**Title:** Selenium and selenium species in the etiology of Alzheimer’s dementia: biases associated withthe case-control study design

**Authors:**

**Affiliations**

1CREAGEN - Environmental, Genetic, and Nutritional Epidemiology Research Center, and 2Center for Neurosciences and Neurotechnology, Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, 287 Via Campi, Modena 41125, Italy;

3Department of Epidemiology, Boston University School of Public Health, 715 Albany Street, Boston, MA 02118, USA;

4Department of Neurosciences, Azienda Ospedaliero-Universitaria di Modena, via del Pozzo 71, Modena, Italy;

5Helmholtz Center Munich – German Research Center for Environmental Health GmbH, Research Unit Analytical BioGeoChemistry, 1 Ingolstaedter Landstrasse, Neuherberg 85764, Germany

**Corresponding author:** Marco Vinceti. Environmental, Genetic, and Nutritional Epidemiology Research Center - CREAGEN, Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, 287 Via Campi, Modena 41125, Italy. (Tel. +39-059-2055481; fax +39-059-2055483; e-mail: marco.vinceti@unimore.it)

**Running head:** Selenium, Alzheimer’s dementia, case-control study

**Abstract**

Background: Selenium is a trace element of strong interest for both nutritional and toxicologic properties. The possibility that selenium species may influence the onset of neurological disease, including Alzheimer’s dementia (AD), has been suggested on the basis of several human studies. However, conflicting and even opposite association between exposure and risk has been reported, possibly due to biases in exposure assessment .

Methods. After having detected an excess AD risk associated to higher levels of a selenium species, the inorganic hexavalent one, in subjects with mild cognitive impairment (MCI) using a cohort study design, we investigated the relation between selenium and AD using a case-control study design, We assessed risk of AD associated with cerebrospinal fluid levels of selenium species in 56 MCI participants and 33 patients with AD.

Results: AD risk inversely correlated with cerebrospinal fluid selenium content, as well as with inorganic selenium and with the organic form bound to selenoprotein P. Overall organic-bound selenium (sum of different organo-Se-species) was not associated with disease risk, while the selenium bound to other organo-Se-species positively correlated with AD risk, possibly due to compensatory selenoprotein upregulation following increased oxidative stress.

Conclusions: This case-control study yielded entirely different results compared with those generated by a recent cohort study including a part of the study population. Study findings indicate that the case-control design does not allow to reliably assess the role of selenium exposure in Alzheimer’s dementia etiology, falsely suggesting an etiologic role of selenium deficiency likely due to reverse causation. The case-control study design may instead allow insights into the pathologic process underlying disease progression, suggesting the occurrence of selenoprotein upregulation during disease progression.

**Keywords:** Mild cognitive impairment, Alzheimer’s disease, dementia, selenium, selenium species, cerebrospinal fluid.

**Introduction**

There is consensus that the trace element selenium (Se), whose exposure is generally around 20-100 µg/day in most populations worldwide, is an element of considerable interest under both a toxicological and a nutritional perspective, with a very narrow range of safe exposure (Vinceti, Filippini et al. 2017, Brigelius-Flohe and Arner 2018, Vinceti, Filippini et al. 2018). However, the range of exposure considered to be safe differs across studies and regulatory agencies, and recent evidence has highlighted the likely occurrence of side effects at exposure levels previously deemed to be safe (Vinceti, Filippini et al. 2018, Vinceti, Filippini et al. 2018, Vinceti, Filippini et al. 2018, Yarmolinsky, Bonilla et al. 2018, Yarmolinsky, Wade et al. 2018), while its possible beneficial effects in chronic disease prevention has substantially failed to be demonstrated (Rees, Hartley et al. 2013, Vinceti, Burlingame et al. 2016, Vinceti, Filippini et al. 2018). Among the various disease ascribed to both inadequate or excess Se intake there are neurodegenerative disease, and specifically Alzheimer’s dementia (AD), a possible connection currently subject to considerable attention and active investigation (Cicero, Mostile et al. 2017, Reddy, Bukke et al. 2017, Solovyev, Drobyshev et al. 2018, Varikasuvu, Prasad et al. 2018). Some evidence has been provided for a role of Se in AD etiology (Loef, Schrauzer et al. 2011, Killin, Starr et al. 2016, Cicero, Mostile et al. 2017, Reddy, Bukke et al. 2017, Varikasuvu, Prasad et al. 2018), though en effect of Se has been suggested to be either beneficial or adverse (Morris, Brockman et al. 2016, Cardoso, Hare et al. 2017, Vinceti, Chiari et al. 2017, Solovyev, Drobyshev et al. 2018, Yang, Liou et al. 2018), as also suggested for other neurodegenerative disease (Vinceti, Mandrioli et al. 2014, Cardoso, Roberts et al. 2015, Solovyev 2015, Ellwanger, Franke et al. 2016, Cicero, Mostile et al. 2017, Oliveira, Piccoli et al. 2017, Maass, Michalke et al. 2018, Rae, Kitley et al. 2018). Only one experimental study, a randomized controlled trial carried out within the large selenium and vitamin E cohort intervention study (SELECT), has been carried out on this issue (Kryscio, Abner et al. 2017), showing little effect on Alzheimer’s dementia risk by 200 µg/day organic selenium supplementation and therefore considered to be substantially negative . In addition, Se has been recently suggested as a potential drug to decrease AD progression, though at extremely high doses (Cardoso, Roberts et al. 2018).

Consistently with the evidence yielded by the epidemiologic studies, biological plausibility for both a toxic and a beneficial role of Se has been provided. Such evidence concerns for instance its effects of on brain cortex, cognitive performance and Alzheimer’s disease related changes (Naderi, Salahinejad et al. 2017, Naderi, Salahinejad et al. 2018) (Jin, Zhu et al. 2017, Zheng, Zhang et al. 2017), its pro-oxidant activity couple with the antioxidant properties of selenoproteins (Vinceti, Maraldi et al. 2009, Hatfield, Tsuji et al. 2014, Labunskyy, Hatfield et al. 2014, Jablonska and Vinceti 2015).

In addition to a clarification about the real role of Se in AD pathology, if any, many methodological aspects remain to be elucidated. Among these, the window of exposure relevant to such relation, the most suitable indicators of Se exposure in the central nervous system, and which are the Se chemical forms involved in AD onset. The latter issue is particularly relevant also on the basis of recent studies, which have shown both the strong differences in biological activities of Se species (Weekley and Harris 2013, Jablonska and Vinceti 2015, Michalke, Willkommena et al. 2018, Vinceti, Filippini et al. 2018), and their uneven distribution in the human body as well as in environmental sources, including diet (Filippini, Michalke et al. 2018). Moreover, the study design is of utmost importance, since the usual case-control approach characterizing most of the studies might suffer from exposure misclassification and reverse causality, therefore yielding biased effect estimates.

We have recently carried out a nonexperimental longitudinal study to investigate the risk of AD occurrence in a cohort of Italian participants affected by mild cognitive impairment (MCI), according to the baseline levels of the various selenium chemical forms as detected in a central nervous system indicator, cerebrospinal fluid. We found that selenium species did not influence and/or predict AD risk except for the inorganic hexavalent form (selenate), which was positively and strongly associated with subsequent dementia occurrence (Vinceti, Chiari et al. 2017). Conversely, there have been a number of case-control studies investigating this issue, based in most cases on peripheral indicators of exposure (such as serum/plasma selenium levels) or less frequently on cerebrospinal fluid selenium levels, and never including a full speciation analysis (Loef, Schrauzer et al. 2011, Reddy, Bukke et al. 2017, Varikasuvu, Prasad et al. 2018). The case-control studies generally showed no association or an inverse association between to assess the relation between this element and AD risk, though positive associations were also reported (Loef, Schrauzer et al. 2011, Reddy, Bukke et al. 2017, Varikasuvu, Prasad et al. 2018).

An hypothesis to explain these conflicting results of case-control studies on the role of selenium in AD etiology, also taking into account the results of our recent cohort study, might be misclassification of long-term selenium exposure due to the alterations in nutritional status or metabolism in these patients following disease progression (Vinceti, Crespi et al. 2013, Vinceti, Mandrioli et al. 2014), as well as the inability of overall selenium indicators to reflect levels of single selenium species (Vinceti, Grill et al. 2015). To investigate these issues and particularly to assess these potential biases, we carried out a case-control study partially overlapping with our previous cohort investigation.

**Methods**

*Study cohort*

The flowchart reported in Figure 1 shows the study we undertook after the Modena Ethics Committee approval. We considered as eligible for our original cohort study a consecutive series of subjects who received a clinical diagnosis of either amnestic MCI (single domain or multiple domain) or non-amnestic MCI of non-vascular origin admitted 2008 through 2014 to the Modena and Reggio Emilia Neurology Memory Clinic of Policlinico University Hospital (former Sant’Agostino-Estense) (Tondelli, Bedin et al. 2015, Vinceti, Chiari et al. 2017). We further restricted participation in this study to the 56 MCI subjects who underwent a lumbar puncture if required for diagnostic purposes and had 1 ml of cerebrospinal fluid available for research purposes. In the present study, these MCI participants constituted the referent group. In addition to them, we recruited the 33 subjects who received a diagnosis of AD in the 2008-2014 period at the same clinic, underwent a lumbar puncture for diagnostic purposes and had 1 ml of cerebrospinal fluid still available for research purposes.

We had available a routine blood tests, the neurological and neuropsychological examinations, and brain MRI for all participants at the time of either MCI and AD diagnosis. Analytical determinations routinely performed in the cerebrospinal fluid and available for this study were levels of Aβ1-42 (β-amyloid) and of the tau protein, as the total (t-tau) and the phosphorylated (p-tau) form. APOE ε4 allele status was available in 64 participants.

*Analytical determinations*

Sampling of CSF and selenium speciation refers to Mandrioli, Michalke et al. 2017. In short terms: Standardized lumbar punctures were performed minimizing the risk of biological and chemical contamination (Mandrioli, Michalke et al. 2017). After collection we transported CSF to the adjacent laboratory within 30 minutes, centrifuged (15 min, 2700 g, 20 oC) and aliquoted samples into polypropylene storage tubes. CSF β-amyloid, t-tau, and p-tau 181 were measured as previously described (Tondelli, Bedin et al. 2015). The remaining, anonymized aliquots were immediately stored at -80°C and were transported in dry ice to the element speciation laboratory at the Helmholtz Zentrum München.

We determined total selenium by inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS) and the selenium species: selenite (Se(IV)), selenate (Se(VI)), selenomethionine-bound Se (Se-Met), selenocysteine-bound Se (Se-Cys), thioredoxin reductase-bound Se (Se-TXNRD), glutathione-peroxidase-bound Se (Se-GPX), selenoprotein P-bound Se (Se-SELENOP) and human serum albumin-bound Se (Se-HSA), in CSF samples by ion exchange chromatography (IEC) coupled with ICP-DRC-MS, according to (Michalke and Berthele 2011, Solovyev, Berthele et al. 2013). For total Se determination, CSF samples were diluted 1/10 with Milli-Q water + 1 µg/L Rh as internal standard, whereas Se-speciation used a Knauer 1100 Smartline inert Series gradient HPLC system with an ion exchange column AS-11 (250 x 4 mm I.D.) from Thermo Fischer Scientific Inc. (Sunnyvale, CA, USA) for species separation. was. Samples (undiluted CSF, a´ 20 µl) were determined in duplicate. Mobile phases were A= 3.33 mM Tris-HAc buffer, 5% methanol, pH 8.0, and B= 10 mM Tris-HAc buffer, 500 mM ammonium acetate, 5% Methanol, pH 8.0, using gradient elution specified in Mandrioli, Michalke et al. 2017. The experimental settings for ICP-DRC-MS (NexIon 300 D, Perkin Elmer) were: radio frequency power: 1250 W; plasma gas flow: 15 L Ar/min; auxiliary gas flow: 1.05 L Ar/min; nebulizer gas flow: 0.92 L Ar/min; daily optimized, dwell time 300 ms; ions monitored: 77Se, 78Se, 80Se, 103Rh; DRC reaction gas: CH4 reaction at 0.58 ml/min; DRC rejection parameter q: 0.6. Five-point calibration curves between blanks and 5000 ng Se/L were linear with very good r² for monitored Se isotopes (>0.999881). Data files from selenium chromatograms were processed with PeakfitTM software for peak area integration. Analytical figures of merit: limit of detection (LOD) = 19.5 ng Se/L for Se-species. Accuracy of selenium determination and selenium species quantification was checked by analyzing control materials and a certified reference material: quality control (QC) for total Se determination was performed by analyzing control materials ‘human serum’ and ‘urine’ from Recipe, Munich. Accuracy values were 98.4 ± 3.8% (serum) and 102.1 ± 5.4% (urine).

The certified reference material NIST 1950 (National Institute of Standards and Technology, Gaithersburgh, MD, USA) was used for QC regarding total Se, Se-SELENOP, Se-GPX and Se-HSA. Accuracy values were 103 ± 5.1% (Se-SELENOP, target value=100%: 50.2 ± 4.3 µg/kg), 93 ± 3.1 % (Se-GPX, target value=100%: 23.6 ± 1.3 µg/kg), and 97 ± 1.7 % (Se-HSA, target value=100%: 28.2 ± 2.6 µg/kg).

*Data analysis*

We used for our analysis half the threshold of values which fell below the limit of detection (LOD) (US Environmental Protection Agency 2000, Croghan and Egeghy September 22-24, 2003). Most participants had Se species above the LOD (96%, 87%, 100%, 98%, 30%, 54% and 99% for Se(IV), Se(VI), Se-SELENOP, Se-Met, Se-Cys, Se-GPX and Se-HSA, respectively). We used linear regression analysis to assess the correlation between log-transformed CSF concentrations and either β-amyloid or p-tau at baseline, excluding form that analysis subjects having values below the LOD. We computed a crude and adjusted odds ratio of Alzheimer’s dementia by using bivariate and multivariable logistic regression analysis, respectively, in the entire study population and in subgroups according to sex, age and APOE ɛ4 status, as well as to a cutpoint of β-amyloid of 557 pg/mL based on the distribution of this biomarker in the AD group and on the literature (Zwan, Rinne et al. 2016). In such analysis, exposure to Se and Se species were considered either as dichotomous (plus/minus the median as computed in referents only) or as continuous variables. In multivariable analysis, we adjusted for potential factors hypothesized or known to be associated with exposure and/or to the outcome, such as age, sex, education (years) and length (years) of storage of the cerebrospinal fluid sample. We run an additional multivariable analysis by adding to the model β-amyloid and p-tau, in order to test the association between Se and AD independently from changes in these predictive & etiologic biomarkers.

**Results**

Table 1 summarizes the characteristics of our case (AD) and referent (MCI) participants at diagnosis, and the concentrations of Se, Se species, and biomarkers of amyloidosis and neurodegeneration in cerebrospinal fluid. Some characteristics differed among the two populations, namely age, education and APOE ɛ4 status, and all these factors were controlled for in the analysis. Concerning biomarkers, MCI subjects showed higher levels of overall Se, inorganic Se and HSA-Se, while there was limited difference concerning summed organic Se species between the two groups. However, when looking at the single organic selenium compounds, Se-SELENOP levels were higher and Se-Met and Se-GPX concentrations were lower in MCI patients compared with controls. Levels of β-amyloid were lower in AD patients compared with MCI subjects, while the opposite was true for t-tau and p-tau. Results were substantially consistent in subgroup analyses according to sex and age group (Supplemental Table 1 and 2). When results in AD patients were compared to each subgroup of referents, results were comparable to those obtained when dealing with the entire referent population, though MCI participants with lower β-amyloid levels had higher levels of overall Se, inorganic hexavalent Se (Se(VI)), Se-HSA and the markers of neurodegeneration t-tau and p-tau, while levels of organic Se and of the two major constituents of this category, SELENOP and Se-Met, were lower (Table 1).

In multiple regression analysis (Table 2), there was little evidence of any association of β-amyloid with Se species, with the exception of a positive relation with Se-SELENOP and particularly Se-Met and Se-Cys in MCI participants, while in AD patients there was only a slighter association with Se-Met. Little evidence of an association between Se species and p-tau emerged, with the exception of a slight association of organic Se and particularly Se-SELENOP and (less precisely) Se-Met in AD subjects. Overall Se was very slightly and positively associated with β-amyloid and p-tau in the AD group, as was with p-tau in MCI subjects.

Odds ratios (OR) for AD according to median of overall Se and the single Se species levels are reported in Table 3, using as cutpoint the median level computed for the corresponding MCI population, i.e. all referents in the two subgroups identified according to the β-amyloid value. Both crude estimates and those adjusted for sex, age years of sample storage and education are reported. OR associated with overall Se were less than 0.5 in both crude (0.43, 95% CI 0.18-1.08) and adjusted (0.46, 95% CI 0.17-1.22) analysis, and they further decreased for inorganic Se, due to both low OR for tetravalent Se (Se(IV) and hexavalent Se (Se(VI)). OR for organic Se were close to the unit (adjusted OR 0.96, 95% CI 0.33-2.77), but this was due to opposite patterns for Se-SELENOP (0.44, 95% 0.15-1.28) and for the remaining organic forms Se-Met, Se-Cys and Se-GPX, characterized by very high ORs. OR for Se-HSA was 0.31 in the adjusted analysis (95% CI 0.11-0.87). These estimates were substantially confirmed when the referent population was limited to participants with higher β-amyloid cerebrospinal fluid concentrations, less likely to be affected by subclinical Alzheimer’s disease compared with MCI participants with lower β-amyloid levels, except for OR for overall organic Se (well above the unity) and for Se-SELENOP, which were not lower but above the unity. After stratifying the analysis according to APOE ε4 status, effect estimates were more statistically unstable due to the limited number of subjects in each category (Supplemental Table 3). Results for the overall Se were not substantially different, while OR for inorganic Se was much lower in APOE ε4 carriers, particularly in the adjusted analysis. In such analysis and in the latter subgroup, OR for Se-Met, Se-Cys and Se-GPX were much increased compared with non APOE ε4 carriers, and this also increased the overall OR for organic Se. The OR associated with Se-HSA was also higher in the APOE ε4 carriers.

Adjusted ORs of AD for 1-unit continuous increase in exposure to Se and Se species are reported in Table 4, also taking into account the two subgroups of referents defined according to β-amyloid values, and the APOE ε4 carrier status. Results showed a substantially comparable pattern to the aforementioned estimates based on dichotomous exposure categories based on the median value, showing a high consistency between these two analyses. The only differences according to referent subgroup was the higher OR associated to Se-Cys when MCI subjects in the lower β-amyloid category were considered as referents. Further breakdown of study population according to sex or age group and based on continuous Se levels (Supplemental Tables 4 and 5) yielded little evidence of substantial differences, with some exceptions. Women had lower ORs for overall and inorganic Se, and higher OR for organic Se compared with men, and differences across sexes were even increased when single Se species were considered (such as Se(IV), Se-SELENOP, Se-Cys and Se-GPX). Older subjects had higher ORs for AD compared with younger participants for most Se categories and species, and this was particularly true for organic Se. Comparable results were obtained when OR calculations were based on dichotomous exposure categories, i.e. above or below the median level of selenium and of the single selenium species.

We eventually performed a calculation of the ORs for AD performing an additional adjustment in the multivariable analysis, i.e. for β-amyloid and p-tau alongside with sex, age, storage time and education (Table 5). Results of such most adjusted model were substantially the same than the those computed without this additional adjustment and reported in Table 3, except for a considerably higher OR for organic Se (1.94, 95% CI 0.51-7.41) driven by very high ORs for Se-Met, Se-Cys and Se-GPX (but not Se-SELENOP).

**Discussion**

We found that a case-control approach to assess the relation between Se status in the central nervous system and AD risk, including participants with established AD and referents with MCI, showed an inverse association between overall Se exposure and the disease. This was also true when exposure assessment was limited to inorganic Se, while risk positively correlated with exposure to some Se species, most of which bound to selenoproteins. These results were markedly different and even opposite to those generated by a longitudinal study carried out in a part of this study population, since the follow-up of the referent group (MCI participants) we found a positive relation between baseline levels of inorganic hexavalent Se and subsequent dementia occurrence, and no relation for the other Se species (Vinceti, Chiari et al. 2017). Therefore, the comparative assessment of these results show the potential for bias of case-control study assessing Se status at the time of the study, despite the use of a central nervous system indicator and a comprehensive analysis of all Se chemical forms, and independently of the likelihood of AD-related pathological changes in the referent population we used.

These findings indicate that progression to the AD modifies the levels of Se species in cerebrospinal fluid, and that these changes markedly influence the assessed of selenium-related relative risk, incorrectly indicating an inverse association between exposure and AD. Assuming therefore that case-control study design generates misleading results on the relation between Se and AD, we may hypothesize that previous case-control studies on this issue, independently from the referent population used and from the biomarker used (blood, urine and cerebrospinal fluid Se levels) or diet itself, most likely suffered from this issue of reverse causation. Such bias may have therefore incorrectly suggested Se deficiency as underpinning disease status, based on the lower levels of overall Se detected in AD patients, a hypothesis which in turn generated interest in Se supplementation to prevent AD (Pitts, Byrns et al. 2014, Cardoso, Roberts et al. 2015, Solovyev 2015). A longitudinal study design appears to be therefore needed to investigate AD etiology as related to exposure to Se and its species.

Our findings also appear to confirm the findings and the hypothesis generated by the two studies investigating selenoprotein P levels in cerebrospinal fluid and post-mortem tissues from AD patients (Bellinger, He et al. 2008, Rueli, Parubrub et al. 2015). Rueli et al. who found elevated selenoprotein P levels in choroid plexus and cerebrospinal fluid of AD patients compared with controls (Rueli, Parubrub et al. 2015), consistently with previous results by Bellinger et al. obtained in post-mortem specimens of brain cortex (Bellinger, He et al. 2008). The authors suggested that those findings could reflect a compensatory response to oxidative stress characterizing dementia progression through the upregulation of Se-containing and non-Se-containing antioxidant enzymes (Bellinger, He et al. 2008, Rueli, Parubrub et al. 2015), though increased levels of selenoproteins found in AD patients might alternatively be associated with harmful effects *per se* (Bellinger, He et al. 2008), as suggested in other diseases (Hatfield, Tsuji et al. 2014, Mita, Nakayama et al. 2017, Peters, Carlson et al. 2018, Vinceti, Filippini et al. 2018, Vinceti, Filippini et al. 2018). Bellinger et al. and Rueli et al. therefore concluded that the increased selenoprotein P levels found in cases could reflect a upregulation of this antioxidant enzyme accompanying disease progression (Bellinger, He et al. 2008, Rueli, Parubrub et al. 2015). Accordingly, in our study the high ORs associated with some organic Se species cannot be interpreted as indicating an excess AD risk associated with these forms but appear to be a reverse-causation effect, also since no change in organic Se levels at baseline in our MCI participants who later progressed to AD could be detected in the cohort study (Vinceti, Chiari et al. 2017). The increased organic Se levels we observed in AD patients was likely a consequence of the oxidative stress accompanying (and possibly favoring) dementia progression and leading to selenoprotein upregulation. Consistently with these findings and hypotheses, increased selenoprotein activity or Se levels in blood or cerebrospinal fluid in AD patients have been reported in other studies (Anneren, Gardner et al. 1986, Perrin, Briancon et al. 1990, Basun, Forssell et al. 1991, Ceballos-Picot, Merad-Boudia et al. 1996, Meseguer, Molina et al. 1999, Martin-Aragon, Bermejo-Bescos et al. 2009, Krishnan and Rani 2014, Ramos, Santos et al. 2015, Rueli, Parubrub et al. 2015), while other studies yielded different results (Loef, Schrauzer et al. 2011, Reddy, Bukke et al. 2017). In addition, in post-mortem brain samples of participants to the Chicago Memory and Aging Project a positive association between Se brain levels and neurofibrillary tangle severity, one of the neuropthological fingerprints of Alzheimer disease, has also emerged (Morris, Brockman et al. 2016). In this study, the higher Se content may be a consequence of disease progression too, or alternatively it may have an etiologic relevance. Accordingly, Bellinger te al. found evidence for an association between immunoreactivity to selenoprotein P and intraneuronal neurofibrillary tangles, and for co-localization of amyloid-beta protein and selenoprotein P, in post-mortem brain tissues from patients with Alzheimer’s disease (Bellinger, He et al. 2008).

Our results highlight the relevance of Se speciation in addressing the relation between Se and AD (Vinceti, Chiari et al. 2017). Growing evidence has been provided to show how speciation may influence the relation of exposure to heavy metals and other trace elements, Se in particular, with neurodegenerative disease. (Michalke, Halbach et al. 2009, Michalke, Willkommena et al. 2018) Se species have different toxicological an nutritional activities, due to their relevant differences in biological reactivity and function (Fairweather-Tait, Collings et al. 2010, Weekley and Harris 2013, Jablonska and Vinceti 2015, Vinceti, Grill et al. 2015), still far from being entirely elucidated but under active investigation (Solovyev, Berthele et al. 2013, Vinceti, Mandrioli et al. 2014, Oliveira, Piccoli et al. 2017, Solovyev, Vinceti et al. 2017, Michalke, Willkommena et al. 2018). The present study is the first case-control investigation, to the best of our knowledge, investigating the specific relation between all the full spectrum of Se species and AD risk. The relevance of Se speciation when investigating the involvement of Se in neurodegenerative disease etiology and progression has been recently highlighted (Vinceti, Solovyev et al. 2013, Mandrioli, Michalke et al. 2017, Vinceti, Chiari et al. 2017, Maass and Lingor 2018, Maass, Michalke et al. 2018, Michalke, Willkommena et al. 2018). Interestingly, however, none of the Se species escaped the risk of bias due to reverse causation in our population, since the ORs in the present case-control study markedly differ from those generated by our cohort investigation for almost all inorganic and organic species, and for overall Se as well (Vinceti, Chiari et al. 2017).

Our study was based on a central nervous system indicator of Se exposure, cerebrospinal fluid levels, and not of peripheral biomarkers such as selenium concentrations serum, plasma, urine or nail selenium levels, or an assessment of its dietary intake. We used cerebrospinal fluid levels since there is evidence of complex regulatory systems in exchange of selenium between blood and central nervous system, and for some Se species, namely the inorganic ones, no correlation may exist between these two compartments (Schweizer, Streckfuss et al. 2005, Scharpf, Schweizer et al. 2007, Zhang, Zhou et al. 2008, Solovyev, Berthele et al. 2013, Burk, Hill et al. 2014, Michalke, Solovyev et al. 2017), possibly due to the specific features of transfer and metabolism of Se species across the blood-brain barrier, and the relative independence of their central nervous system levels (Schweizer, Streckfuss et al. 2005, Scharpf, Schweizer et al. 2007, Zhang, Zhou et al. 2008, Solovyev, Berthele et al. 2013, Burk, Hill et al. 2014, Michalke, Solovyev et al. 2017). Therefore, the use of a target tissue when addressing the neurological effects of Se may allow to avoid a serious exposure misclassification arising from the use of circulating Se levels as a proxy of brain Se content. The inherent limitations in using peripheral biomarkers of exposure have been extensively addressed (Ashton, Hooper et al. 2009, Fairweather-Tait, Bao et al. 2011, Jablonska and Vinceti 2015, Vinceti, Filippini et al. 2017, Vinceti, Filippini et al. 2018), and unfortunately no indicator reflecting the very long-term exposure to Se, and in addition exposure to specific Se species, has been so far identified. Three case-control studies have used cerebrospinal fluid levels to assess the relation between selenium exposure and AD, generally finding in patients lower levels compared with controls (Meseguer, Molina et al. 1999, Gerhardsson, Blennow et al. 2009, Cardoso, Hare et al. 2017), consistently with our findings.

We used in our study, as referents, MCI participants instead of ‘healthy subjects’, mainly for ethical reasons, since in these subjects lumbar puncture may be part of the standard diagnostic process and therefore cerebrospinal specimens become available. The possibility that this referent population may not be adequate in reflecting ‘control’ levels of Se species in cerebrospinal fluid must be considered. For this reason, we also restricted our assessment by using only a part of the referent group, the one in the highest category of cerebrospinal fluid β-amyloid, for which subclinical Alzheimer’s disease was less likely to have occurred. Lack of substantial changes in AD relative risk estimates indicated that a bias due to an incorrect choice of the control group unlikely occurred.

The reasons underlying the lower Se status found in our AD patients compared with referents are difficult to evaluate. We speculate that an impairment of nutritional status, as frequently occurring even subtly in AD patients (Cardoso, Ong et al. 2010), may have been the source of a decreased intake and therefore of the lower overall Se status we detected. In addition, progression the disease status may affect Se delivery to the brain, and/or impair its transport, excretion and utilization, and these changes may unevenly affect Se species. Findings form case-control studies recruiting subjects defined as ‘healthy’ and carriers of subjective memory complaints, mild cognitive impairment, and AD found decreasing blood Se levels across these subgroups of increasing disease severity (Cardoso, Silva Bandeira et al. 2014, Olde Rikkert, Verhey et al. 2014, Paglia, Miedico et al. 2016, Vaz, Fermino et al. 2017), suggesting impairment in nutrient status already at early disease stages (Olde Rikkert, Verhey et al. 2014, Lee, Thomas et al. 2015). On the converse, as previously mentioned increased selenoprotein levels in blood, brain and cerebrospinal fluid have been detected in some studies carried out in established AD (Loef, Schrauzer et al. 2011, Rueli, Parubrub et al. 2015, Vinceti, Chiari et al. 2017), possibly due to oxidative stress-driven upregulation of antioxidant enzymes including selenoproteins, determining higher levels of organic-bound Se and even overall Se (Bellinger, He et al. 2008, Vinceti, Maraldi et al. 2009, Jablonska and Vinceti 2015, Rueli, Parubrub et al. 2015). Overall, it is therefore possible that AD risk associated with Se species may be biased in opposite directions, depending on the balance between impaired Se intake or metabolism and selenoprotein upregulation.

In our study, we found that OR for AD were not substantially modified for overall Se and inorganic Se by an additional adjustment for biomarkers of amyloidosis and neurodegeneration, i.e. β-amyloid and p-tau, two parameters we did not account for in the main analysis since they could be intermediate factors in any relation between selenium and dementia onset. Conversely, the OR associated with organic Se was increased compared to that found in the less adjusted analysis. This further suggest that Alzheimer’s disease is associated, even at the same levels of biomarkers of neurodegeneration, with an upregulation of selenoproteins in the central nervous system, likely as a consequence of increased oxidative stress (Calabrese, Sultana et al. 2006, Pohanka 2014)\(Nesi, Sestito et al. 2017). We also observed some differences in the ORs associated with some Se species according to such as sex, age and APOE ε4 carriership (a factor potentially interacting with or influencing Se status (Gao, Jin et al. 2009, Cardoso, Hare et al. 2017)). Among these, we found a high OR associated with organic Se in the oldest subjects, opposite to what we detected in the youngest participants.

Some limitations may well have affected the results of the present study. First, study size was rather small, due to both the limited number of MCI and AD patients needed cerebrospinal fluid sampling for clinical purposes and the analytical complexity of Se speciation analyses. This clearly increased the statistical imprecision of our effect estimates, as reflected by their confidence intervals (Rothman and Greenland 2018). Another limitation is inherent in the nonexperimental nature of our study, the possibility of unmeasured confounding, due to other chemicals of nutritional and/or toxicological importance covariating with Se species. However, experimental studies encompassing Se administration are likely to be impossible for ethical reasons, due to the serious adverse effects emerged in trials encompassing selective administration of organic or inorganic Se species (Vinceti, Filippini et al. 2017, Vinceti, Filippini et al. 2018, Vinceti, Filippini et al. 2018, Yarmolinsky, Bonilla et al. 2018). Therefore, only nonexperimental cohort studies may offer future opportunities to further investigate this issue, attempting to control for potential confounders. Alternatively, secondary analyses of Se trials such as that recently published for SELECT (Kryscio, Abner et al. 2017) may be of strong interest to test the effects of single Se chemical forms (or sources, such as in the case of selenized yeast) in AD etiology. While the association of Se status with the etiology of chronic disease such as cardiovascular disease, cancer and diabetes has been substantially elucidated also owing to the large number of studies including randomized trials and the consistency of the results (Rees, Hartley et al. 2013, Vinceti, Filippini et al. 2018, Vinceti, Filippini et al. 2018), its relation with neurodegenerative disease including AD still needs to be elucidated, based on recent experimental and nonexperimental human studies and by laboratory investigations.

**List of abbreviations**

AD: Alzheimer’s dementia

CI: confidence interval

CSF: cerebrospinal fluid

FTD: frontotemporal dementia

HR: hazard ratio

IQR: interquartile range

LBD: Lewy body disease

LOD: Limit of detection

MCI: mild cognitive impairment

Se(IV): selenite

Se(VI): selenate

Se-SELENOP: selenoprotein P-bound Se

Se-Met: selenomethionine-bound Se

Se-Cys: selenocysteine-bound Se

Se-GPX: glutathione-peroxidase-bound Se

Se-TXNRD: thioredoxin reductase-bound Se

Se-HSA: human serum albumin selenium-bound Se

**Declarations**

**Competing interests**

The authors declare no conflict of interest.

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Figure 1. Flowchart of the case-control study on selenium species in cerebrospinal fluid of patients with Alzheimer’s dementia (AD) and with mild cognitive impairment (MCI).

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**Table 1.** Characteristics of study population and distribution of levels of Se species (as μg Se/L CSF) and of β-amyloid, total (t-tau) and phosphorylated (p-tau) tau proteins (pg/mL) in cerebrospinal fluid of the study population

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | AD (N=33) |  | MCI (N=56) |  | MCI with amyloid > 557 (N=29) |  | MCI with amyloid < 557 (N=27) |
|  | N | (%) |  | N | (% |  | N | (% |  | N | (% |
| Sex |  |  |  |  |  |  |  |  |  |  |  |
| Males | 16 | (48.5) |  | 30 | (53.6) |  | 16 | (55.2) |  | 14 | (51.9) |
| Females | 17 | (51.5) |  | 26 | (46.4) |  | 13 | (44.8) |  | 13 | (48.1) |
| Age at entry |  |  |  |  |  |  |  |  |  |  |  |
| <65 years | 21 | (63.6) |  | 24 | (42.9) |  | 14 | (48.3) |  | 10 | (37.0) |
| ≥65 years | 12 | (36.4) |  | 32 | (57.1) |  | 15 | (51.7) |  | 17 | (63.0) |
| Education |  |  |  |  |  |  |  |  |  |  |  |
| <8 years | 6 | (18.2) |  | 18 | (32.1) |  | 11 | (37.9) |  | 7 | (26.0) |
| 8-12 years | 12 | (36.4) |  | 16 | (28.6) |  | 6 | (20.7) |  | 10 | (37.0) |
| ≥13 years | 15 | (45.4) |  | 22 | (39.3) |  | 12 | (41.4) |  | 10 | (37.0) |
| APOE ɛ4  |  |  |  |  |  |  |  |  |  |  |  |
| Non-carriers | 11 | (33.3) |  | 21 | (37.5) |  | 16 | (55.2) |  | 5 | (18.5) |
| Carriers | 14 | (42.4) |  | 18 | (32.1) |  | 6 | (20.7) |  | 12 | (44.4) |
| Missing | 8 | (24.3) |  | 17 | (30.4) |  | 7 | (24.1) |  | 10 | (37.1) |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | AD (N=33) |  | MCI (N=56) |  | MCI with amyloid > 557 (N=29) |  | MCI with amyloid < 557 (N=27) |
|  | 50th | (IQR) |  | 50th | (IQR) |  | 50th | (IQR) |  | 50th | (IQR) |
| Total Se | 3.68 | (2.91 - 4.31) |  | 4.17 | (3.17 - 4.62) |  | 4.16 | (3.73 - 4.51) |  | 4.40 | (3.62 - 5.08) |
| Inorganic Se | 0.44 | (0.34 - 0.60) |  | 0.64 | (0.46 - 0.77 ) |  | 0.63 | (0.48 - 0.73) |  | 0.69 | (0.46 - 0.91) |
| Se(IV) | 0.34 | (0.24 - 0.42) |  | 0.41 | (0.32 - 0.63) |  | 0.41 | (0.33 - 0.55) |  | 0.45 | (0.30 - 0.66) |
| Se(VI) | 0.12 | (0.06 - 0.23) |  | 0.14 | (0.09 - 0.32 ) |  | 0.12 | (0.09 - 0.26) |  | 0.20 | (0.11 - 0.40) |
| Organic Se | 1.84 | (1.19 - 2.25) |  | 1.82 | (1.20 - 2.29) |  | 1.84 | (1.37 - 2.40) |  | 1.60 | (1.03 - 2.18) |
| Se-SELENOP | 1.52 | (0.84 - 1.91) |  | 1.58 | (1.07 - 2.01) |  | 1.63 | (1.16 - 2.04) |  | 1.45 | (0.94 - 1.87) |
| Se-Met | 0.18 | (0.10 - 0.23) |  | 0.14 | (0.08 - 0.22) |  | 0.17 | (0.10 - 0.23) |  | 0.12 | (0.06 - 0.16) |
| Se-Cys | 0.01 | (0.01 - 0.08) |  | 0.01 | (0.01 - 0.01) |  | 0.01 | (0.01 - 0.01) |  | 0.01 | (0.01 - 0.01) |
| Se-GPX | 0.05 | (0.01 - 0.12) |  | 0.01 | (0.01 - 0.08) |  | 0.01 | (0.01 - 0.09) |  | 0.01 | (0.01 - 0.06) |
| Se-HSA  | 1.26 | (0.86 - 1.52) |  | 1.54 | (1.13 - 1.83) |  | 1.53 | (1.08 - 1.80) |  | 1.60 | (1.16 - 1.97) |
| Unknown | 0.12 | (0.04 - 0.36) |  | 0.25 | (0.14 - 0.39) |  | 0.28 | (0.12 - 0.38) |  | 0.24 | (0.15 - 0.43) |
| β-amyloid | 452 | (385 - 499) |  | 596 | (449 - 798) |  | 789 | (691 - 1012) |  | 441 | (370 - 509) |
| t-tau | 597 | (440 - 791) |  | 372 | (220 - 619) |  | 255 | (198 - 374) |  | 511 | (304 - 769) |
| p-tau | 96 | (77 - 118) |  | 69 | (50 - 88) |  | 56 | (48 - 80) |  | 86 | (62 - 128) |

Abbreviations: MCI, mild cognitive impairment; IQR, interquartile range; AD, Alzheimer’s disease; Se(IV), selenite; Se(VI), selenate; Se-SelenoP, selenoprotein P-bound Se; Se-Met, selenomethionine-bound Se; Se-Cys, selenocysteine-bound Se; Se-GPX, glutathione-peroxidase-bound Se; Se-HSA, human serum albumin selenium-bound Se.

**Table 2.** Linear regression analysis of CSF Se species versus log-transformed values of biomarkers of AD pathology (β amyloid and phosphorylated (p-tau) tau protein as dependent variables) in the 33 AD and the 56 MCI study participants. Adjusted for sex, age at entry, years of storage

|  |  |  |  |
| --- | --- | --- | --- |
|  | 33 AD study participants |  | 56 MCI study participants |
| Se species | β | 95% CI | P |  | β | 95% CI | P |
| *β-amyloid* |  |  |  |  |  |  |  |
| Total Se | 0.08 | (-0.02 to 0.17) | 0.125 |  | 0.03 | (-0.10 to 0.15) | 0.683 |
| Inorganic Se | 0.03 | (-0.32 to 0.37) | 0.881 |  | -0.25 | (-0.65 to 0.15) | 0.217 |
| Se(IV) | -0.00 | (-0.64 to 0.64) | 0.999 |  | -0.06 | (-0.72 to 0.61) | 0.865 |
| Se(VI) | 0.13 | (-0.60 to 0.86) | 0.716 |  | -0.80 | (-1.72 to 0.13) | 0.089 |
| Organic Se | 0.09 | (-0.06 to 0.24) | 0.218 |  | 0.176 | (0.01 to 0.34) | 0.034 |
| Se-SELENOP | 0.11 | (-0.05 to 0.27) | 0.167 |  | 0.17 | (-0.01 to 0.35) | 0.070 |
| Se-Met | 0.59 | (-0.19 to 1.37) | 0.135 |  | 2.31 | (0.84 to 3.78) | 0.003 |
| Se-Cys | 0.34 | (-2.18 to 2.87) | 0.768 |  | 2.94 | (-0.75 to 6.63) | 0.101 |
| Se-GPX | -0.00 | (-0.39 to 0.39) | 0.988 |  | 0.39 | (-1.62 to 2.39) | 0.692 |
| Se-HSA | 0.08 | (-0.10 to 0.26) | 0.364 |  | 0.013 | (-0.22 to 0.24) | 0.910 |
| Unknown | 0.13 | (-0.29 to 0.56) | 0.526 |  | -0.11 | (-0.66 to 0.45) | 0.698 |
| *P-tau* |  |  |  |  |  |  |  |
| Total Se | 0.11 | (-0.08 to 0.29) | 0.239 |  | 0.09 | (-0.04 to 0.23) | 0.175 |
| Inorganic Se | -0.05 | (-0.69 to 0.60) | 0.885 |  | -0.08 | (-0.52 to 0.35) | 0.701 |
| Se(IV) | -0.45 | (-1.63 to 0.72) | 0.437 |  | -0.53 | (-1.23 to 0.16) | 0.129 |
| Se(VI) | 0.28 | (-1.11 to 1.66) | 0.683 |  | 0.24 | (-0.73 to 1.20) | 0.624 |
| Organic Se | 0.28 | (0.01 to 0.54) | 0.041 |  | 0.10 | (-0.08 to 0.28) | 0.278 |
| Se-SELENOP | 0.37 | (0.09 to 0.64) | 0.011 |  | 0.13 | (-0.08 to 0.33) | 0.213 |
| Se-Met | 0.67 | (-0.82 to 2.17) | 0.364 |  | 0.11 | (-1.59 to 1.81) | 0.899 |
| Se-Cys | 0.80 | (-3.70 to 5.31) | 0.699 |  | -2.32 | (-8.62 to 3.98) | 0.413 |
| Se-GPX | -0.08 | (-1.06 to 0.91) | 0.874 |  | -0.116 | (-2.68 to 2.45) | 0.926 |
| Se-HSA | -0.00 | (-0.35 to 0.34) | 0.989 |  | 0.04 | (-0.22 to 0.30) | 0.760 |
| Unknown | -0.16 | (-0.97 to 0.64) | 0.683 |  | 0.74 | (0.18 to 1.30) | 0.011 |

**Table 3**.Crude and adjusteda *odds ratios* (OR) of developing AD according to cerebrospinal fluid Se species levels, according to different referent groups. Selenium exposure status defined as 0 (below or equal) and 1 (above) withreference to the median value in the control (MCI) participants.

Crude analysis

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | All referent |  | Referents with amyloid >557 pg/mL |  | Referents with amyloid <557 pg/mL |
| Se species | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |
| Total Se | 0.43 | (0.18 - 1.08) |  | 0.54 | (0.19 - 1.50) |  | 0.25 | (0.08 - 0.77) |
| Inorganic Se | 0.32 | (0.12 – 0.83) |  | 0.34 | (0.12 - 1.01) |  | 0.25 | (0.08 - 0.77) |
| Se(IV) | 0.38 | (0.15 – 0.95) |  | 0.35 | (0.12 - 1.01) |  | 0.30 | (0.10 - 0.89) |
| Se(VI) | 0.53 | (0.22 – 1.29) |  | 0.88 | (0.32 - 2.38) |  | 0.35 | (0.12 - 1.02) |
| Organic Se | 1.06 | (0.45 - 2.51) |  | 0.99 | (0.37 - 2.69) |  | 1.66 | (0.59 - 4.63) |
| Se-SELENOP | 0.65 | (0.27 - 1.56) |  | 0.53 | (0.19 - 1.47) |  | 1.29 | (0.47 - 3.58) |
| Se-Met | 2.47 | (1.00 – 6.13) |  | 1.12 | (0.41 - 3.04) |  | 2.14 | (0.74 - 6.16) |
| Se-Cys | 3.06 | (1.20 - 7.79) |  | 2.62 | (0.88 - 7.81) |  | 3.67 | (1.12 - 12.03) |
| Se-GPX | 3.56 | (1.40 - 9.02) |  | 3.28 | (1.14 - 9.47) |  | 3.88 | (1.31 - 11.47) |
| Se-HSA | 0.32 | (0.12 - 0.83) |  | 0.30 | (0.10 - 0.88) |  | 0.25 | (0.08 - 0.77) |
| Unknown | 0.57 | (0.24 - 1.38) |  | 0.47 | (0.17 - 1.30) |  | 0.62 | (0.22 - 1.73) |

Adjusted analysis

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Total Se | 0.46 | (0.17 - 1.22) |  | 0.55 | (0.17 - 1.84) |  | 0.23 | (0.06 - 0.81) |
| Inorganic Se | 0.37 | (0.14 – 1.01) |  | 0.36 | (0.11 - 1.16) |  | 0.28 | (0.08 - 0.98) |
| Se(IV) | 0.45 | (0.17 – 1.20) |  | 0.38 | (0.12 - 1.14) |  | 0.37 | (0.11 - 1.23) |
| Se(VI) | 0.64 | (0.25 – 1.62) |  | 0.97 | (0.33 - 2.81) |  | 0.24 | (0.07 - 0.86) |
| Organic Se | 0.96 | (0.33 - 2.77) |  | 0.97 | (0.28 - 3.39) |  | 2.17 | (0.51 - 9.22) |
| Se-SELENOP | 0.44 | (0.15 - 1.28) |  | 0.33 | (0.09 - 1.20) |  | 1.12 | (0.28 - 4.48) |
| Se-Met | 3.15 | (1.15 – 8.63) |  | 1.47 | (0.49 - 4.42) |  | 2.35 | (0.69 - 7.93) |
| Se-Cys | 4.00 | (1.36 – 11.77) |  | 3.58 | (1.03 - 12.46) |  | 5.81 | (1.41 - 23.96) |
| Se-GPX | 4.05 | (1.44 – 11.38) |  | 3.64 | (1.16 - 11.41) |  | 4.53 | (1.30 - 15.74) |
| Se-HSA | 0.31 | (0.11 - 0.87) |  | 0.34 | (0.11 - 1.04) |  | 0.13 | (0.03 - 0.56) |
| Unknown | 0.57 | (0.21 - 1.54) |  | 0.51 | (0.16 - 1.59) |  | 0.62 | (0.19 - 1.98) |

aAdjusted for sex, age at entry, years of storage and years of education

**Table 4**.Adjusteda *odds ratios* (OR) of developing AD according to increasing levels (0.1 mcg/L) of cerebrospinal fluid Se species, according to the different referent populations

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ALL SUBJECT | All referent |  | Referents with amyloid >557 |  | Referents with amyloid <557 |
| Se species | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |
| Total Se | 0.95 | (0.90 - 1.00) |  | 0.95 | (0.89 - 1.02) |  | 0.93 | (0.87 - 0.99) |
| Inorganic Se | 0.85 | (0.72 - 1.01) |  | 0.90 | (0.72 - 1.11) |  | 0.77 | (0.62 - 0.96) |
| Se(IV) | 0.77 | (0.60 - 1.00) |  | 0.78 | (0.56 - 1.07) |  | 0.72 | (0.53 - 0.99) |
| Se(VI) | 0.86 | (0.63 - 1.18) |  | 1.04 | (0.71 - 1.53) |  | 0.65 | (0.43 - 0.96) |
| Organic Se | 0.98 | (0.91 - 1.05) |  | 0.96 | (0.87 - 1.05) |  | 1.00 | (0.91 - 1.09) |
| Se-SELENOP | 0.92 | (0.85 - 1.00) |  | 0.89 | (0.80 - 1.00) |  | 0.93 | (0.84 - 1.03) |
| Se-Met | 1.87 | (1.08 – 3.26) |  | 1.55 | (0.84 – 2.85) |  | 2.33 | (1.09 – 4.97) |
| Se-Cys | 2.18 | (1.08 – 4.41) |  | 1.76 | (0.81 – 3.80) |  | 3.23 | (1.14 – 3.51) |
| Se-GPX | 1.72 | (1.08 – 2.75) |  | 1.72 | (0.97 – 3.03) |  | 1.90 | (1.03 – 3.51) |
| Se-HSA | 0.92 | (0.84 - 1.02) |  | 0.95 | (0.85 - 1.06) |  | 0.86 | (0.75 - 0.99) |
| Unknown | 0.85 | (0.66 - 1.09) |  | 0.89 | (0.66 - 1.18) |  | 0.84 | (0.64 - 1.11) |

aAdjusted for sex, age at entry, years of storage and years of education

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| APOE ɛ4 carriers |  |  |  |  |  |  |  |  |
| Total Se | 0.93 | (0.84 - 1.03) |  | 0.88 | (0.66 - 1.19) |  | 0.93 | (0.84 - 1.03) |
| Inorganic Se | 0.70 | (0.48 - 1.00) |  | 0.02 | (0.00 - 12.81) |  | 0.73 | (0.51 - 1.04) |
| Se(IV) | 0.65 | (0.40 - 1.05) |  | 0.16 | (0.01 - 3.14) |  | 0.69 | (0.43 - 1.09) |
| Se(VI) | 0.66 | (0.38 - 1.13) |  | 0.66 | (0.28 - 1.57) |  | 0.67 | (0.39 - 1.16) |
| Organic Se | 0.99 | (0.88 - 1.11) |  | 1.37 | (0.82 - 2.27) |  | 0.97 | (0.86 - 1.10) |
| Se-SELENOP | 0.92 | (0.79 - 1.07) |  | 1.04 | (0.70 - 1.56) |  | 0.89 | (0.75 - 1.06) |
| Se-Met | 1.95 | (0.75 – 5.09) |  | 1.36 | (0.29 – 6.40) |  | 2.37 | (0.72 – 7.80) |
| Se-Cys | 4.04 | (0.76 – 21.60) |  | 2.42 | (0.27 – 21.96) |  | - | - |
| Se-GPX | 1.54 | (0.84 – 2.81) |  | 1.57 | (0.58 – 4.22) |  | 1.80 | (0.74 – 4.42) |
| Se-HSA | 0.90 | (0.76 - 1.08) |  | 0.80 | (0.51 - 1.25) |  | 0.94 | (0.76 - 1.16) |
| Unknown | 0.83 | (0.55 - 1.25) |  | 1.20 | (0.53 - 2.75) |  | 0.75 | (0.48 - 1.18) |

aAdjusted for sex, age at entry, years of storage and years of education

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| APOE ɛ4 noncarriers |  |  |  |  |  |  |  |
| Total Se | 0.96 | (0.87 - 1.07) |  | 0.94 | (0.84 - 1.06) |  | 1.26 | (0.73 - 2.16) |
| Inorganic Se | 0.87 | (0.64 - 1.20) |  | 0.91 | (0.65 - 1.29) |  | 0.90 | (0.48 - 1.69) |
| Se(IV) | 0.84 | (0.55 - 1.30) |  | 0.87 | (0.53 - 1.38) |  | 1.26 | (0.49 - 3.25) |
| Se(VI) | 0.82 | (0.42 - 1.58) |  | 0.95 | (0.46 - 1.95) |  | 0.30 | (0.03 - 2.65) |
| Organic Se | 0.99 | (0.88 - 1.11) |  | 0.91 | (0.78 - 1.07) |  | 0.92 | (0.59 - 1.44) |
| Se-SELENOP | 0.94 | (0.82 - 1.09) |  | 0.89 | (0.74 - 1.06) |  | 0.78 | (0.43 - 1.42) |
| Se-Met | 0.91 | (0.77 – 1.08) |  | 1.30 | (0.43 – 3.96) |  | - | - |
| Se-Cys | 1.75 | (0.59 – 5.44) |  | 1.70 | (0.55 – 5.28) |  | 3.31 | (0.01 – too high) |
| Se-GPX | 0.70 | (0.14 – 3.39) |  | 0.67 | (0.14 – 3.18) |  | 0.18 | (0.00 – 80.99) |
| Se-HSA | 1.04 | (0.90 - 1.22) |  | 1.04 | (0.88 - 1.22) |  | 1.32 | (0.64 - 2.70) |
| Unknown | 0.58 | (0.29 - 1.14) |  | 0.48 | (0.21 - 1.08) |  | - | - |

aAdjusted for sex, age at entry, years of storage and years of education

**Table 5**.Adjusteda *odds ratios* (OR) of developing AD according selenium exposure above the median

value in the MCI participants (subjects below the median composing the referent category).

|  |  |  |
| --- | --- | --- |
|  | Selenium exposure status defined as 0 (below or equal) and 1 (above) withreference to the median value |  |
| Se species | OR | 95% CI |  |
| Total Se | 0.44 | 0.14 - 1.43 |  |
| Inorganic Se | 0.31 | 0.09 - 1.01 |  |
| Se(IV) | 0.46 | 0.14 - 1.48 |  |
| Se(VI) | 0.39 | 0.12 - 1.22 |  |
| Organic Se | 1.94 | 0.51 - 7.41 |  |
| Se-SELENOP | 0.72 | 0.19 - 2.78 |  |
| Se-Met | 5.55 | 1.56 - 19.78 |  |
| Se-Cys | 7.41 | 1.83 - 30.09 |  |
| Se-GPX | 3.88 | 1.15 - 13.06 |  |
| Se-HSA | 0.26 | 0.07 - 0.92 |  |
| Unknown | 0.81 | 0.25 - 2.62 |  |

aAdjusted for sex, age at entry, years of storage and years of education,

β-amyloid and p-tau

**Supplemental Table 1.** Distribution of levels of Se species (μg/L) and of β-amyloid, total (t-tau) and phosphorylated (p-tau) tau proteins (pg/mL) in cerebrospinal fluid of study population by sex

|  |  |  |  |
| --- | --- | --- | --- |
|  | Men (N=46) |  | Women (N=43) |
|  | MCI (N=30) |  | AD (N=16) |  | MCI (N=26) |  | AD (N=17) |
|  | 50th | IQR |  | 50th | IQR |  | 50th | IQR |  | 50th | IQR |
| Total Se | 4.20 | 3.76 - 4.87 |  | 4.16 | 3.18 - 4.41 |  | 3.97 | 3.57 - 4.51 |  | 3.46 | 2.86 - 3.98 |
| Inorganic Se | 0.60 | 0.41 - 0.77 |  | 0.47 | 0.35 - 0.90 |  | 0.67 | 0.53 - 0.79 |  | 0.44 | 0.31 - 0.55 |
| Se(IV) | 0.39 | 0.30 - 0.54 |  | 0.36 | 0.24 - 0.48 |  | 0.48 | 0.39 - 0.66 |  | 0.33 | 0.21 - 0.36 |
| Se(VI) | 0.16 | 0.11 - 0.31 |  | 0.12 | 0.04 - 0.36 |  | 0.14 | 0.06 - 0.32 |  | 0.10 | 0.07 - 0.19 |
| Organic Se | 1.92 | 1.21 - 2.40 |  | 1.87 | 1.63 - 2.31 |  | 1.61 | 1.06 - 2.04 |  | 1.30 | 1.11 - 2.12 |
| Se-SELENOP | 1.68 | 1.14 - 2.07 |  | 1.55 | 1.16 - 1.82 |  | 1.33 | 0.86 - 1.80 |  | 0.93 | 0.83 - 1.91 |
| Se-Met | 0.16 | 0.09 - 0.23 |  | 0.18 | 0.11 - 0.22 |  | 0.13 | 0.06 - 0.18 |  | 0.17 | 0.10 - 0.26 |
| Se-Cys | 0.01 | 0.01 - 0.01 |  | 0.01 | 0.01 - 0.12 |  | 0.01 | 0.01 - 0.01 |  | 0.01 | 0.01 - 0.07 |
| Se-GPX | 0.03 | 0.01 - 0.10 |  | 0.11 | 0.01 - 0.28 |  | 0.01 | 0.01 - 0.06 |  | 0.04 | 0.02 - 0.06 |
| Se-HSA  | 1.62 | 1.16 - 1.81 |  | 1.21 | 1.05 - 1.48 |  | 1.42 | 1.06 - 1.89 |  | 1.26 | 0.80 - 1.52 |
| Unknown species | 0.29 | 0.17 - 0.41 |  | 0.12 | 0.07 - 0.45 |  | 0.20 | 0.12 - 0.38 |  | 0.12 | 0.04 - 0.31 |
| β-amyloid | 612 | 462 - 806 |  | 435 | 354 - 473 |  | 551 | 417 - 789 |  | 463 | 410 - 507 |
| t-tau | 296 | 219 - 511 |  | 668 | 442 - 847 |  | 415 | 221 - 697 |  | 570 | 433 - 791 |
| p-tau | 59 | 49 - 83 |  | 99 | 82 - 122 |  | 85 | 50 - 111 |  | 92 | 70 - 105 |

**Supplemental Table 2.** Distribution of levels of Se species (μg/L) and of β-amyloid, total (t-tau) and phosphorylated (p-tau) tau proteins (pg/mL) in cerebrospinal fluid of study population by age

|  |  |  |  |
| --- | --- | --- | --- |
|  | <65 years (N=45) |  | ≥65 years (N=44) |
|  | MCI (N=24) |  | AD (N=21) |  | MCI (N=32) |  | AD (N=12) |
|  | 50th | IQR |  | 50th | IQR |  | 50th | IQR |  | 50th | IQR |
| Total Se | 4.35 | 3.75 - 4.85 |  | 3.41 | 2.90 - 4.16 |  | 4.07 | 3.68 - 4.48 |  | 4.16 | 3.33 - 4.43 |
| Inorganic Se | 0.65 | 0.46 - 0.79 |  | 0.44 | 0.31 - 0.56 |  | 0.63 | 0.46 - 0.74 |  | 0.47 | 0.38 - 0.75 |
| Se(IV) | 0.46 | 0.35 - 0.64 |  | 0.33 | 0.21 - 0.42 |  | 0.41 | 0.31 - 0.63 |  | 0.35 | 0.31 - 0.48 |
| Se(VI) | 0.12 | 0.07 - 0.37 |  | 0.12 | 0.05 - 0.23 |  | 0.18 | 0.11 - 0.28 |  | 0.11 | 0.07 - 0.23 |
| Organic Se | 1.87 | 1.39 - 2.20 |  | 1.57 | 1.18 - 2.24 |  | 1.74 | 1.18 - 2.35 |  | 2.01 | 1.78 - 2.31 |
| Se-SELENOP | 1.63 | 1.17 - 1.96 |  | 1.12 | 0.84 - 2.06 |  | 1.52 | 0.95 - 2.04 |  | 1.58 | 1.03 - 1.81 |
| Se-Met | 0.12 | 0.08 - 0.18 |  | 0.17 | 0.11 - 0.23 |  | 0.15 | 0.07 - 0.22 |  | 0.20 | 0.09 - 0.30 |
| Se-Cys | 0.01 | 0.01 - 0.08 |  | 0.01 | 0.01 - 0.07 |  | 0.01 | 0.01 - 0.01 |  | 0.05 | 0.01 - 0.12 |
| Se-GPX | 0.01 | 0.01 - 0.09 |  | 0.04 | 0.01 - 0.07 |  | 0.01 | 0.01 - 0.07 |  | 0.11 | 0.02 - 0.29 |
| Se-HSA  | 1.60 | 1.15 - 1.86 |  | 1.07 | 0.83 - 1.60 |  | 1.53 | 1.09 - 1.79 |  | 1.39 | 1.11 - 1.52 |
| Unknown | 0.29 | 0.15 - 0.43 |  | 0.12 | 0.04 - 0.39 |  | 0.24 | 0.14 - 0.37 |  | 0.11 | 0.05 - 0.35 |
| β-amyloid | 667 | 507 - 797 |  | 452 | 386 - 507 |  | 526 | 427 - 790 |  | 446 | 355 - 497 |
| t-tau | 282 | 166 - 543 |  | 570 | 527 - 782 |  | 394 | 255 - 651 |  | 614 | 437 - 852 |
| p-tau | 67 | 45 - 100 |  | 95 | 77 - 130 |  | 69 | 55 - 85 |  | 98 | 78 - 113 |

**Supplemental Table 3**.Crude and adjusted *odds ratios* (OR) of developing AD according to Se species cerebrospinal fluid levels by APOE ɛ4 carriership status. Selenium exposure status defined as 0 (below or equal) and 1 (above) withreference to the median value.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Crude |  | Adjusteda |
|  | APOE ɛ4 non-carriers(N=32) |  | APOE ɛ4 carriers(N=32) |  | APOE ɛ4 non-carriers(N=32) |  | APOE ɛ4 carriers(N=32) |
| Se species | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |
| Total Se | 0.50 | (0.10 - 2.44) |  | 0.44 | (0.11 - 1.87) |  | 0.63 | (0.12 – 3.43) |  | 0.52 | (0.10 – 2.77) |
| Inorganic Se | 0.41 | (0.09 - 2.00) |  | 0.13 | (0.02 - 0.78) |  | 0.60 | (0.11 - 3.38) |  | 0.10 | (0.01 - 0.81) |
| Se(IV) | 0.63 | (0.14 - 2.81) |  | 0.26 | (0.04 - 1.54) |  | 1.02 | (0.19 - 5.48) |  | 0.21 | (0.03 - 1.62) |
| Se(VI) | 0.41 | (0.09 - 2.00) |  | 0.48 | (0.12 - 1.98) |  | 0.59 | (0.11 - 3.25) |  | 0.57 | (0.11 - 3.08) |
| Organic Se | 0.63 | (0.14 - 2.71) |  | 1.07 | (0.26 - 4.36) |  | 0.41 | (0.07 - 2.52) |  | 0.76 | (0.10 - 5.96) |
| Se-SELENOP | 0.63 | (0.14 - 2.71) |  | 0.75 | (0.18 - 3.06) |  | 0.41 | (0.07 - 2.52) |  | 0.35 | (0.05 - 2.43) |
| Se-Met | 2.00 | (0.41 - 9.74) |  | 3.12 | (0.71 - 13.81) |  | 2.41 | (0.42 - 13.81) |  | 5.74 | (0.82 - 40.14) |
| Se-Cys | 2.43 | (0.47 - 12.54) |  | 6.00 | (0.98 - 36.71) |  | 2.49 | (0.39 - 15.85) |  | 237.10 | (2.50 - to high) |
| Se-GPX | 2.33 | (0.52 - 10.48) |  | 3.12 | (0.71 - 13.81) |  | 2.28 | (0.39 - 13.21) |  | 6.42 | (0.93 - 44.13) |
| Se-HSA | 0.75 | (0.15 - 3.74) |  | 0.25 | (0.06 - 1.14) |  | 0.81 | (0.13 - 5.13) |  | 0.32 | (0.05 - 1.83) |
| Unknown | 0.20 | (0.03 - 1.17) |  | 0.48 | (0.12 - 1.98) |  | 0.24 | (0.03 - 1.76) |  | 0.74 | (0.12 - 4.43) |

aAdjusted for sex, age at entry, years of storage, years of education

**Supplemental Table 4**.Crude and adjusted *odds ratios* (OR) of developing AD according to increasing levels (0.1 mcg/L) of cerebrospinal fluid Se species by sex.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Crude |  | Adjusteda |
|  | Men (N=46) |  | Women (N=43) |  | Men (N=46) |  | Women (N=43) |
| Se species | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |
| Total Se | 0.94 | (0.87 - 1.02) |  | 0.94 | (0.88 - 1.01) |  | 0.94 | (0.86 - 1.02) |  | 0.95 | (0.88 - 1.02) |
| Inorganic Se | 0.93 | (0.75 - 1.15) |  | 0.67 | (0.49 - 0.92) |  | 0.92 | (0.74 - 1.14) |  | 0.71 | (0.51 - 0.99) |
| Se(IV) | 0.88 | (0.64 - 1.21) |  | 0.56 | (0.37 - 0.87) |  | 0.89 | (0.64 - 1.23) |  | 0.61 | (0.39 - 0.95) |
| Se(VI) | 0.95 | (0.63 - 1.44) |  | 0.75 | (0.47 - 1.20) |  | 0.89 | (0.58 - 1.38) |  | 0.76 | (0.47 - 1.25) |
| Organic Se | 0.99 | (0.91 - 1.08) |  | 1.01 | (0.93 - 1.10) |  | 0.96 | (0.87 - 1.07) |  | 0.99 | (0.86 - 1.10) |
| Se-SELENOP | 0.95 | (0.86 - 1.05) |  | 0.99 | (0.90 - 1.08) |  | 0.89 | (0.78 - 1.01) |  | 0.95 | (0.84 - 1.07) |
| Se-Met | 1.35 | (0.72 – 2.54) |  | 2.14 | (0.99 – 4.60) |  | 1.68 | (0.74 – 3.77) |  | 2.16 | (0.93 – 5.02) |
| Se-Cys | 1.69 | (0.76 – 3.77) |  | 1.65 | (0.61 – 4.46) |  | 2.72 | (0.99 – 7.47) |  | 1.78 | (0.61 – 5.17) |
| Se-GPX | 1.62 | (0.96 – 2.75) |  | 1.30 | (0.69 – 2.47) |  | 1.86 | (0.91 – 3.82) |  | 1.59 | (0.79 – 3.18) |
| Se-HSA | 0.86 | (0.74 - 1.00) |  | 0.93 | (0.83 - 1.03) |  | 0.87 | (0.74 - 1.03) |  | 0.95 | (0.84 - 1.07) |
| Unknown | 0.85 | (0.63 - 1.14) |  | 0.81 | (0.56 - 1.15) |  | 0.93 | (0.66 - 1.30) |  | 0.78 | (0.53 - 1.15) |

aAdjusted for age at entry, years of storage and years of education

**Supplemental Table 5** Crude and adjusted *odds ratios* (OR) of developing AD according to increasing levels (0.1 mcg/L) of cerebrospinal fluid Se species by age.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Crude |  | Adjusteda |
|  | < 65 years (N=45) |  | ≥ 65 years (N=44) |  | < 65 years (N=45) |  | ≥ 65 years (N=44) |
| Se species | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |  | OR | 95% CI |
| Total Se | 0.93 | 0.87 - 0.99 |  | 0.97 | 0.89 - 1.06 |  | 0.95 | 0.89 - 1.02 |  | 0.99 | 0.89 - 1.10 |
| Inorganic Se | 0.83 | 0.67 - 1.02 |  | 0.82 | 0.60 - 1.12 |  | 0.88 | 0.70 - 1.09 |  | 0.83 | 0.60 - 1.16 |
| Se(IV) | 0.70 | 0.51 - 0.97 |  | 0.80 | 0.54 - 1.18 |  | 0.75 | 0.53 - 1.07 |  | 0.80 | 0.50 - 1.28 |
| Se(VI) | 0.86 | 0.60 - 1.23 |  | 0.82 | 0.46 - 1.46 |  | 0.95 | 0.63 - 1.41 |  | 0.75 | 0.39 - 1.44 |
| Organic Se | 0.96 | 0.89 - 1.05 |  | 1.04 | 0.95 - 1.15 |  | 0.95 | 0.85 - 1.06 |  | 1.04 | 0.92 - 1.17 |
| Se-SELENOP | 0.95 | 0.87 - 1.03 |  | 0.99 | 0.89 - 1.09 |  | 0.92 | 0.82 - 1.03 |  | 0.93 | 0.80 - 1.08 |
| Se-Met | 2.04 | 0.90 - 4.60 |  | 1.59 | 0.83 - 3.02 |  | 2.37 | 0.93 - 6.08 |  | 1.94 | 0.82 - 4.60 |
| Se-Cys | 1.14 | 0.46 - 2.84 |  | 2.30 | 0.95 - 5.55 |  | 1.68 | 0.58 - 4.90 |  | 4.91 | 1.32 - 18.23 |
| Se-GPX | 1.08 | 0.66 - 1.79 |  | 2.35 | 1.08 - 5.10 |  | 1.35 | 0.74 - 2.47 |  | 4.19 | 1.44 - 12.21 |
| Se-HSA | 0.90 | 0.81 - 1.01 |  | 0.90 | 0.78 - 1.05 |  | 0.95 | 0.83 - 1.08 |  | 0.94 | 0.79 - 1.13 |
| Unknown | 0.81 | 0.59 - 1.09 |  | 0.83 | 0.57 - 1.20 |  | 0.84 | 0.60 - 1.18 |  | 0.99 | 0.66 - 1.48 |

aAdjusted for sex, age at entry, years of storage and years of education

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