trace elements (TrEL) are essential components in maintaining the development and health of humans [1]. Zinc (Zn), copper (Cu) and selenium (Se) have numerous and important metabolic functions including normal growth, immunological functions, energy metabolism, antioxidative systems etc. [2]. Normally, the physiological concentration of TrELs in serum is very low ("trace amount", μmol/L), and both the deficiency and the toxicity of TrELs may have serious clinical consequences and give raise to public health problems. Therefore, correct measurements of TrELs are essential for appropriate nutritional assessments and treatment of patients. To ensure safe clinical application of the TrELs, reliable biological variation (BV) data are required [3, 4]. BV has two main components; within-subject BV (within-subject biological variation [CV_I]) which is described as the random fluctuation around a homeostatic set point of an analyte and between-subject BV (between-subject biological variation [CV_G]), which is the variation between the homeostatic set points of an analyte among different individuals [5]. BV data is widely used in laboratory medicine including: (1) the setting analytical performance specifications (APS) of measurement systems [5–7], (2) the calculation of reference change value (RCV), which is used to evaluate the significance between consecutive measurements, (3) the index of individuality (II) to evaluate the utility of population based reference intervals (popRI), and (4) personalized reference intervals (prRI) [7].

Limited data are available for the BV of TrELs [8], and most of the existing data were obtained in the 1980s and 1990s [9], not adhering to today's standards for reporting of BV studies. The European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Working Group (BV-WG) has defined a checklist identifying the key elements to be reported in BV studies [10] and have also, in collaboration with the EFLM BV Task group on the Biological Variation Database, developed the Biological Variation Data Critical Appraisal Checklist (BIVAC), which can be used to evaluate the quality of published data, prior to meta-analysis and inclusion in the EFLM BV Database [11]. Moreover, the BV-WG set up the European Biological Variation Study (EuBIVAS), a BIVAC compliant multicenter study, which has delivered updated BV data for numerous measurands [12] and in which the pre-analytical and analytical phases were standardized, as published in detail in [13].

In this study, we followed the EuBIVAS protocol [13] and the EuBIVAS statistical approaches, the "Røraas method" [14], to report BV estimates for Cu, Zn and Se in agreement with the EFLM checklist [10] and BIVAC criteria [11] and used this data to deliver updated APS and RCV and to assess the II for these markers.

Materials and methods

Study population and protocol

The study was conducted at Acibadem Labmed Clinical Laboratories and Acibadem Mehmet Ali Aydınlar University, Istanbul, Turkey. In total, 106 apparently healthy subjects (males and females) were recruited from university staff, students and laboratory workers. The study protocol was approved by Institutional Ethical Review Board of Acibadem Mehmet Ali Aydınlar University in agreement with the World Medical Association Declaration of Helsinki. Prior to inclusion in the study, all subjects provided written informed consent and completed questionnaires to provide information on their lifestyle and health status including physical activity, eating habits, dietary supplements and use of smoking and alcohol. The inclusion and exclusion criteria were based on the EuBIVAS [13], with exclusion criteria including diagnosis of diabetes mellitus, dyslipidemia, history of chronic kidney and liver diseases, thalassemia and hemoglobinopathies, known carrier for hepatitis B, C and HIV and female subjects who were pregnant or breastfeeding. In the first week, a group of tests were performed in all participants to provide data to assess the exclusion criteria. Subjects with any of the following results; whose fasting serum glucose >6.94 mmol/L (126 mg/dL), gamma-glutamyl transferase (GGT) >150 U/L and total cholesterol >6.47 mmol/L (250 mg/dL) were excluded. In addition, subjects reporting taking TrEl as dietary supplements were also excluded. Additionally, to follow the health status of the subjects throughout the study, serum C-reactive protein (CRP), alanine aminotransferase (ALT), triglycerides and creatine kinase (CK) were measured every week and drugs and dietary supplement taken by subjects recorded. Subjects whose CK activity was higher than the upper limit of the reference interval, but who reported performing regular exercise, were not excluded from the study.

Based on the initial evaluation, two subjects were excluded from the study as one subject had high body mass index (BMI) (34 kg/m^2) and one was breastfeeding. Nineteen subjects withdrew from the study without donating samples. The data of 17 subjects, for which less than four samples were collected, were also excluded from the study. Additionally, two samples of the subjects taking dietary supplements were excluded from the study. Two subjects who were vegetarians and two subjects on diet of the sports program were not excluded from the study.

Sample collection and handling

Morning fasting blood samples were drawn from all participants once weekly, on the same day (Tuesdays–Fridays), for 10 consecutive weeks from February to April 2018. If it was not possible to obtain a sample from a subject due to personal reasons, blood was drawn on the same day the following week. All samples were drawn by the same phlebotomist. Blood samples were collected into deionized tubes (BD royal blue). All samples were centrifuged at 3,000 *g*. Serum samples were stored at −80 °C until analysis (July 2019).

Analytical methods

All analyses were performed in duplicate on inductively-coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies 7700 Series ICP-MS). Due to a high number of samples and the limited capacity of ICP-MS, all samples were analyzed in 13 runs. All analyzes were completed within 3.5 months (July–October 2019). The same lots of standards, internal quality control (IQC) materials and consumables were used throughout the study. No significant deviations or drift were detected in both IQC and EQAS during the entire analysis period.

Data analysis

The data analysis was performed as previously described [15, 16]. Firstly, outlier subjects were identified based on the Dixon-Q test and excluded. In the second step the homogeneity of within-subject and analytical variabilities (replicate measurements) were verified on CV-transformed [14] data using Bartlett and Cochran tests, respectively. In case of heterogeneity for the analytical component, the one most different of the duplicate measurement results was excluded as a first step. If the heterogeneity persisted, then the second measurement result was excluded as well. Larger individual systematic changes were identified by the homogeneity test of the CV_I (Cochran test). For each measurand, to examine if individuals were in steady-state, the linear regression was calculated as previously described in EuBIVAS publications for other measurands [17, 18]. The linear regression of the means of the 136 duplicate analyses from every blood drawing 1, 2 … 10 (pooled mean group sample concentrations) vs. the blood drawing number (1–10) was calculated for each TrEL. Subjects were considered in steady state if the 95% CI of the slope of the regression line included zero and if not, the data were adjusted by applying the inverse equation of the regression to all measurement results at each week.

The Røraas method, a CV-ANOVA method, was used to derive the estimates of CV_I of TrELs [14]. BV estimates were calculated for the overall study population and subgroups of males and females.

The CV_G of the measurands were estimated using ANOVA on natural log-transformed data. The Kolmogorov Smirnov test was used to verify the normality of the distributions of the mean values. The Burdick and Graybill method was used to calculate the 95% CIs of BV estimates [19], and the difference between BV estimates of subgroups (female and male) was evaluated based on the overlap, or lack of overlap, of their CIs. The correlation between the TrELs concentrations and subjects' ages were analyzed by Spearman r tests.

APS for analytical imprecision (CV_{APS}), analytical bias (B_{APS}), II and the number of samples required to estimate the homeostatic set points (NHSPs) were calculated using the following equations.

$$CV_{APS} = 0.5 \times CV_I \quad (1)$$

$$Bias_{APS} = 0.25 \times \sqrt{CV_I^2 + CV_G^2} \quad (2)$$

$$II = \frac{CV_I}{CV_G} \quad (3)$$

The RCV for increase and decrease were calculated separately using the equations given below:

$$SD^2_{A,\log} = Log_e\left(CV_A^2 + 1\right) \quad (4)$$

$$SD^2_{I,\log} = Log_e\left(CV_I^2 + 1\right) \quad (5)$$

$$SD^* = \sqrt{SD^2_{A,\log} + SD^2_{I,\log}} \quad (6)$$

$$RCV\% = 100\% \times e^{\left(\left(\pm Z_a \times \sqrt{2} \times SD^*\right) - 1\right)} \quad (7)$$

where $SD_{A,log}$ is the analytical SD; the $SD_{I,log}$, is the within-subject SD; and the SD^* is the combination of the $SD_{A,log}$ and $SD_{I,log}$. For the significant unidirectional change (probability level at 95%), a z value of 1.65 was used.

The NHSP for each TrEl was calculated using the following equation:

$$NHSP = \left(z \times \frac{\sqrt{CV_I^2 + CV_A^2}}{D}\right)^2 \quad (8)$$

where D is the percentage deviated from the true homeostatic set point. Using the CV_A calculated from the duplicate analysis of individuals' samples, NHSP were estimated for 5, 10 and 20% deviation from the true homeostatic set points.

Results

The final population used to derive the BV of TrELs, consisted of 68 subjects (36 females and 32 males). The median ages of female and male subjects were 23 (range, 18–51) and 29.5 years (range, 19–50) respectively. Physical activity, eating habits, dietary supplements, smoking and alcohol intake of the study population are summarized in Supplementary Table 1.

The number of results identified as outliers by Dixon-Q, Bartlett and Cochran tests are given in Table 1. Data were excluded mainly due to analytical heterogeneity, indicating differences between the results of the duplicate measurements. For the total study population, the highest number of excluded data was for Zn, at 3.4%, leaving 1,132 results from 68 individuals to estimate the CV_I.

Positive significant slopes were found for Zn (male), Cu (all, male and female) and Se (all, male and female) and the trends were corrected for each TrEL prior to estimating the BV components.

Table 1: The number of subjects and results excluded prior to calculation of CV_I estimates of trace elements (TrEL).

Measurands		Numbers of excluded results/subjects				Data used to estimate CV_I		Total % of outliers
		Homogeneity (Bartlett and Cochran tests)			Dixon-q test			
		Replicate (analytical homogeneity)	Results (within homogeneity)	Subjects (within homogeneity)	Subjects (between)	Number of results	Number of subjects	
Copper	All	13	0	0	0	1,160	68	1.1%
	M	9	0	0	0	557	32	1.6%
	F	0	0	0	0	607	36	0.0%
Zinc	All	36	4	0	0	1,132	68	3.4%
	M	21	0	0	0	543	32	3.7%
	F	13	4	0	0	591	36	2.8%
Selenium	All	6	2	0	0	1,163	68	0.8%
	M	0	2	0	0	562	32	0.4%
	F	8	0	0	0	599	36	1.3%
Median (all subjects) (min–max)								**1.1% (0.8–3.4%)**

CV_I, within-subject biological variation; TrEL, trace elements.

The measurement results ordered in accordance with age and sex for Zn, Cu and Se are given in Figures 1–3. A weak correlation was observed between the median concentration and age of subjects (r=0.26; p=0.036) for Se, but not for Zn and Cu (Supplementary Figures 1–3).

The mean concentrations, BV estimates and NHSP (5, 10 and 20% deviation from the actual value) of Zn, Cu and Se for all subjects and females and males are given in Table 2. For all TrELs, significant differences between the mean concentrations of females and males were observed, whereas the BV estimates were not significantly different between genders (Table 2). For overall study population, the NHSP within 20% of the true homeostatic set point was one for all three TrEl.

The APS, RCV and II of TrELs are presented in Table 3. Marked individuality were observed for both Cu (II=0.53) and Se (II=0.63).

Discussion

An adequate supply of TrELs is critical for normal development and health and to be able to correctly determine the concentration of TrEls is important. In previous BV studies,

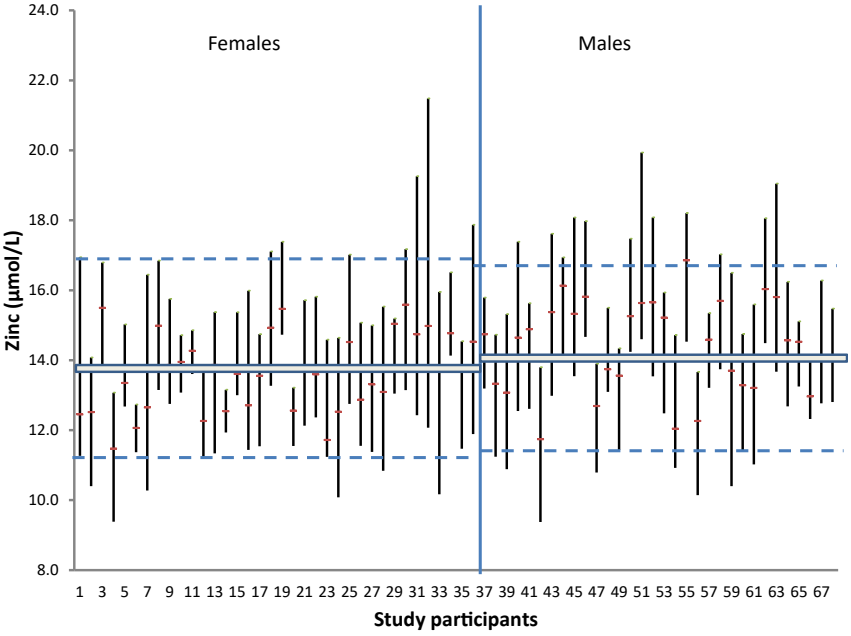

Figure 1: Median values with range (minimum–maximum) of zinc (Zn) concentrations for individuals based on weekly samplings for 10 weeks. The continuous line is the median value with 95% CIs. Dashed lines indicate 5th and 95th percentiles data included in the study.

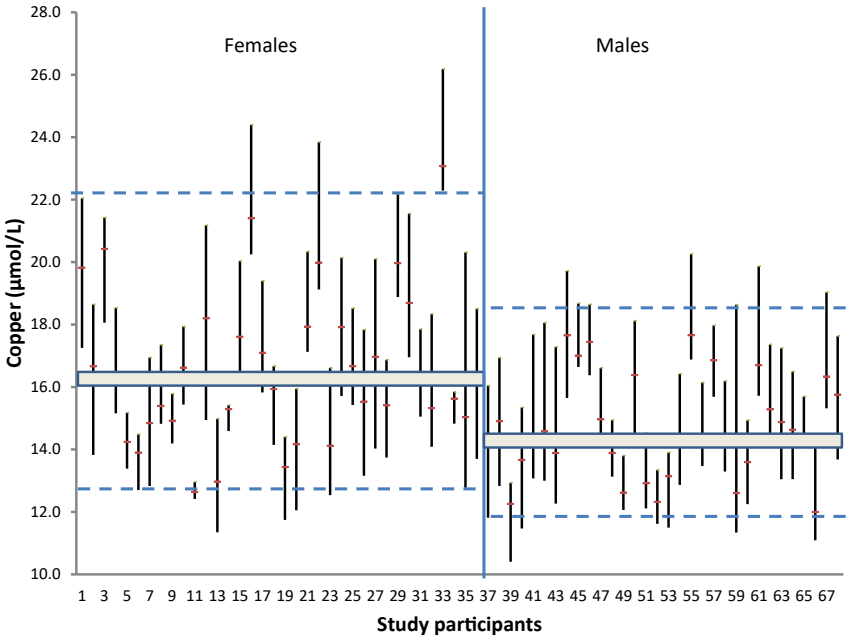

Figure 2: Median values with range (minimum–maximum) of copper (Cu) concentrations for individuals based on weekly samplings for 10 weeks.
The continuous line is the median value with 95% CIs. Dashed lines indicate 5th and 95th percentiles data included in the study.

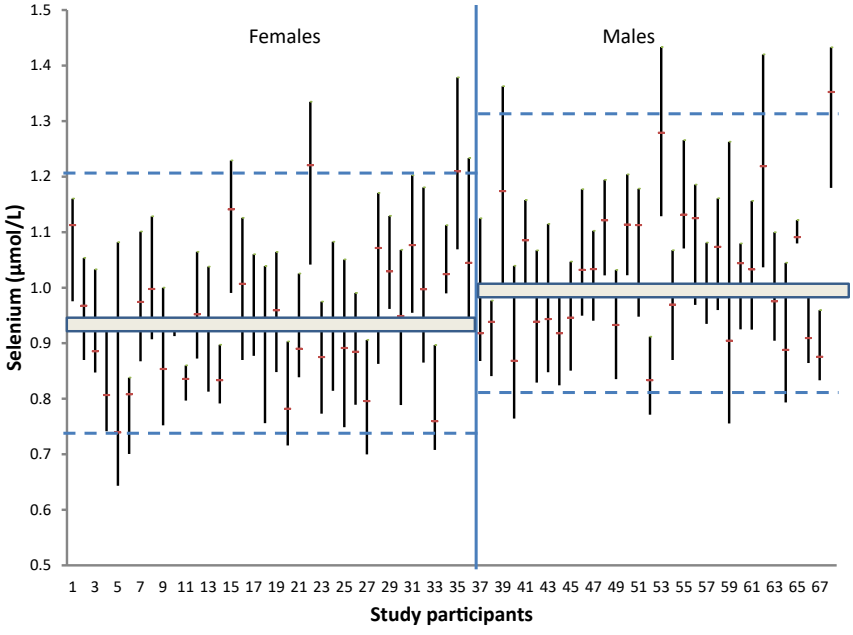

Figure 3: Median values with range (minimum–maximum) of selenium (Se) concentrations for individuals based on weekly samplings for 10 weeks.
The continuous line is the median value with 95% CIs. Dashed lines indicate 5th and 95th percentiles data included in the study.

TrEIs concentrations were measured by different types of atomic absorption spectrophotometry (AAS) [20–24] and colorimetric methods [25]. This study reports for the first time BV data based on TrELs analysis by ICP-MS. The new generation of ICP-MS provides fast and accurate measurements of multiple TrEL in a single run [26] and is superior to older methods. In addition to improved interpretation of test results, reliable BV data for TrELs enables the identification of the required APS for measurement systems. Data of this study show that the desirable APS for imprecision based on BV is met by ICP-MS (Table 2). However, it should be noted that the APS for imprecision and bias are interrelated and a significant bias will diminish the imprecision error budget and *vice versa*. In this study, for Zn, 3.1% data were excluded to fulfill statistical criteria for the analytical component, indicating that there were differences between the results of the duplicate analysis, likely associated with the low concentrations seen in healthy individuals. For AAS, different

Table 2: Within- and between-subject BV estimates for zinc (Zn), copper (Cu) and selenium (Se) with 95% CIs and the number of samples required to estimate the homeostatic set points (NHSP) at 5, 10 and 20%.

Measurands		Number of individuals	Total number of results	Mean number of samples/individuals	Mean number of replicates/samples	Mean concentration (95% CI)	CV_A% (95% CI)[b]	CV_I % (95% CI)	CV_G % (95% CI)[a]	NHSP, 5%	NHSP, 10%	NHSP, 20%
Zinc, µmol/L	All	68	1,132	8.60	1.88	13.9 (13.9–14.1)	2.4 (2.2–2.5)	8.8 (8.2–9.3)	8.3 (6.9–10.2)	13	4	1
	M	32	543	8.81	1.86	14.1 (13.9–14.2)*		8.4 (7.7–9.3)	8.8 (6.9–12.1)	12	3	1
	F	36	591	8.42	1.90	13.6 (13.5–13.8)		9.4 (8.6–10.2)	**7.2 (5.5–9.9)**	15	4	1
Copper, µmol/L	All	68	1,160	8.63	1.95	15.2 (15.1–15.4)	2.2 (2.1–2.4)	7.8 (7.3–8.3)	14.7 (12.5–17.8)	11	3	1
	M	32	557	8.84	1.94	14.2 (14.0–14.4)*		7.8 (7.1–8.5)	**11.9 (9.3–15.8)**	11	3	1
	F	36	607	8.44	1.99	16.2 (16.0–16.4)		8.0 (7.3–8.7)	14.5 (11.9–19.6)	11	3	1
Selenium, µmol/L	All	68	1,163	8.60	1.98	0.96 (0.96–0.97)	2.3 (2.2–2.4)	7.7 (7.2–8.2)	12.3 (10.3–14.8)	10	3	1
	M	32	562	8.78	2.00	1.00 (0.98–1.01)*		7.2 (6.6–7.9)	**11.6 (8.9–15.2)**	9	3	1
	F	36	599	8.44	1.94	0.94 (0.92–0.95)		8.2 (7.5–9.0)	12.0 (9.4–15.7)	12	3	1

Results in bold indicate that these estimates have been used for calculations of APS (see Table 2). [a]Normality assumption was not fulfilled for all individuals based on Kolmogorov Smirnov test ($p>0.05$). [b]Analytical variation (CV_A) estimates represent duplicate analysis of all study samples (CV-ANOVA). Zn, zinc; Cu, copper; Se, selenium; NHSP, the number of samples required to estimate the homeostatic set points. *indicates significantly different from female.

Table 3: Analytical performance specifications (APS) for imprecision (CV_{APS}) and for bias ($Bias_{APS}$), asymmetrical reference change values (RCV) for decrease and increase and index of individuality (II) of zinc (Zn), copper (Cu) and selenium (Se) derived using the data given in Table 2.

Measurands	CV_{APS}, %	$Bias_{APS}$ %	RCV, % decrease; increase	II
Zinc	4.4	3.02	−19.1; 23.6	1.06
Copper	3.9	4.2	−17.2; 20.7	0.53
Selenium	3.8	3.63	−17.0; 20.5	0.63

CV_{APS}, APS for analytical imprecision; Zn, zinc; Cu, copper; Se, selenium; RCV, reference change values.

CV_A estimates have been reported for Zn (ranging from 1.4 to 15.4%) [22, 24], Cu (ranging from 0.9 to 8.4%) [24, 27] and Se (ranging from 3.0 to 7.6%) [21, 27] highlighting that in some studies desirable APS based on BV were not met by AAS.

Similar to most other biochemical routine analytes [9], individuality, as expressed by II, is characteristic for the TrELs included in our study (Table 3). A high II, particularly if higher than 1.4, indicates that the conventional popRI is of some value when interpreting the result of an individual. The II of Cu, Zn and Se calculated from the data of this study were found to be lower than 1.4, which suggests that conventional population based reference intervals (popRIs) will be of little utility for these TrELs. The RCV provides a basis for evaluating the significance of changes between consecutive measurements based on estimates of analytical variation and CV_I. TrELs are consumed by food; thus, nutritional status, eating habits, dietary intake and supplements have great influence on serum TrELs level. Therefore, the popRIs may not be the 'reference' for every individual. Instead of popRI, a reference interval that is specific to individual, i.e., prRI [7] would be more useful as long as the individuals are at steady state.

PrRI of the measurands can be estimated from CV_Is and previous measurements results of an individual [7]. The homeostatic set point for a specific measurand may be estimated by taking the mean of an individual's previous measurements results obtained in a steady state situation. In this regard, the deviation from the true homeostatic set point, and consequently the NHSP, become the critical parameter for reliable prRIs. The higher the NHSP, the more accurately the homeostatic set point is estimated. The data of this study shows that, at least four samples for Zn and three samples for Cu and Se are needed to estimate the true homeostatic set point within 10% deviation.

In this study, we found higher levels of Zn and Se and lower level of Cu in males than females (Table 2). Similar results have been reported previously [28–30]. For example Komarova et al. [30] measured whole blood and plasma TrELs concentrations and found higher levels of whole blood Zn and plasma Se but lower level of plasma Cu in males than females (n=120).

In the following, the different TrELs and available BV data for these markers are discussed in detail.

Zinc

Zn is ubiquitous in all living organisms and is, after iron, the most abundant TrEL in the human body [31]. It is essential for the function of more than 300 metalloenzymes. Due to the multiple biochemical functions, the clinical presentation of the Zn deficiency varies greatly, ranging from growth retardation to impairment of immunity [31]. Zn deficiency is a global public health problem; it affects approximately one third of the world's population, particularly in the developing countries [32]. The interpretation and accurately measurement of Zn is crucial for the diagnosis and monitoring of patients. In the literature, only a few studies have reported the BV of Zn in healthy subjects. González-Revaldería et al. [24] recruited 15 healthy subjects (age 25–40 years) and collected weekly samples for four weeks. The authors measured Zn by AAS and reported the CV_I and CV_G as 9.3 and 9.4%, respectively. Yucel et al. [23] collected weekly samples from 20 healthy subjects (age 23–54 years) for 10 weeks and reported the CV_I and CV_G of Zn as 6.3% (4.8–9.1) and 23.3% (16.0–42.2), respectively. Lux et al. [21] collected weekly (12 weeks) and monthly (three months) serum samples from 12 healthy subjects (four females and eight males, aged 23–45 years) and reported CV_I and CV_G for Zn as 11 and 14%, respectively. The participants of these studies were not on a special diet, but Gallagher et al. [20] collected daily, weekly and monthly samples from "free-living" (n=5) and restricted diet (n=5) subjects and measured the BV of Zn. The authors reported a higher CV_I (9.0%) for free-living subjects, and a lower CV_I (5.5%) for the restricted diet subjects, both with monthly samplings. Additionally Giles et al. [22] measured the BV of Zn in elderly subjects using daily and weekly finger-prick samples from 36 subjects (age 60–88 years) and reported the CV_I and CV_G as 35 and 28.6%, respectively. These estimates are in a higher range than the other studies and could be related to the different study design and/or study population.

In our study, we found CV_I and CV_G estimates for Zn in all subjects of 8.8% (8.2–9.3) and 8.3% (6.9–10.2), respectively, with no difference between males and females. These are in line with most previous publications,

and in adult subjects, the reported CV_I for Zn, regardless of sampling interval is lower than 10%, for all the available studies. However, there is a lack of studies focusing on factors such as age, nutritional status, states of health and sample type, which might be decisive factors when determining the BV of Zn. In our study, only four subjects followed uncommon diet and thus, we could not assess the effect of nutritional status on the BV of Zn.

Copper

Cu is a TrEL that is associated with numerous enzymes and proteins in humans. These have various functions including energy metabolism, connective tissue formation, angiogenesis, iron metabolism etc. [31]. Similar to Zn, only a few papers have reported the BV of Cu, in small sample sized groups with maximum 20 subjects [23]. Although the CV_G varies, the CV_I estimates reported in all paper including the present study are <10%. For example Lux et al. [21] reported CV_I and CV_G for Cu as 8 and 19%, respectively, and Yucel et al. [23] reported similar BV data for CV_I and CV_G of Cu as 6.05% (4.60–8.83) and 19.64% (13.51–35.85), respectively. Bal et al. [27] reported higher CV_I and lower CV_G for Cu as 7.1% (6.4–8.1) and 4.3% (2.6–5.6), respectively.

Gallagher et al. [20] measured the BV of Cu in subjects on free-living and restricted diets. In contrast to Zn, the authors reported the highest CV_I (9.2%) for monthly sampling subjects on restricted diet, whereas the CV_I in free-living subjects was found to be 4.9%. They observed a similar pattern for the BV of ceruloplasmin, a major Cu carrying protein which has ferroxidase activity, and reported the CV_I for ceruloplasmin for restricted diet and free-living subjects as 12.9 and 4.5%, respectively [20].

Braga et al. [25] recruited 19 healthy subjects (10 men and nine women; age range, 23–48 years) and collected samples every two weeks for two months. The authors measured serum Cu level using colorimetric method and reported the CV_I and CV_G for Cu as 5.8 and 14.5%, respectively. As expected, they found a similar pattern for the BV of ceruloplasmin and reported the CV_I and CV_G as 6.2 and 14.4%, respectively [25].

In our study we obtained similar CV_I and CV_G of Cu as 7.8% (7.3–8.3) and 14.7% (12.5–17.8), respectively, and no difference between males and females. The data of the literature and this study emphasize the marked individuality for this marker (II=0.53), indicating the limitation in using population based reference limits for the diagnosis and monitoring of patients.

Selenium

Se is the constituent of various enzymes and proteins including glutathione peroxidase, iodothyronine deiodinase, tioredoxin reductase etc. [31]. It has crucial functions in antioxidative systems and the conversion of thyroid hormones i.e., thyroxin to active triiodothyronine. Se deficiency has been reported in various clinical situation such as Keshan disease, Kashin-back disease and hypothyroidism [31], highlighting the importance of understanding the variation affecting patients' results. In the literature, only two studies have reported the BV of Se. Lux et al. [21] collected weekly (12 weeks) and monthly (three months) serum samples from 12 healthy subjects and reported CV_I and CV_G estimates for Se as 12 and 14%, respectively. Bal et al. [27] recruited 15 individuals (six male and nine female), collected weekly samples for 10 weeks and reported the CV_I and CV_G for Se as 2.5% (2.3–2.9) and 6.9% (6.6–7.2), respectively. Due to the lack of CI in the study by Lux et al., it is not possible to perform a statistical comparison. However, compared to the study of Bal et al. [27] we found higher CV_I 7.7% (7.2–8.2), and CV_G 12.3% (10.3–14.8). Similar to Cu, a high individuality (II=0.63) is characteristic for Se, highlighting the limitations of using population based reference limits for diagnosis and monitoring of patients.

Limitations of the study

Serum TrEls levels are influenced by diets and nutritional statues. Results from the Turkish population therefore risk not being transferable to other populations. However, the fact that the results obtained in the present study essentially confirm the findings of earlier studies from other populations, support a notion that the data may be relevant for other populations. The study population consisted of young adult subjects and lacks data for BV of TrELs in children and elderly subjects.

Conclusions

In this study, samples were obtained under strict preanalytical protocol and serum TrELs concentrations were measured using state of the art methodology, ICP-MS, to deliver reliable BV data for TrELs. The study thus provides updated BV data for Zn, Cu and Se, based on a BIVAC compliant approach [11]. Our data shows that Cu and Se are characterized by marked individuality, and population-

based reference limits should therefore not be used in the monitoring of patients.

Acknowledgment: The authors thank BD (Bacton Dickinson) for uncoditional scientific supporting of trace element tubes and Acibadem Labmed Clinical Laboratory staff for the technical assistance: Julide Coskun, Nese Yilmaz and Serife Yildiz.
Research funding: Acıbadem Mehmet Ali Aydınlar University Scientific Research Projects Commission, Project number: 2019/03/01.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: The study protocol was approved by Institutional Ethical Review Board of Acibadem Mehmet Ali Aydınlar University in agreement with the World Medical Association Declaration of Helsinki (2019-3/39).

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2021-0886).