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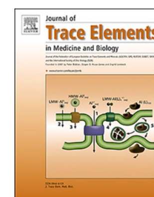

Human serum albumin-bound selenium (Se-HSA) in serum and its correlation with other selenium species

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A R T I C L E I N F O

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A B S T R A C T

Introduction: Selenium (Se) is a trace element with different toxicological and nutritional properties according to its chemical forms. Among the wide range of selenium species, human serum albumin-bound selenium (Se-HSA) has still uncertain composition in terms of organic or inorganic selenium species. This study aimed at investigating the relation between Se-HSA levels with total selenium and the specific organic and inorganic selenium species.

Methods: We determined levels of total selenium and selenium species in serum of participants enrolled in two populations of the Emilia-Romagna region, in Northern Italy. Anion exchange chromatography coupled with inductively coupled plasma dynamic reaction cell mass spectrometry was used as quantification method. Correlations between Se-HSA and the other selenium compounds were analyzed using linear regression and restricted cubic spline regression models, adjusted for potential confounders.

Results: The first cohort comprised 50 participants (men/women: 26/24) with median (interquartile range, IQR) age 50 (55−62) years, while the second was composed of 104 participants (M/W: 50/54), median (IQR) age 48 (44−53) years. Median (IQR) levels of total selenium were 118.5 (109−136) μg/L and 116.5 (106−128) μg/L, respectively, while Se-HSA was 25.5 μg/L (16.2–51.5) and 1.1 (0.03–3.1) μg/L, respectively. In both populations, Se-HSA was positively associated with inorganic selenium species. Conversely, Se-HSA was inversely associated with organic selenium, especially with selenoprotein P-bound-Se (Se-SELENOP) and less strongly with selenomethionine-bound-Se (Se-Met), while the relation was null or even positive with other organic species. Evaluation of non-linear trends showed a substantially positive association with inorganic selenium, particularly selenite, until a concentration of 30 μg/L, above which a plateau was reached. The association with Se-SELENOP was inverse and strong until 100 μg/L, while it was almost null at higher levels.

Conclusions: Our findings seem to indicate that Se-HSA incorporates more selenium when circulating levels of inorganic compounds are higher, thus supporting its mainly inorganic nature, particularly at high circulating levels of selenite.

1. Introduction

Selenium (Se) is a metalloid demonstrating an intriguing relation with human health depending on the dose and the chemical species. In particular, selenium speciation represents a key issue for both nutritional and toxicological implications, taking into account the inorganic or organic features of the various selenium compounds [1–5].

The main source of selenium is diet, particularly cereals, meat and fish products [6,7]. Approximately about 80 % of selenium is absorbed from food although high variations are present depending on the species considered [8,9]. Dietary selenium is available in both organic and inorganic forms. Among the organic forms, selenomethionine represents

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the main form of Se present in the diet, especially cereals and it is absorbed by the same mechanism as methionine [6]. Similarly, selenomethionine (SeM) and selenocysteine (SeC) are the major forms of selenium in animal food due to their incorporation on muscle protein. Finally, fish appears to be the main source of inorganic forms selenite (Se-IV) and selenate (Se-VI) though high variation has been found in fish and seafood, also contributing to the intake of organic species [10,11]. Among the inorganic forms of Se, Se-VI has a good bioavailability, although most of the absorbed Se-VI is rapidly excreted through urine unchanged [12]. Conversely, the absorption of Se-IV is more variable, generally around 50 %, and enters in the systemic selenium pool [12, 13].

After adsorption, most of selenium is then made available for the synthesis of new selenoproteins through a trans-sulfuration mechanism for synthesis of the amino acid selenocysteine to be subsequentely incorporated in selenoproteins [14]. Selenoprotein P is the most abundant selenoprotein expressed in many tissues, but it is mainly produced in the liver and then secreted into the plasma (about 30–60 %). Other selenoproteins include the glutathione peroxidase family and thioredoxin reductases involved in the cellular redox homeostasis [14,15]. Transport of selenium into the bloodstream is mainly through selenoprotein P (50 %), followed by extracellular glutathione peroxidase, selenomethionine in albumin and other minor compounds [16].

Several advances have been implemented in the search field of selenium speciation and their relation with human health [17]. Nonetheless, the features of some species are still unclear, in particular about the nature and physiological (and toxic) functions of human serum albumin-bound selenium (Se-HSA). Se-HSA is a species with still uncertain composition in terms of inorganic and organic selenium content [18] and with unclear physiological significance, being apparently unrelated to physiological function, and with an entirely uncertain toxicity pattern.

For these reasons, we aimed at investigating the epidemiologic and biological features of this species by exploring the correlation of Se-HSA levels with total selenium and other selenium species, focusing in particular on the different association with organic and inorganic compounds.

2. Methods

2.1. Study populations

We included in the present study two Italian populations from Northern Italy in which we collected serum samples and measured total selenium and selenium species [19,20]. Both study populations were originally recruited with the aim to perform selenium speciation analysis with advanced techniques and to assess factors influencing circulating levels of Se species in the human. With reference to the latter issue, there seems to be very little if any data about the relation between Se-HSA and the other circulating selenium species. Briefly, we recruited the first study population using a random sampling method stratified by age and sex, in order to obtain a representative sample of the general population aged 35–70 years of Modena municipality in the period 2011–2013 [19]. The study was approved by Modena Policlinico Ethics Committee (approval no. 71/11) and all subjects who agreed to participate signed a written consent form and were invited to the Health Unit Center of Modena municipality in the morning, to give a fasting venous blood sample. Similarly, we recruited in a second study a blood donor population from Reggio Emilia province referred to the Transfusion Medicine Center 'Casa del Dono' of the Reggio Emilia Local Health Unit [20]. We recruited non-smoking subjects aged 30–60 years residing in Reggio Emilia province in the period 2017–2019. The study was approved by the Reggio Emilia Ethical Committee (approval no. 2016/0022799) and all subjects who agreed to participate signed a written consent form and were invited to give a fasting blood venous samples at the same Transfusion Medicine Unit.

In both study participants, we collected a blood sample in plastic tube (BD Vacutainer®, Becton Dickinson, Milan, Italy), immediately centrifuged for 10 min at 1000 g and then serum aliquots of 1 ml (at least two per subjects) were stored at −15/20 °C and kept continuously frozen until shipping to the Helmholtz Center Munich- National Research Center for Environmental Health (Research Unit Analytical BioGeoChemistry, Neuherberg, Germany) for speciation analysis. In addition, all participants completed a tailored self-administered questionnaire collecting demographic, educational and lifestyle information.

2.2. Sample analysis

2.2.1. Chemical, standard and sample preparation for Se-speciation

Supra pure grade chemicals and reagents were used throughout. Selenite, selenate, Selenomethionine (Se-Met), Selenocystine (Se-Cys), thioredoxin reductase-bound selenium (Se-TXNRD) (EC 232–749–6), glutathione peroxidase-bound selenium (Se-GPX), HSA and Tris buffer were ordered from Sigma–Aldrich, Deisenhofen, Germany, while selenoprotein P (Se-SELENOP) standard was prepared from serum using affinity chromatography (AFC) with methods reported in detail elsewhere [21]. Certified selenium stock standards (1000 mg/L) were ordered from CPI International, Santa Rosa, CA, USA. Ammonium acetate (NH_4Ac) and acetic acid (HAc) were from Merck, Darmstadt, Germany. $Argon_{liq}$ and methane (99.999 % purity) were purchased from Air Liquide, Kleve, Germany. Preparation of selenite and selenate species stock solutions were performed at concentration of 1000 mg Se/L by dissolution in Milli-Q water (18.2 MΩ cm, Milli-Q system, Millipore, Bedford, MA, USA). Se-HSA was prepared at a concentration of 1000 mg/L, by mixing 10 mg Se/l selenite with this stock solution and incubating for at least 14 days. Using the stock standard solutions by appropriate dilution with Milli-Q H_2O, working standards of selenium species were prepared daily. A chromatogram of a Se-HSA standard (including oxidation of excess Se-IV to Se-VI in this standard) is presented in Supplemental Fig. S1.

Whole blood samples were collected in vacutainer tubes and centrifuged at 4000 rpm for 10 min at room temperature to isolate serum. 1 ml of serum was then aliquoted and stored at −20 °C. Samples were transported deep frozen in dry ice to the Munich laboratory by air courier and kept continuously frozen until analysis. Before analysis, each sample was slowly thawed in refrigerator at 4 °C, and vortexed.

2.2.2. Selenium speciation analysis

Full details of the speciation analysis were reported in the previous papers [19,20]. To summarize, based on a previous established methodology [22,23] strong anion exchange was performed to separate selenium species. The ion exchange chromatography (IEC) system was directly hyphenated to inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) used as the element specific chromatographic detector. With this coupled system the selenium species Se-HSA, Se-IV, Se-VI, Se-Cys, Se-Met, Se-SELENOP, Se-GPX, and Se-TXNRD were determined. Briefly, a Knauer 1100 Smartline inert Series gradient HPLC system equipped with an anion exchange column (AG 11 precolumn, 50 mm × 4 mm, + AS 11 analytical column, 250 mm × 4 mm I.D., from Thermo-Dionex, Idstein, Germany) was used for species separation. Sample volume used was 100 μL. The mobile phases were as follows: eluent A: 10 mM Tris–HAc buffer, pH 8.0; and eluent B: A + 500 mM NH_4Ac, pH 8.0. Gradient elution expressed as % were: eluent A: 0–3 min 100 %; 3–10 min 100–60 %; 10–23 min 60–45 %; 23–26 min 45–43 %; 26–28 min 43–0 %; 28–52 min 0 %; 52–60 min 100 %. The flow rate was 0.70 ml/min. For internal standardization we mixed the column effluent with 1 μg/l Rh (final concentration, Rh flow rate: 0.1 ml/min) and directed to ICP-MS.

The experimental settings chosen for the ICP-DRC-MS (Perkin Elmer NexIon 300 D) were slightly different for the two studies. For the first one, they were the following: radio frequency power: 1250 W, plasma gas flow: 15 L Ar/min auxiliary gas flow: 1.05 L Ar/min, nebulizer gas

flow: 0.94 L Ar/min, daily optimized, dwell time 300 ms, ions monitored: ^{78}Se, ^{80}Se, ^{103}Rh, DRC reaction gas: CH_4 reaction at 0.58 ml/min, DRC rejection parameter q: 0.6. For the second study: radio frequency power: 1300 W; plasma gas flow: 16 L Ar/min; auxiliary gas flow: 1.05 L Ar/min; nebulizer gas flow: 0.98 L Ar/min, daily optimized; dwell time: 300 ms; ions monitored: ^{78}Se, ^{80}Se; DRC reaction gas: CH_4 Reaction at 0.58 ml/min; DRC rejection parameter q: 0.6. Measurement of total selenium differed among the two studies. In the first it was analyzed by graphite furnace atomic absorption spectrometry (GFAAS) based on the method of the MAK collection-biomonitoring methods [24]. In the second study, it was quantified using ICP-sf-MS (ELEMENT II, Thermo Scientific). The experimental settings were as follows: radio frequency power: 1260 W; plasma gas flow: 16 L Ar/min; auxiliary gas flow: 0.85 L Ar/min; nebulizer gas flow: 1.085 L Ar/min, daily optimized; ions monitored: ^{77}Se and ^{78}Se, both in high-resolution mode.

Peak quantification was performed by comparing peak areas with peak area calibration curves. Standard addition method for standard-retention-time matched identification of selenium species and as QC means in quantification was used. Species identity was then confirmed using a 2-D approach of IEC-capillary electrophoresis (CE)-ICP-DRC-MS. If species matched the standard compounds with both chromatography/electrophoretic techniques, the identification was regarded as acceptable. Rh and selenium data files were exported from the NexIon "Syngistics" software and processed with Peakfit™ software for peak area integration (Systat Software, Inc - Inpixon HQ, Palo Alto, CA). The limit of detection for all selenium species was 0.02 μg/L.

2.3. Data analysis

For this study, we considered 50 participants from the first study (hereinafter Study-A) [19] and 104 from the second one (hereinafter Study-B) [20], respectively.

We used number and percentages (%) for categorical variables along with median and interquartile range (IQR) for continuous variables. In order to minimize influence of outliers in data analysis, we winsorized extremes values at the 1st or 99th percentiles.

We performed Pearson's correlations between Se-HSA and main possible confounders (namely age, sex, body mass index, and storage time) as well as total selenium and selenium compounds. We also analyzed the association between Se-HSA and overall selenium and its compounds using linear and nonlinear regression models adjusted for potential confounders. For nonlinear associations, we used restricted cubic splines models with three knots at fixed percentiles (10th, 50th and 90th). For all estimates, we assessed statistical imprecision through calculation of 95 % confidence interval (CI). We used the routines 'pwcorr', 'mkspline', 'regress', 'xbcrsplinei', and 'winsor' of Stata 17.0 (Stata Corp., College Station, TX, USA, 2021) for data analysis.

3. Results

Table 1 presents characteristics of study participants. Overall, sex distribution was similar in both studies, with median age slightly lower in Study-B compared to Study-A. Body mass index (BMI) was substantially similar on median values, but Study-A showed higher percentages of overweight and obese participants. Smoking status showed similar percentage of former smokers in both studies. Study-A showed 18 % of current smokers, while they were not present in Study-B because excluded as per inclusion criteria. Storage time was considerably higher in the first Study-A, as in Study-B samples were stored for a median of 6 months before speciation analysis compared to 36 months of the Study-A.

Table 2 showed distribution of total selenium and selenium compounds in both populations, demonstrating similar total levels, but marked differences in organic selenium levels: inorganic selenium is double in Study-A compared to Study-B, almost entirely driven by Se-IV (15.8 vs 4.4 μg7L) while Se-VI levels are similar. Conversely, organic

Table 1
Characteristics of study populations.

	Study-A N	(%)	Study-B N	(%)
Total population	50	(100)	104	(100)
Sex				
Males	26	(52.0)	50	(48.1)
Females	24	(48.0)	54	(51.0)
Age				
Median (IQR)	50	(44–62)	48	(44–53)
< 50 years	23	(46.0)	60	(57.7)
≥ 50 years	27	(54.0)	44	(42.3)
BMI				
Median (IQR)	25.3	(23.1–28.4)	24.6	(22.4–26.9)
< 25	22	(44.0)	56	(53.8)
≥ 25-< 30	21	(42.0)	40	(38.5)
≥ 30	7	(14.0)	8	(7.7)
Education				
Primary school	3	(6.0)	2	(1.9)
Middle school	10	(20.0)	15	(14.4)
High school	23	(46.0)	54	(51.9)
College or more	14	(28.0)	33	(31.7)
Smoking status				
Never	26	(52.0)	76	(73.1)
Former	15	(30.0)	28	(26.9)
Current	9	(18.0)	-	
Storage time (months)	36.1	(22.6–45.1)	6.4	(4.3–9.1)
Urinary cotinine (μg/L)	-		0.4	(0.1–1.1)

selenium species showed higher levels in Study-B, due to higher contribution of Se-SELENOP. Conversely, Se-GPX and Se-TXNRD were higher in Study-A, as did Se-HSA (25.5 μg/L vs. 1.1 μg/L in Study-A and Study-B, respectively).

Further assessment of Se-HSA levels by subgroups showed higher levels in subjects older than 50 years, while male and normal-weighted individuals showed higher levels in Study-A, while the opposite was true for Study-B (Table 3). Also the higher Se-HSA levels characterizing former smokers in Study-A were not replicated in Study-B.

Supplemental Table S1 shows Pearson's correlation analysis between Se-HSA and main confounders showing strong correlation with age and storage time in both studies, while sex and smoking habits demonstrated almost null relation. BMI showed inverse correlation in Study-A, but not in Study-B. As a consequence, we decided to include sex, age, BMI and storage time as adjustment variables in the multivariable model.

Pearson's correlation analysis between Se-HSA and selenium species is shown in Supplemental Table S2. For both studies, we found a substantially positive correlation of Se-HSA with total selenium and inorganic species, though stronger in Study-A. Similarly, the inverse relation with organic species is found in both studies, again stronger for Study-A. Interestingly, we found a positive relation with Se-Cys in Study-A, while the relation was null in Study-B, while it was the opposite for Se-GPX and Se-TXNRD showing positive relation in Study-B, while negative and null, respectively, in Study-A. The 'Unknown species', which had be assessed only in Study-A, showed a positive association with Se-HSA. The aforementioned associations were substantially confirmed in the linear regression multivariable analysis (Supplemental Table S3), generally showing higher estimates for Study-A compared to Study-B.

Spline regression analysis between total selenium and Se-HSA showed a substantially positive association, more evident for Study-A characterized by higher Se-HSA levels (Fig. 1). Association with organic and inorganic species are showed in Figs. 2 and 3. The association with organic species was positive in the first study, while the opposite was true in Study-B. Conversely, in both studies the association with Se-SELENOP was negative until 100 μg/L, while it was almost null at higher levels. Se-Cys showed an almost null association, while Se-GPX demonstrated a U-shape relation in Study-A, but a substantially positive in Study-B. Similarly, Se-TXNRD showed a null relation in the first study, but a slightly positive one in Study-B. For inorganic selenium species, the relation with Se-HSA was positive till approximately 30 μg/L above

Table 2
Distribution of total selenium (Se) and species in serum of study subjects. Data are in μg/L. IQR: interquartile range; Se-IV: selenite; Se-IV: selenate; Se-SELENOP: selenoprotein P; Se-Met: selenomethionine; Se-Cys: selenocystine; Se-GPX: glutathione peroxidase-bound selenium; Se-TXNRD: thioredoxin reductase-bound selenium (Se-TXNRD); Se-HSA: human serum albumin-bound selenium.

	N = 50 Median	Study-A (IQR)	% total Se	N = 104 Median	Study-B (IQR)	% total Se
Total Se	118.5	(109.0–136.0)	100	116.5	(106.0 – 128.0)	100
Inorganic Se	21.1	(8.9–34.8)	17.8	9.7	(3.7–22.9)	8.3
Se-IV	15.8	(6.6–29.2)	13.3	4.4	(1.6–13.8)	3.8
Se-VI	3.1	(1.5–7.3)	2.6	3.3	(1.0–8.2)	2.8
Organic Se	66.5	(36.2–89.3)	56.1	102.2	(84.5–113.9)	87.7
Se-SELENOP	26.9	(3.4–48.8)	22.7	84.8	(64.2–104)	72.8
Se-Met	0.01	(0.01–2.0)	0.01	2.7	(1.4–5.2)	2.3
Se-Cys	2.6	(0.8–4.7)	2.2	1.9	(0.5–3.6)	1.6
Se-GPX	17.1	(11.1–24.0)	14.4	5.5	(2.1–8.9)	4.7
Se-TXNRD	6.0	(3.7–10.1)	5.1	0.4	(0.03–1.5)	0.3
Se-HSA	**25.5**	**(16.2–51.5)**	21.5	**1.1**	**(0.03–3.1)**	0.9
Unknown	0.01	(0.01–3.2)	0.01	-	-	

Table 3
Human serum albumin-bound selenium (Se-HSA) for all subjects and in specific subgroups. BMI; body mass index; IQR: interquartile range.

	n	Median	Study-A IQR	n	Median	Study-B IQR
Total subjects	50	25.5	(16.2–51.5)	104	1.1	(0.03–3.1)
Sex						
Males	26	28.6	(16.2–48.1)	50	1.1	(0.03–4.0)
Females	24	23.2	(16.7–53.0)	54	1.2	(0.03–2.9)
Age						
< 50 years	23	16.2	(13.3–23.1)	60	1.0	(0.03–2.5)
≥ 50 years	27	39.8	(25.5–57.9)	44	1.6	(0.3–4.1)
BMI						
< 25 BMI	22	24.9	(16.0–54.5)	56	1.0	(0.03–3.4)
≥ 25 BMI	28	25.5	(18.7–41.4)	48	1.5	(0.03–3.1)
Smoking habits						
Non-smokers	26	24.0	(16.2–43.0)	76	1.2	(0.03–3.5)
Ex-smokers	15	30.7	(17.9–54.5)	28	0.9	(0.03–2.5)
Smokers	9	25.5	(15.3–39.8)	-	-	-

which a plateau is reached. Similar pattern can be noted for both Se-IV and Se-VI although the relation was null for Se-VI in Study-A, and almost entirely positive for Se-IV in the Study-B.

4. Discussion

The assessment of selenium speciation is a topic of utmost importance for an adequate exposure assessment considering the different relation between individual selenium species and human health [25–28]. In particular, organic compounds are generally considered less toxic compared to inorganic ones due to their biological role in the redox balance and selenium transportation. For these reasons, it is important to evaluate levels and biological features of selenium species with still uncertain role in humans. In addition and in particular, very little is known about a peculiar selenium compound that is serum human albumin-bound selenium, both about it composition in terms of selenium species and about its physiological and pathological forms. Previous studies indicated that human serum albumin may bound from 10 up to 60 % of the total circulating selenium [21,29–32]. In our study, Se-HSA represented approximately 20 % of total selenium in the first study (Study-A), within the range of previous reports, while in our second study Se-HSA represented approximately 1 % of total selenium, an unexpectedly low content. These contrasting findings may be related to the different storage time of samples before the analysis, as in both studies we found a positive correlation between storage time and Se-HSA levels, stronger for Study-A than for Study-B and possibly indicating a progressive degradation with shift of selenium from inorganic species to HSA. As a matter of fact, previous studies already reported that human serum albumin is involved in selenium handling [16]. On one hand the main mechanism for Se-HSA synthesis is thought to be the non-specific incorporation of selenium into selenomethionine or selenocysteine residues, thus randomly replacing methionine [33,34], on the other thiol exchange-mediated transfer of selenite through formation of selenotrisulfide in different proteins including hemoglobin [35] and human serum albumin [18] has been suggested to occur. In addition, two experimental studies conducted in rats showed that selenite effluxed from red blood cells was reduced in the form of selenide and

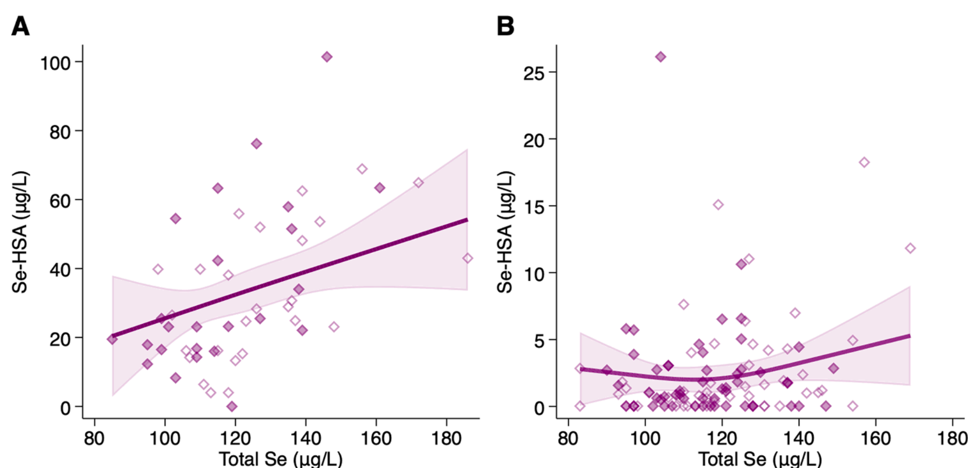

Fig. 1. Spline analysis between serum total selenium levels and human serum albumin-bound selenium (Se-HSA) in Study-A and Study-B.

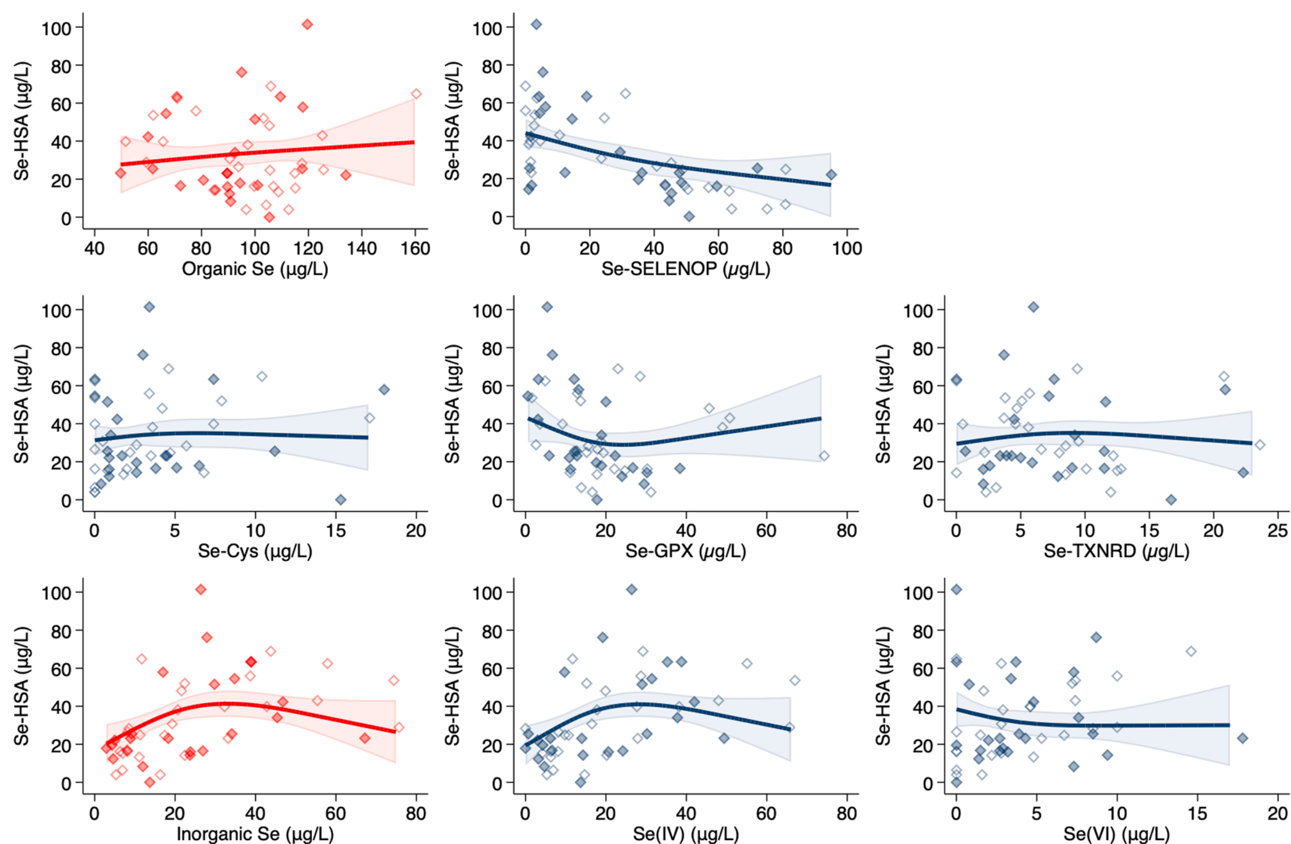

Fig. 2. Spline analysis between serum selenium species and human serum albumin-bound selenium (Se-HSA) in Study-A (N = 50).

then bound selectively to albumin. The binding site of albumin to selenium was hypothesized to be a disulfide group [36,37]. Unfortunately, the analytical method we used could not discriminate between selenium incorporated as selenomethionine or selenocysteine, or other species including the inorganic ones. Storage time may have also affected levels of other selenoproteins: in particular we noticed an inverse relation between organic selenium species, mainly due to Se-SELENOP in Study-A (adjusted Beta coefficient: −1.46, 95 % CI −2.44 to −0.48) [19]. Conversely, a positive association could be noted between Se-SELENOP and storage time in Study-B (adjusted Beta coefficient: 1.54, 95 % CI −0.14 to 3.21 within a multivariable model including sex, age, BMI and smoking as confounding factors). This issue may partially explain the very different Se-HSA levels between the two studies. In addition, we cannot rule out that other analytical factors and analytical instability of selenium species may have affected the serum selenium-containing protein levels [38,39], suggesting caution in the interpretation of the results, particularly from Study-B reporting much lower level of Se-HSA compared to the previous literature.

Another issue that should be taken into account is the influence of dietary habits and overall selenium status [6]. Despite the investigated populations showed substantial comparable total selenium levels, we cannot rule out that some differences in dietary habits could have affected the Se-HSA/total selenium ratio. We previously assessed the relation between Se-HSA and dietary habits in Study-A population and we found a positive association with intake of cereals and fresh fruits in line with total selenium and particularly Se-Cys. Conversely, a negative association was found between Se-HSA and intake of milk and dairy products, similarly to Se-IV. In addition, Se-HSA showed a negative association with intake of eggs, an opposite trend compared to that characterizing both organic and inorganic species [40]. Unfortunately, we did not assess the relation of selenium species with dietary habits in Study-B, but overall selenium intake was higher in Study-A (median intake 92.0 µg/day, IQR: 68.8–116.3 µg/day) [40] compared with Study-B (median value 76.1 µg/day, IQR: 60.6–97.8 µg/day) [41]. This could be due to the lower intake of meat products and the higher intake of cereals and vegetables in this latter group [40,42], though a thoughtful assessment would be needed to clarify the actual relation with dietary habits.

The positive association between Se-HSA and inorganic selenium species is also supported by previous studies reporting comparable high levels of both Se-HSA and inorganic selenium in cerebrospinal fluid (CSF) [1,22]. Despite the different biological matrix may hamper the comparability of such results, Se-HSA in serum and CSF demonstrated to be linearly correlated as showed in studies investing paired samples [21, 43].

To the best of our knowledge, no studies have so far assessed the correlation of Se-HSA with the other selenium serum species, thus hampering the evaluation of the biological role of this compound and its relation with human health. The topic is of considerable interest in medicine and biology, due to the different and sometimes opposite effects of the different selenium species on target tissues. This is particularly true for oxidative stress, since different selenium compounds may alter redox balance in opposite ways, e.g. beneficial effects in the case of some organic selenium species, and negative effects for inorganic selenium forms [17,44–48].

Some limitations of the present study must be acknowledged. Despite we assessed such relation using data from two investigations, the overall sample size remains limited, thus yielding statistical unstable associations and the possible lack of representativeness of the study samples with reference to the general population, particularly for Study-B population that was recruited from blood donors. However, this should not be entirely true for Study-A population who was randomly selected to obtain a representative sample of the Modena municipality general population [19]. Another limitation of the present study relates to the its observational design, which did not allow us to rule out the occurrence of residual confounding. In particular, we were unable to take into

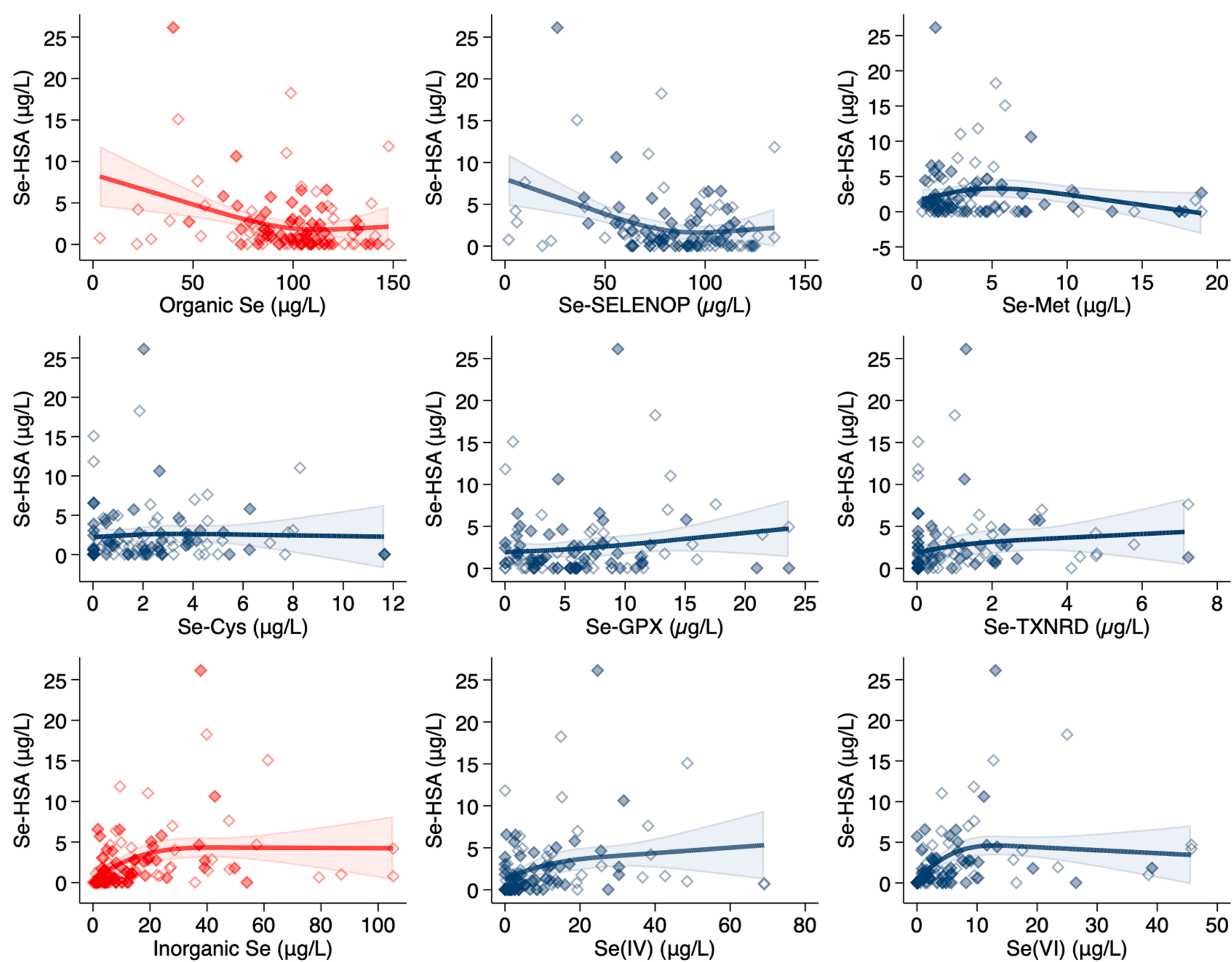

Fig. 3. Spline analysis between serum selenium species and human serum albumin-bound selenium (Se-HSA) in Study-B (N = 104).

account other sources of selenium exposure which may have affected the circulating levels of individual selenium species such as ambient air and drinking water, previously suggested to contribute to inorganic selenium compounds, e.g. as particulate selenium for atmospheric air [49] and selenite in drinking water [27,50]. In addition, the circulating levels of selenium species may not necessarily reflect their dietary intake due to their different absorption, metabolism, retention in body tissues and more generally bioavailability [6], thus affecting the correlation between selenium compounds. Finally, we cannot rule out that the different kinetics of proteins and the small-size selenium species may have hampered the adequacy of determination of circulating levels of selenium species, and as a consequence of the investigated relation with Se-HSA.

5. Conclusions

Our findings reported a positive association between Se-HSA and inorganic selenium species and suggest higher Se-HSA concentrations when levels of inorganic selenium are higher, particularly of selenite, thus supporting its mainly inorganic nature.

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CRediT authorship contribution statement

TF and MV conceived the study. TU, PG, CM, AF, BM and TF extracted and analyzed data. All authors interpreted the data. TU and TF prepared the first draft of the manuscript with contribution of BM and MV. All authors read and approved the final manuscript.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtemb.2023.127266.

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