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Selenium exposure and urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine: Major effects of chemical species and sex

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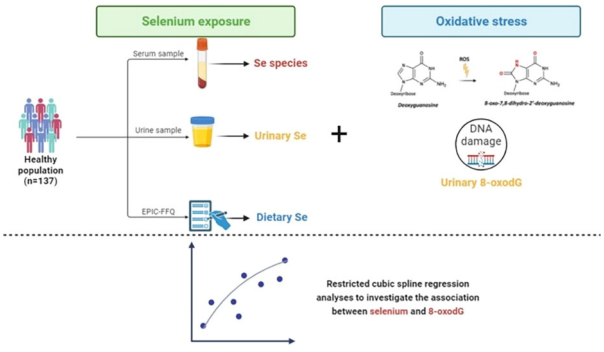
HIGHLIGHTS

- Overall selenium was not associated with 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG).
- Selected selenium species were associated with the biomarker of oxidative stress.
- Effect modification by sex was encountered.
- Selenomethionine-bound selenium was strongly associated with 8-oxodG.

GRAPHICAL ABSTRACT



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ABSTRACT

Selenium is an element present in trace amounts and different chemical forms. It may exert both beneficial and adverse effects on cellular redox status and on the generation of reactive oxygen species. 8-oxo-7,8-dihydro-2′deoxyguanosine (8-oxodG) is an oxidized derivative of deoxyguanosine, and a sensitive biomarker of oxidative stress and genotoxicity. The present study assessed the extent to which selenium status was associated with urinary 8-oxodG concentrations in a Northern Italian population.

We recruited healthy, non-smoking blood donors living in the Reggio Emilia province during 2017–2019. We measured urinary 8-oxodG concentrations and used restricted cubic spline regression analyses to investigate the association between selenium status (estimated using food frequency questionnaires, urinary concentrations, and serum concentrations of selenium and selenium species) and 8-oxodG/g creatinine.

Among 137 participants aged 30–60 years, median urinary selenium and 8-oxodG concentrations were 22.02 μg/L and 3.21 μg/g creatinine, respectively. Serum samples and selenium speciation analyses were available for 104 participants. Median total serum selenium levels and dietary intake were 116.5 μg/L and 78.7 μg/day, respectively. In spline

Abbreviations: β, Beta coefficients; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; BMI, body mass index; CI, confidence interval; EPIC, European Prospective into Cancer and Nutrition; FFQ, food-frequency questionnaire; ICP-MS, inductively coupled plasma mass spectrometry; IQR, interquartile range; LoQ, limit of quantification; QC, quality control; ROS, reactive oxygen species; Se (IV), selenite; Se (VI), selenate; Se-Cys, selenocysteine-bound selenium; Se-GPX, glutathione peroxidase-bound selenium; Se-HSA, human serum albumin-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se-TXNRD, thioredoxin reductase-bound selenium; SELECT, Selenium and Vitamin E Cancer Prevention Trial.

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regression analysis, there was little association between dietary, serum, or urinary selenium with 8-oxodG concentrations. In sex-specific analyses, urinary selenium showed a positive association with the endpoint among males. For single selenium species, we observed positive associations with urinary 8-oxodG for serum organic selenium species, and negative associations for inorganic selenium forms. In the most adjusted analysis, urinary 8-oxodG concentrations showed a strong positive association with selenomethione-bound selenium (Se-Met) and a negative association with inorganic tetravalent selenium, selenite. In sex-specific analyses, these associations were considerably stronger in males than in females.

Overall, study findings indicate that selenium species exhibited very different patterns of associations with the biomarker of oxidative stress, and that these associations also depended on sex. Background exposure to Se-Met appears to be strongly and positively associated with oxidative stress.

1. Introduction

Selenium is a micronutrient and element present in trace amounts in living organisms. Its properties range from essentiality to toxicity based on the dose and species considered (Vinceti et al., 2016). For these reasons, there is ongoing debate concerning its role in human health (Vinceti et al., 2017b; Vinceti et al., 2018). Its antioxidant and pro-oxidant properties have not been fully elucidated (Lee and Jeong, 2012; Vinceti et al., 2022b). Selenium may adversely affect cellular redox status via direct oxidation of thiol groups and indirect generation of reactive oxygen species (ROS) (Duffield-Lillico et al., 2003; Misra et al., 2015). In addition, selenoproteins may have adverse metabolic effects on glucose and lipid metabolism (Saito, 2020b; Saito, 2021; Tinkov et al., 2020; Vinceti et al., 2022b). Conversely, selenoproteins are involved in redox homeostasis and protection from apoptotic cell death induced by oxidative stressors (Fairweather-Tait et al., 2011; Holmgren, 2000; Zoidis et al., 2018). Overall, there is evidence that both insufficient and excess selenium exposure can adversely affect human health, with a very narrow safe range of intake (Vinceti et al., 2018). In addition to the dual influence on redox status, selenium has been found to both promote and counteract genotoxicity, depending on the specific selenium compound, the amount of exposure, and the biological system (Brozmanova et al., 2010; Cemeli et al., 2006; Gobi et al., 2018; Lazard et al., 2017; Santos et al., 2009; Schwerdtle et al., 2010; Valdiglesias et al., 2010).

8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is an oxidized derivative of deoxyguanosine, extensively used as a sensitive biomarker of oxidative stress in humans (Gorini et al., 2021). Moreover, it is frequently used to assess oxidative stress induced by heavy metals exposure and it is considered a sensitive marker of DNA damage that may lead to mutagenicity and cancer promotion (Valavanidis et al., 2009; Vinceti et al., 2017b). In humans, some factors may influence susceptibility to the biological effects of selenium and its compounds, including their genotoxic and antigenotoxic properties (Valdiglesias et al., 2010), such as sex, age, and other environmental chemicals (Tower et al., 2020). The extent to which selenium status influences oxidative stress in humans has been subject of a limited number of studies.

We performed a cross-sectional study to examine the associations between selenium and selenium compound status and oxidative stress, as measured using 8-oxodG biomarker concentrations, in healthy Italian blood donors.

2. Methods

2.1. Study population

We conducted a cross-sectional study in a group of blood donors from Reggio Emilia province, Northern Italy, during 2017–2019. The study population and data collection were previously described in detail (Urbano et al., 2021a; Urbano et al., 2021b). Briefly, 148 healthy blood donors from the Transfusion Medicine Center 'Casa del Dono' of the Reggio Emilia Local Health Unit gave written informed consent to participate, approved by the Reggio Emilia Ethical Committee (no. 2016/0022799). Inclusion criteria for study participants were age 30–60 years, being non-smokers, unaffected by major disease or clinical condition, and residing in the Reggio Emilia province. Participants were asked to give urinary and fasting blood samples for the study. Of the 148 originally-enrolled participants, four withdrew before the completion of the study, while seven were excluded because of high cotinine levels (>30 μg/L) consistent with active smoking. The final study population comprised 137 subjects. At enrolment, all completed a previously validated food-frequency questionnaire (FFQ) (European Prospective into Cancer and Nutrition, EPIC), specifically developed for a Northern Italian population (Filippini et al., 2018b; Pala et al., 2003).

2.2. Laboratory determinations

2.2.1. Blood selenium and selenium speciation measurements

For selenium speciation, we equipped a NexSar gradient HPLC system (Perkin Elmer) with an anion exchange column AG 11 (precolumn 50 × 4 mm) + AS 11 (analytical column 250 × 2 mm I.D.) from Thermo (Dionex Idstein, Germany). The sample volume was 50 μL, column temperature set at 28 °C. The mobile phases were: eluent A: 10 mM Tris-HAc, pH 8.0; and eluent B: 10 mM NH4CO3, pH 9.0. Gradient elution expressed as %-eluent A: 0–3 min 100 %; 3–10 min 100–60 %; 10–21 min 60–0 %; 21–23 min 0 %; 23–25 min 0–100 %. The flow rate was 0.85 mL min^{-1}. The experimental settings at dynamic reaction cell for inductively coupled plasma mass spectrometry (ICP-DRC-MS) (Perkin Elmer NexIon) after optimization were: radio frequency power: 1250 W, plasma gas flow: 16 L Ar/min auxiliary gas flow: 1.05 L Ar/min, nebulizer gas flow: 0.94 L Ar/min, daily optimized, dwell time 100 ms, ions monitored: 78Se, 80Se, 82Se, DRC reaction gas: CH4 reaction at 0.58 mL/min, DRC rejection parameter q: 0.6.

For total selenium determination, samples were diluted 1/5 with Milli-Q water + Rh as internal standard (1 μg/L final Rh concentration) and determined with an ELEMENT II ICP-sf-MS instrument (Thermo Fisher Scientific, Bremen, Germany). The experimental settings for ICP-sf-MS were: 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, dwell time 300 ms, ions monitored: 77Se, 78Se, in high resolution mode.

2.2.2. Urinary selenium measurements

Before analysis, urine samples were thawed at room temperature for 2 h. Each sample was mixed and heated at 37 °C for 30 min to dissolve the sediment. An aliquot of 600 μL was transferred into a 10 mL polyethylene tube and added to 2.4 mL of an aqueous solution of nitric acid 0.05 % *v*/v prepared by diluting ultrapure nitric acid (69 % TraceSelect, Fluka, France), containing 7.5 μg/L of Scandium-45 (45Sc), Yttrium-89 (89Y) and Indium-111 (111In) as internal standards (Inorganic Ventures, Inc., Lakewood, NJ, USA). All solutions were prepared using Milli-Q® ultrapure water (conductivity 0.056 μS/cm) (Merck, Darmstadt, Germany). The urine samples were analyzed by ICP-MS X Series II (Thermo Electron Corporation, Rodano, Italy). The instrument was operated in collision cell mode (CCT-Ked), with 3.7 mL/min of helium used to reduce interference. For each sample, three replicates were run. The calibration curve was in the range of 0.2–70 μg/L. The calibration solutions were obtained by diluting a selenous acid standard solution containing selenium at 1 mg/mL (BDH,

VWR International, Milano) with an aqueous solution of nitric acid 0.05 % v/v in the presence of internal standards. The calibration curve was linear with a correlation coefficient ≥0.999. The limits of quantification (LOQs), calculated as ten times the standard deviation of the blank, amounted to 1.2 μg/L. Internal quality assurance was performed using two quality controls (QCs) for metals in urine: Lyphocheck Urine Metals Control, Level-1 (Bio-Rad Laboratories, Anaheim, CA, USA), and Seronorm® Level-1 (Sero AS, Billingstad, Norway). Before analysis, QCs were reconstituted in accordance with manufacturers' instructions. QC accuracy was between 90 % and 110 % and precision ranged between 7 % and 11 %.

2.2.3. Urinary 8-oxodG

We analyzed urinary 8-oxodG concentrations using liquid chromatography coupled with triple quadrupole mass spectrometry, as previously described (Hanchi et al., 2017). Briefly, 500 μL of urine was mixed in a polyethylene tube with 500 μL of MilliQ-purified water and 5 μL of internal standards ((15 N5)-2′-deoxyguanosine 1 mg/L and cotinine-d310 mg/L). The solution was centrifuged for 10 min and the supernatant was filtered directly in the vial for the analysis. An aliquot of 10 μL was injected into the liquid chromatography equipped with a reverse phase column Gemini-NX C18 110A (100-mm length, 2.00-mm internal diameter, 3-μm particle size; Phenomenex, Bologna, Italy). The chromatographic separation was performed at room temperature using a gradient mixture of 1.5 mM ammonium formate with 0.1 % HCOOH in methanol and 1.5 mM ammonium formate with 0.1 % HCOOH in water at 0.25 mL/min, for a total run time of 10 min. Detections were performed using a triple quadrupole mass spectrometer (TSQ Quantum Access, Thermo Scientific, Rodano, Italy) equipped with a heated electrospray ionization source operating in positive ion mode. Quantification was based on selective reaction monitoring. A linear calibration curve for both analytes was obtained in urine by adding aqueous solutions of 8-oxodG at different concentrations. The limit of quantification, calculated as the lowest point in the calibration curve that gave a reproducible analytical response with a precision value, was 0.12 μg/L for 8-oxodG. The recovery rate of the analytical procedure was 98 % and the analytical imprecision was <10 %.

2.2.4. Data collection and analysis

Standard in-person interviews were performed to collect data on medical history, sociodemographic information, and lifestyle habits of each subject through ad hoc designed questionnaires. Usual diet including intake of the trace element selenium was assessed using the EPIC-FFQ (Filippini et al., 2018a). In brief, each participant completed the questionnaire, composed of 188 questions on food items consumption and quantities. Total daily exposure to selenium was assessed using a combination of analytical results on food and dietary habits assessed through the EPIC-FFQ (Filippini et al., 2018b).

The associations between urinary, dietary and total serum selenium content were previously investigated (Urbano et al., 2021b). Crude and adjusted models were used to perform linear and spline regression analyses for the association between selenium in the three matrices and urinary content of 8-oxodG and 8-oxodG/g creatinine. In the adjusted models, sex as binary variable, together with age, body mass index (BMI) and urinary cotinine as continuous variables, were used as adjustment covariates. Beta regression linear coefficients with their 95 % confidence intervals (CI) were computed for each regression analysis, to compare the effect strength of each independent variable, the biomarker of selenium exposure, on the dependent variable, either overall 8-oxodG concentrations or 8-oxodG concentrations adjusted for creatinine (g). To evaluate potential non-linearity of our associations, we used regression analyses fitted on a restricted cubic spline model using three knots at fixed percentiles (10th, 50th, and 90th) of selenium content in diet, urine, and total selenium with selenium species concentrations in serum. To handle extreme values, we winsorized data at 99th percentile of the following variables: urinary selenium (89.9 μg/L), thioredoxin reductase-bound selenium (Se-TXNRD) (17.6 μg/L), selenomethionine-bound selenium (Se-Met) (30.4 μg/L), selenocysteine-bound selenium (Se-Cys) (16.4 μg/L), total inorganic selenium (122.8 μg/L), selenite (121.9 μg/L), selenate (66.4 μg/L), and human serum albumin-bound selenium (Se-HSA) (26.1 μg/L), and at 1st percentile of total serum selenium (22.0 μg/L).

We used the 'mkspline', 'regress', 'winsor', and 'xbcrsplinei' routines of the Stata statistical software (version 17.0, Stata Corp., College Station, TX, 2021) to perform all data analyses.

3. Results

Table 1 reports the study population characteristics, divided by sex. Overall median age was 48.2 years, higher in females than in males. Median values of BMI, energy, and alcohol intake were higher among males, while

Table 1
Characteristics of the study population and median (50th) and interquartile range (IQR) concentrations of urinary cotinine and selenium (Se) (n = 137), dietary Se intake (n = 137), total serum, Se species (n = 104), and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) (n = 137) concentrations.

Characteristics	All participants			Males			Females		
	N	Median	IQR	N	Median	IQR	N	Median	IQR
Age (years)	137	48.16	(42.69–52.97)	62	47.71	(41.33–52.55)	75	49.12	(43.58–53.65)
BMI (kg/m^2)	137	24.61	(22.40–26.86)	62	24.85	(23.29–26.57)	75	24.46	(22.04–26.96)
Energy intake (kcal/day)	137	1908	(1555–2308)	62	2051	(1601–561)	75	1832	(1516–2165)
Alcohol intake (g/day)	137	7.35	(1.65–15.08)	62	11.88	(2.72–25.95)	75	4.06	(0.65–13.16)
Urinary cotinine (μg/L)	137	0.27	(0.05–0.86)	62	0.20	(0.05–0.77)	75	0.32	(0.05–0.94)
Urinary Se (μg/L)	137	22.02	(14.64–37.15)	62	24.21	(16.72–39.20)	75	21.30	(13.30–34.66)
Dietary Se (μg/L)	137	78.74	(62.62–101.48)	62	88.37	(69.77–108.28)	75	71.06	(54.77–91.68)
Total serum Se (μg/L)	104	116.5	(106.0–128.0)	50	118.0	(109.0–132.0)	54	115.0	(105.0–125.0)
Organic Se (μg/L)	104	102.24	(84.53–113.86)	50	100.56	(79.28–117.28)	54	102.58	(87.24–112.13)
Se-SELENOP (μg/L)	104	84.81	(64.23–104.05)	50	79.44	(59.88–105.03)	54	86.02	(72.48–101.65)
Se-GPX (μg/L)	104	5.46	(2.12–8.88)	50	3.72	(1.47–9.03)	54	5.74	(2.45–8.16)
Se-TXNRD (μg/L)	104	0.40	(0.03–1.51)	50	0.46	(0.03–1.66)	54	0.12	(0.03–1.31)
Se-Met (μg/L)	104	2.74	(1.41–5.23)	50	2.81	(1.63–5.10)	54	2.55	(1.23–5.67)
Se-Cys (μg/L)	104	1.91	(0.45–3.61)	50	1.93	(0.42–4.06)	54	1.91	(0.49–3.43)
Inorganic Se (μg/L)	104	9.67	(3.73–22.94)	50	10.02	(3.66–28.70)	54	8.74	(3.74–19.28)
Se (IV) (μg/L)	104	4.37	(1.57–13.83)	50	4.38	(1.21–19.09)	54	4.37	(1.77–11.95)
Se (VI) (μg/L)	104	3.30	(1.01–8.17)	50	3.85	(0.97–9.46)	54	3.07	(1.04–7.61)
Se-HSA (μg/L)	104	1.12	(0.03–3.06)	50	1.06	(0.03–4.02)	54	1.24	(0.03–2.85)
8-oxodG (μg/L)	137	3.39	(1.76–6.32)	62	3.48	(2.10–7.61)	75	3.17	(1.60–5.77)
8-oxodG/creatinine (μg/g creatinine)	137	3.21	(2.21–4.80)	62	2.78	(2.09–4.30)	75	3.55	(2.33–5.09)

Abbreviations: BMI, body mass index; IQR, interquartile range; Se-Cys, selenocysteine-bound selenium; Se-GPX, glutathione peroxidase-bound selenium; Se-HSA, human serum albumin-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se-TrxR, thioredoxin reductase-bound selenium; Se (IV), selenite; Se (VI), selenate.

females had higher urinary cotinine levels. Median values of biomarkers of selenium exposure, i.e., total urinary and serum selenium were 22 and 116.5 μg/L, while dietary intake was 78.7 μg/day, with higher values in males than in females. Females had higher median concentrations of total organic selenium compounds (including selenoprotein P-bound selenium, Se-SELENOP and glutathione peroxidase-bound selenium, Se-GPX) and Se-HSA than males, who in turn had higher median concentrations of two organic compounds (Se-TXNRD and Se-Met) and total inorganic selenium compounds (including selenite). Se-Cys and selenite median concentrations were generally similar.

Supplementary Table S1 reports the number and % of samples that were below the LOQ, which showed a very high number of undetermined values for both Se-TXNRD (42 % of samples) and Se-HSA (29 %).

Results of linear regression analyses for the association between total urinary, dietary intake, and serum selenium (thus comprising selected selenium compounds) concentrations versus urinary concentrations of 8-oxodG and their ratio with urinary creatinine are reported in Table 2, both in crude and multivariable analyses. In multivariable analyses, urinary selenium concentrations were positively associated with 8-oxodG, while no such association emerged with dietary selenium intake or total selenium serum concentrations. When 8-oxodG levels were adjusted for urinary creatinine, there was a positive association between urinary selenium and 8-oxodG concentrations, a substantial null association between serum selenium and 8-oxodG, and there was evidence for opposing associations between urinary 8-oxodG and serum selenium species: positive for Se-SELENOP and Se-GPX, inverse for inorganic selenium, selenate, and particularly selenite (Table 2). Serum concentrations of the organic species Se-TXNRD also showed an inverse association with urinary 8-oxodG concentrations, while Se-HSA did not exhibit a clear pattern of association.

Table 2
Linear regression analyses of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxodG/g creatinine versus urinary selenium (Se) concentration, dietary Se intake, total serum Se concentrations, organic, inorganic, and human serum albumin-bound selenium biomarkers as independent variables. Crude and adjusted models. Values are expressed as beta coefficients (β) with 95 % confidence intervals (CI).

	Crude model	Adjusted[a] model
	β (95 % CI)	β (95 % CI)
8-oxoDG (μg/L)		
Urinary Se (μg/L)	0.10 (0.06, 0.14)	0.10 (0.06, 0.14)
Dietary Se (μg/day)	0.01 (−0.01, 0.03)	0.01 (−0.01, 0.03)
Serum Se (μg/L)	−0.02 (−0.06, 0.02)	−0.002 (−0.049, 0.046)
Organic Se (μg/L)	0.01 (−0.02, 0.04)	0.02 (−0.01, 0.05)
Se-SELENOP (μg/L)	0.01 (−0.01, 0.04)	0.02 (−0.01, 0.05)
Se-GPX (μg/L)	−0.01 (−0.16, 0.13)	0.02 (−0.13, 0.17)
Se-TXNRD (μg/L)	−0.12 (−0.65, 0.40)	−0.04 (−0.58, 0.49)
Se-Met (μg/L)	0.001 (−0.166, 0.167)	0.01 (−0.19, 0.17)
Se-Cys (μg/L)	−0.09 (−0.42, 0.24)	−0.03 (−0.36, 0.31)
Inorganic Se (μg/L)	−0.03 (−0.07, 0.01)	−0.03 (−0.07, 0.01)
Se (IV) (μg/L)	−0.07 (−0.13, −0.15)	−0.07 (−0.13, −0.01)
Se (VI) (μg/L)	−0.02 (−0.11, 0.07)	−0.01 (−0.10, 0.08)
Se-HSA (μg/L)	−0.05 (−0.28, 0.18)	−0.02 (−0.23, 0.18)
8-oxoDG/creatinine (μg/g creatinine)		
Urinary Se (μg/L)	0.003 (−0.017, 0.024)	0.10 (−0.06, 0.13)
Dietary Se (μg/day)	−0.002 (−0.013, 0.009)	0.01 (−0.01, 0.03)
Serum Se (μg/L)	−0.004 (−0.024, 0.016)	0.006 (−0.016, 0.276)
Organic Se (μg/L)	0.01 (0.00, 0.03)	0.02 (−0.01, 0.05)
Se-SELENOP (μg/L)	0.01 (0.00, 0.02)	0.02 (−0.01, 0.05)
Se-GPX (μg/L)	0.002 (−0.067, 0.072)	0.02 (−0.13, 0.17)
Se-TXNRD (μg/L)	−0.22 (−0.46, 0.03)	−0.04 (−0.58, 0.49)
Se-Met (μg/L)	0.09 (0.01, 0.18)	0.076 (0.001, 0.151)
Se-Cys (μg/L)	−0.01 (−0.16, 0.15)	−0.03 (−0.36, 0.31)
Inorganic Se (μg/L)	−0.02 (−0.04, 0.00)	−0.016 (−0.033, 0.001)
Se (IV) (μg/L)	−0.04 (−0.06, −0.01)	−0.07 (−0.13, −0.01)
Se (VI) (μg/L)	−0.02 (−0.06, 0.02)	−0.01 (−0.10, 0.08)
Se-HSA (μg/L)	−0.04 (−0.15, 0.07)	−0.01 (−0.11, 0.08)

Abbreviations: Se-Cys, selenocysteine-bound selenium; Se-GPX, glutathione peroxidase-bound selenium; Se-HSA, human serum albumin-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se-TXNRD, thioredoxin reductase-bound selenium; Se (IV), selenite; Se (VI), selenate.

[a] Adjusted for sex, age, body mass index, and urinary cotinine.

In spline regression analyses, urinary selenium was generally linearly and positively associated with urinary 8-oxodG, but the association became slightly and inversely U-shaped when 8-oxodG concentrations were adjusted for urinary creatinine. Specifically, urinary selenium was positively associated with 8-oxodG/g creatinine until 30 μg/L, above which the curve showed a plateau and then an inverse relation. The association of dietary selenium intake with 8-oxodG levels was slightly U-shaped, tending to be inverse under 100 μg/day and positive above such cut-point. Conversely, the association was flattened when 8-oxodG levels were adjusted for creatinine. Associations of serum selenium levels were inversely U-shaped with both 8-oxodG and 8-oxodG/g creatinine with a turning point of the curve at 120 μg/L, though the curve was steeper below than and above that point, and substantially flat when the adjustment for creatinine was performed (Fig. 1).

Concerning the associations between the single serum selenium compounds and creatinine-adjusted urinary 8-oxodG concentrations, for overall organic selenium forms a positive association emerged up to 100 μg/L, where the association flattened and became slightly negative (Fig. 2).

Most organic species, i.e., Se-GPX, Se-Met and Se-Cys, were positively associated with the oxidative stress biomarker apart from Se-SELENOP, for which the pattern of association was inversely U-shaped, similarly to overall organic selenium. Negative associations emerged instead for overall inorganic selenium and consistently for the two inorganic species, selenite and selenite. We excluded from spline regression analyses the Se-TXNRD and Se-HSA species, given the very high percentage of missing values. These results were generally similar when 8-oxodG concentrations were not adjusted for creatinine, with the exception of selenate concentrations, which were not appreciably associated with 8-oxodG, and for the associations for Se-Met and Se-Cys levels, which were substantially attenuated (Supplementary Fig. S1).

We also assessed the relation of each selenium compound with creatinine-adjusted 8oxodG concentrations, by adjusting within the multivariable also for the remaining selenium species (Fig. 3). A positive almost linear association of 8-oxodG with the organic species Se-Met emerged, while there was a slight positive relation with Se-GPX, an inverted U-shaped association with Se-SELENOP, and no relation with Se-Cys. Concerning the inorganic species, both selenate and more strongly selenite were inversely associated with 8-oxodG concentrations.

In subgroup analyses according to sex, total selenium concentrations in urine and serum were positively and linearly correlated with 8-oxodG/g creatinine concentrations among males, although the curves were steeper for urinary selenium and far less steep for serum selenium concentrations. Conversely, dietary selenium showed an inverted U-shaped pattern, with an inflection point around 100 μg of daily selenium intake. Among females, urinary selenium concentrations were positively associated with 8-oxodG/g creatinine levels until 30 μg/L and then an overturn of the curve occurred. The association between dietary selenium and 8-oxodG adjusted for creatinine was weak and inverse, while the pattern for serum selenium was of an inverted U-shaped curve, positive up to 120 μg/L and inverse above this value (Supplementary Fig. S2). In sex-specific analysis, the associations of selenium species and 8-oxodG/g creatinine differed among males and females, with a positive association for total organic selenium and Se-SELENOP concentrations in both subgroups until 80–100 μg/L and then a flattening or a slight decrease of the curve. Se-Met levels were positively correlated with 8-oxodG/g creatinine among males but very little among females, while slight positive associations emerged for Se-GPX and Se-Cys among females and even minimally or null among males. Total inorganic selenium and both inorganic species, selenite and selenate, were inversely associated with 8-oxodG/g creatinine among males, with a steeper relation for selenite, while among females the slight inverse relation for total inorganic selenium and for selenite was not found for selenate.

When we examined the relation of each selenium compound with 8oxodG/g creatinine levels also adjusting for the other selenium species, some differences according to sex emerged (Fig. 4). Among males, three

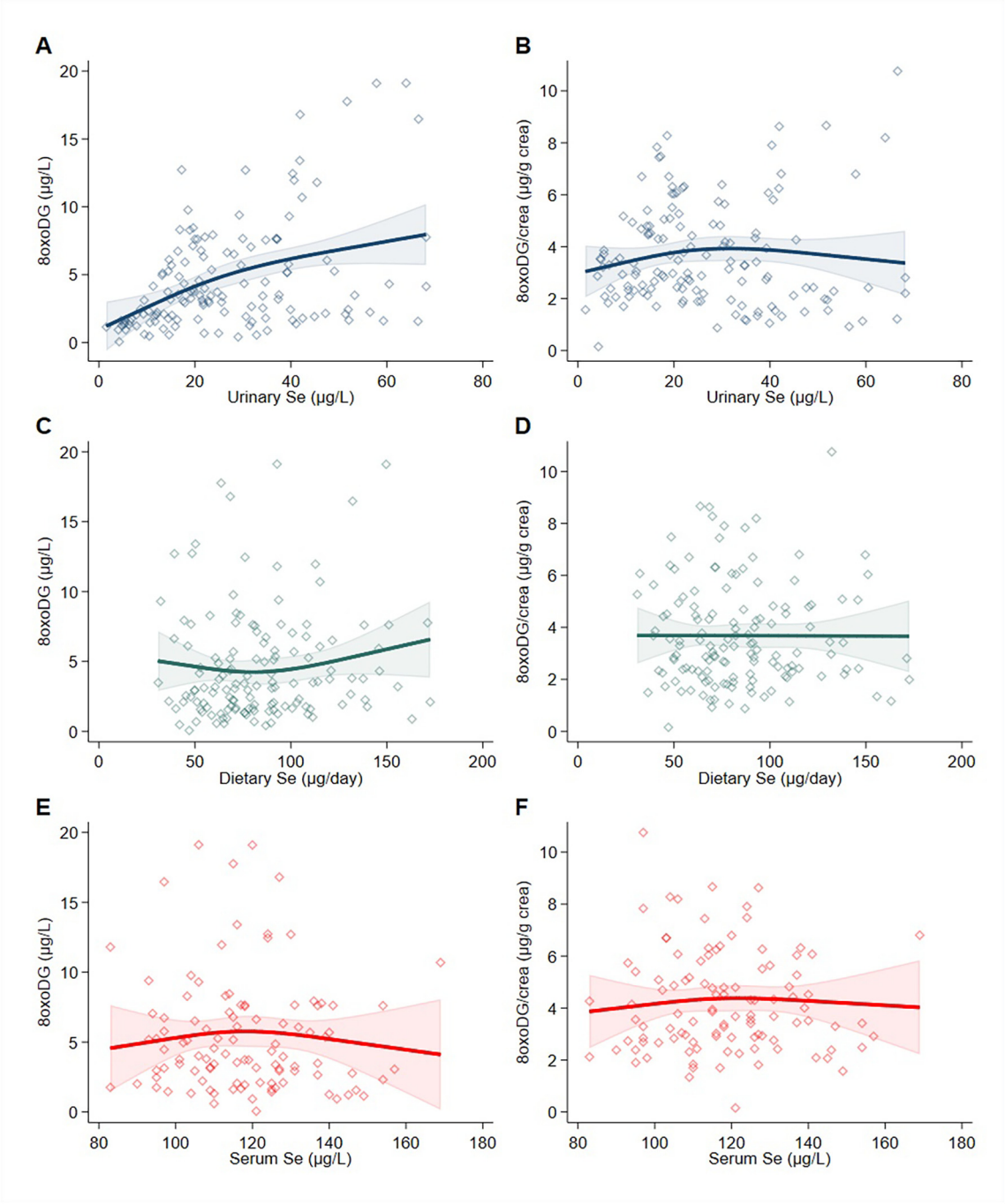
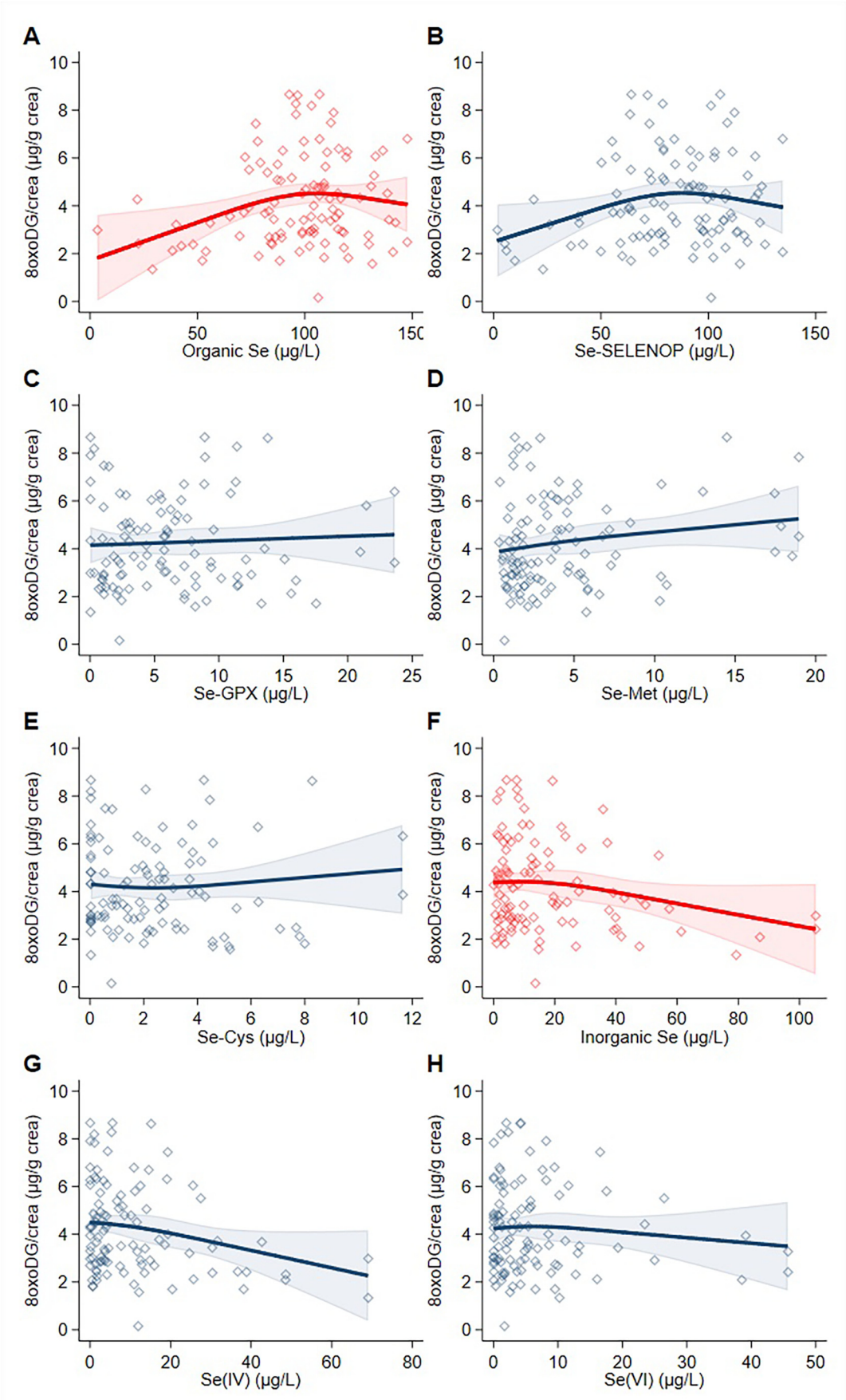


Fig. 1. Spline regression analysis of urinary (n = 137), dietary (n = 137), and total serum (n = 104) selenium (Se) levels, versus 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxodG/g creatinine. The solid line represents the multivariable analysis (adjusted for sex, age, body mass index, and urinary cotinine) with upper and lower confidence interval limits (shaded area). Diamonds represent individual observations.

organic compounds namely Se-SELENOP, Se-GPX, and Se-Met were positively, though not entirely linearly, associated with 8-oxodG/g creatinine levels, with a steeper curve for Se-Met. Inverse associations emerged for Se-Cys, selenate and selenite, particularly for the latter inorganic form. Among females, all associations were strongly attenuated, especially for Se-Cys, apart from Se-GPX for which a slight positive association emerged above 5 µg/L.

4. Discussion

In this cross-sectional study, endogenous concentrations of selected selenium species were associated with biomarkers of oxidative stress, suggesting pronounced and at times opposite effects on redox balance depending on the selenium compounds involved. We found a number of different patterns of association between indicators of exposure to overall selenium or to specific selenium species and 8-oxodG, in some instances even opposite according to data source (urinary and blood selenium and dietary intake), the chemical species considered and the sex of participants. Overall, our results offer little support for a large effect of overall selenium exposure, independently from the method of assessment, on oxidative stress and genotoxicity as implied by increased urinary concentrations of the biomarker we selected as endpoint, 8-oxodG, after adjustment for creatinine excretion. However, for single selenium species, after adjusting for confounders (including the other selenium species), clear evidence


A
8oxoDG/crea (µg/g crea)
Organic Se (µg/L)
B
8oxoDG/crea (µg/g crea)
Se-SELENOP (µg/L)
C
8oxoDG/crea (µg/g crea)
Se-GPX (µg/L)
D
8oxoDG/crea (µg/g crea)
Se-Met (µg/L)
E
8oxoDG/crea (µg/g crea)
Se-Cys (µg/L)
F
8oxoDG/crea (µg/g crea)
Inorganic Se (µg/L)
G
8oxoDG/crea (µg/g crea)
Se(IV) (µg/L)
H
8oxoDG/crea (µg/g crea)
Se(VI) (µg/L)

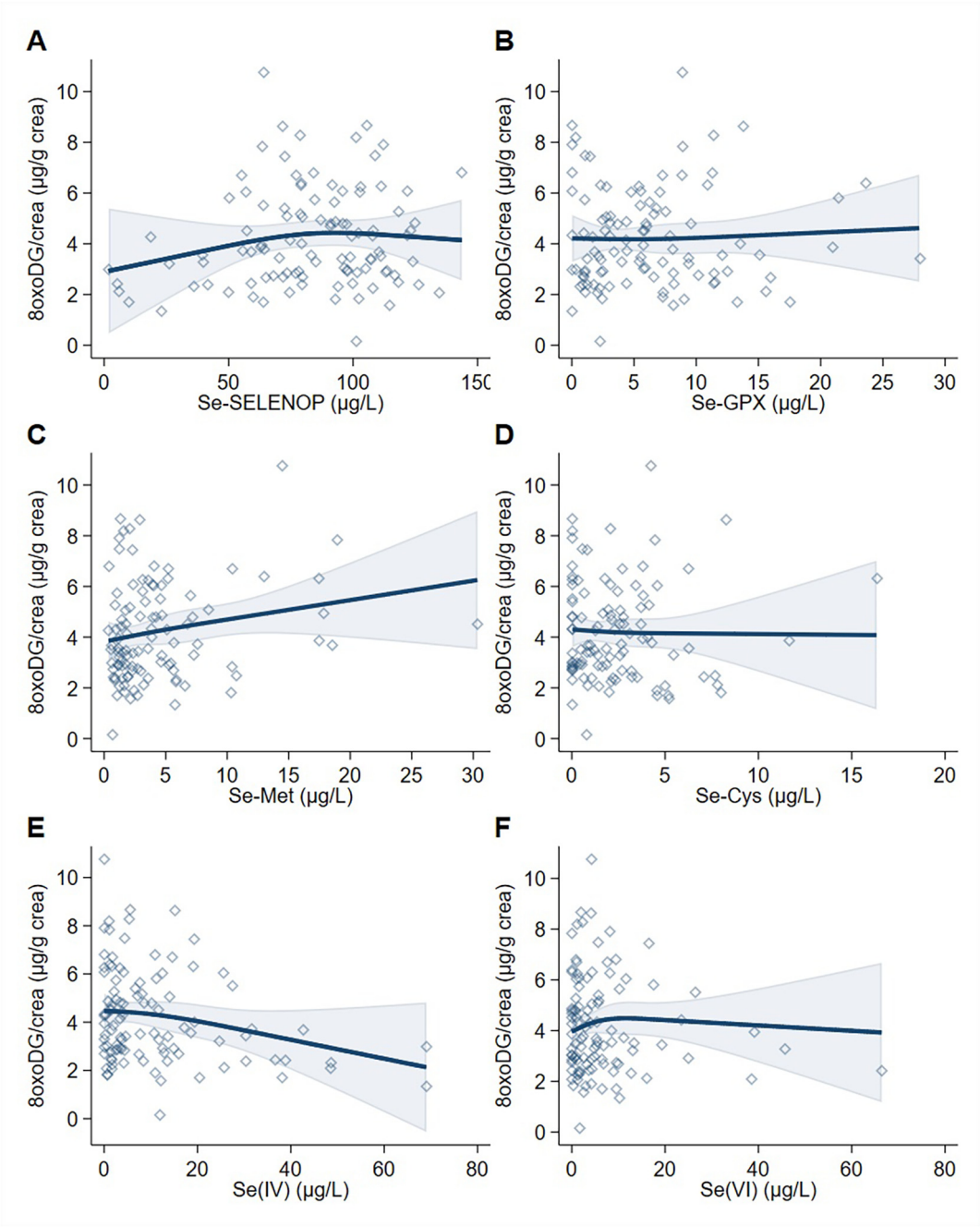


Fig. 3. Spline regression analysis of serum selenium (Se) species levels, versus 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)/g creatinine (n = 104). The solid line represents the multivariable analysis (adjusted for all selenium species and age, sex, body mass index, and urinary cotinine) with upper and lower confidence interval limits (shaded area). Diamonds represent individual observations.
Abbreviations: Se-Cys, selenocysteine-bound selenium; Se-GPX, glutathione peroxidase-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se (IV), selenite; Se (VI), selenate.

emerged that organic selenium species and Se-Met in particular, as well as Se-SELENOP, could elicit a higher synthesis and excretion of 8-oxodG. This is by no means unexpected, given the established capacity of Se-Met to increase oxidative stress in many experimental conditions (Lazard et al., 2017; Malheiros et al., 2020; Maraldi et al., 2019; Maraldi et al., 2011; Naderi et al., 2018; Spallholz, 2019), and also suggests that even low amounts of Se-Met may elicit a pro-oxidant response, a finding of relevance to risk assessment of this selenium species. In fact, while selenoproteins

Fig. 2. Spline regression analysis of serum concentrations of selenium (Se) species versus 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)/g creatinine (n = 104). The solid line represents the multivariable analysis (adjusted for sex, age, body mass index, and urinary cotinine) with upper and lower confidence interval limits (shaded area). Diamonds represent individual observations.
Abbreviations: Se-GPX, glutathione peroxidase-bound selenium; Se-HSA, human serum albumin-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se-TXNRD thioredoxin reductase-bound selenium; Se (IV), selenite; Se (VI), selenate.

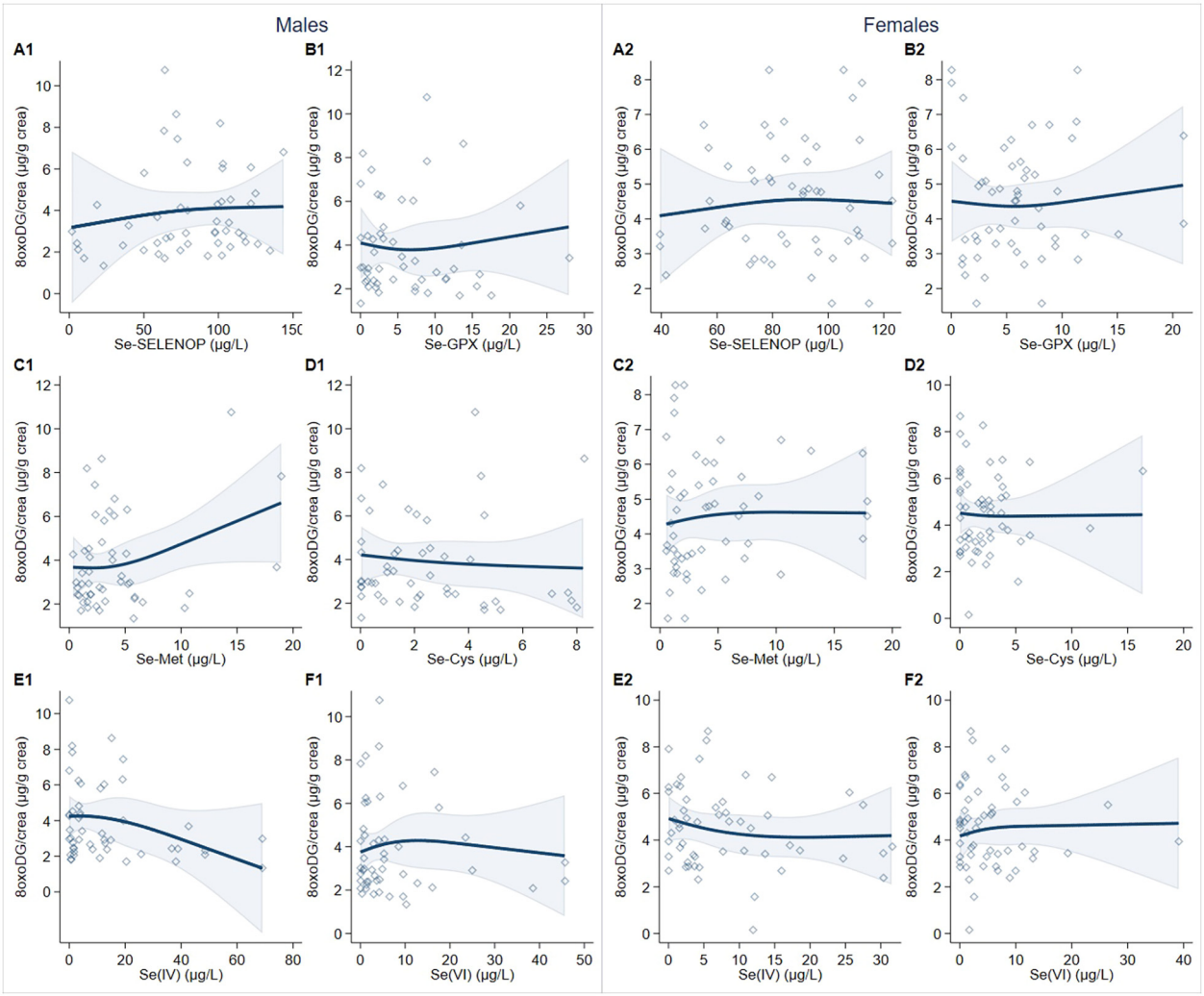


Fig. 4. Spline regression analysis of serum concentrations of selenium (Se) species versus 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)/g creatinine in males (n = 50, A1–F1) and females (n = 54, A2–F2). Multivariable analysis adjusted for all selenium species and age, body mass index, and urinary cotinine. The solid line represents the multivariable analysis with upper and lower confidence interval limits (shaded area). Diamonds represent individual observations.
Abbreviations: Se-Cys, selenocysteine-bound selenium; Se-GPX, glutathione peroxidase-bound selenium; Se-Met, selenomethionine-bound selenium; Se-SELENOP, selenoprotein P-bound selenium; Se (IV), selenite; Se (VI), selenate.

have antioxidant properties, some selenium compounds such as selenomethionine and inorganic selenium forms can promote free radical damage (Banerjee et al., 2022; Jablonska and Vinceti, 2015; Lee and Jeong, 2012; Shamberger, 1985; Spallholz, 1997; Vinceti et al., 2018; Vinceti et al., 2015; Wang et al., 2016; Weekley and Harris, 2013), this in turn being able to trigger a compensatory response by increased synthesis of antioxidant enzymes such as catalase, superoxide dismutase and selenoproteins (Jablonska and Vinceti, 2015; Vinceti et al., 2022b; Vinceti et al., 2009), as shown by laboratory and environmental studies (Kolbert et al., 2019; Lazard et al., 2017; Olsvik et al., 2014; Rigby et al., 2014; Van Hoewyk, 2013). In a trial carried out in a male population, Se-Met supplementation enhanced urinary levels of 8-isoprostane, a biomarker of lipid peroxidation, and slightly of 8-hydroxy-2'-deoxyguanosine (8-OHdG, the non-oxidized precursor of 8-oxodG) compared with placebo, while another source of selenium, selenium-enriched yeast, was associated with a decrease in both biomarkers of oxidative stress (Richie et al., 2014). Interestingly, selenomethionine was used in the largest cancer chemoprevention trial ever conducted, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), showing a lack of efficacy in reducing prostate cancer risk. Instead, selenomethionine supplementation was associated with excess risk of type 2 diabetes mellitus and high-grade prostate cancer (Kristal et al., 2014; Lippman et al., 2009), and adverse metabolic effects for selenoprotein P have also been suggested by experimental laboratory studies and non-experimental human investigations (Saito, 2020a; Saito, 2020b; Vinceti et al., 2022b; Vinceti et al., 2018).

The limited evidence from human studies about selenium and its species as related to oxidative stress is conflicting. Overall selenium exposure, assessed through plasma selenium concentrations and above 110 µg/L, was positively associated with urinary 8-oxodG in a Spanish cross-sectional study (Galan-Chilet et al., 2014), as was urinary selenium with urinary 8-OHdG in a Chinese cohort study (Xiao et al., 2018). Urinary selenium was linearly and positively associated with 8-oxodG in a cross-sectional study (Lu et al., 2016). In a cohort study conducted among pregnant women, urinary selenium was associated with a marked increase in 8-isoprostane and an increase in 8-OHdG (Kim et al., 2019), though there was no mention of adjustment for creatinine. Generally, in observational studies, the selenium species being measured in the body (or the diet) have not been determined, despite the growing awareness that each selenium form has distinctive biochemical properties (Michalke et al., 2018; Vinceti et al., 2018; Vinceti et al., 2015; Weekley and Harris, 2013).

In the present study, we found no appreciable associations between serum concentrations of inorganic selenium overall, or the two inorganic species (selenite and selenate), and urinary 8-oxodG. This finding is not in line with the generally higher toxicity of these compounds compared with

the organic selenium species in a number of laboratory and human studies (Vinceti et al., 2022b; Vinceti et al., 2018; Vinceti et al., 2014), including their genotoxicity (Abul-Hassan et al., 2004; Herrero and Wellinger, 2015; Lazard et al., 2017; McKelvey et al., 2015). Actually, for the inorganic hexavalent form, selenite, we found even evidence of a strong inverse association with 8-oxodG, which was difficult to interpret. Clearly, these observations for both inorganic and organic selenium species apply only to the exposure range of this study population, which is not occupationally exposed neither composed of residents in seleniferous areas (Chawla et al., 2020; Loomba et al., 2020), where different patterns of association might occur.

Taken together, findings of the present study indicate that at average exposure levels in the general 'healthy' population, such as the present one composed by blood donors, indicators of overall selenium intake and status as well as inorganic species are not positively associated with an indicator of oxidative stress, 8-oxodG, but some organic selenium compounds such as Se-Met and Se-SELENOP are, especially among males. When assessed in the context of other human evidence and of its biological plausibility, these observations suggest that in a healthy Western population higher circulating organic selenium species (except for the chemical form that is included in selenoproteins, selenocysteine) are positively associated with increased oxidative stress, and that such association occurs at relatively low amounts of exposure. This also adds to the evidence suggesting that risk assessment of selenium should consider the specific toxicity and the distinctive toxicological and nutritional properties of each selenium compound, as suggested by a large body of evidence (Filippini et al., 2023; Michalke et al., 2018; Vinceti et al., 2022a; Vinceti et al., 2017a; Weekley and Harris, 2013).

We found some evidence for effect modification by sex of the aforementioned associations, which was not unexpected given observations from other studies suggesting differential effects of selenium on disease risk among males and females (Tower et al., 2020). There is evidence that sexual dimorphism can affect selenium biology, i.e., its absorption and excretion, and that associations between selenium and health outcomes can vary by sex (Seale et al., 2018). Overall, urinary selenium was positively associated with urinary 8-oxodG among males but not females, and this was also true for Se-Met. Also, the inverse association observed between serum selenite and urinary 8-oxodG, was much stronger among males. Females could be less susceptible to detrimental effects of selenium overexposure such as type 2 diabetes (Vinceti et al., 2021), and more generally there is evidence for sexual dimorphism in selenium metabolism, retention, and excretion (Combs et al., 2012).

Some inherent limitations of our observational study design must be taken into consideration when interpreting the study findings. Though we investigated a highly selected 'healthy', non-smoking population, thus reducing the risk of bias due to pathologic conditions and adverse lifestyle habits, we cannot rule out the role of unmeasured, residual confounding. However, we made an attempt to control for a number of potential confounders, including smoking, a source of selenium in non-occupationally exposed individuals (Bogden et al., 1981) unexpectedly associated with decreased circulating selenium concentrations probably due to an interaction with cadmium (Jossa et al., 1991; Vinceti et al., 2015), and in addition affecting oxidative stress (Urbano et al., 2022). Inadequate control for smoking can therefore bias the results. However, in our study we enrolled only subjects who declared to be non-smokers, and we also adjusted for urinary metabolite of nicotine cotinine, thus accounting for the influence of passive smoking or undeclared active smoking in our results. Importantly, the cross-sectional design of the present study does not allow to rule out potential for reverse causation, i.e., of the induction of a higher availability and retention of organic selenium species needed for the synthesis of the antioxidant selenium dependent enzymes, like Se-GPX, Se-SELENOP, in the presence of increased oxidative stress. In contrast, under such hypothesis of a rise of organic selenium compounds due to an increased demand of selenoprotein synthesis, we would have expected a positive association between serum Se-Cys and urinary 8-oxodG, which was not observed.

CRediT authorship contribution statement

Conceptualization: MV, TF; Data curation: TU, MM; Formal analysis: TU, SS, EP, SF, BM; Funding acquisition: MV; Investigation: MV, TF, TU; Methodology: MV, TF; Project administration: MV; Resources: MV; Supervision: MV, LAW, SF, BM; Roles/Writing - original draft: TU; Writing - review & editing: All authors.

Data availability

Data will be made available on request.

Declaration of competing interest

Dr. Lauren A. Wise is supported by grants from the National Institutes of Health. She also serves as a fibroid consultant on for AbbVie, Inc. She also receives in-kind donations for primary data collection in Pregnancy Study Online (PRESTO) from Swiss Precision Diagnostics (home pregnancy tests) and Kindara.com (fertility apps). All of these relationships are for work unrelated to this manuscript. The remaining authors declare no conflict of interest.

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Ethical approval

The study was approved by the Ethics Committee of the Reggio Emilia province (protocol approval no. 2016/0022799).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.161584.

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