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## Plasma Selenium Over Time and Cognitive Decline in the Elderly.

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## ABSTRACT

**Background:** Because brain oxidative stress is a cause of cognitive impairment, selenium, which is an antioxidant, may protect against cognitive decline. The aim of the study was to examine whether declining selenium levels over time are associated with cognitive decline in a cohort of community-dwelling French elderly.

**Methods:** During 1991-1993, 1389 subjects (age 60-71 years) were recruited into a nine-year longitudinal study with 6 waves of follow-up. Cognitive functions were evaluated by neuropsychological tests. To take into account the entire set of cognitive measurements and the within-subject correlations between measures, we analyzed mixed linear and logistic models to study associations between selenium change and cognitive decline.

**Results:** After controlling for potential confounders, cognitive decline was associated with decreases of plasma selenium over time. Among subjects who had a decrease of their plasma selenium levels, the greater the decrease in plasma selenium, the higher the probability of cognitive decline. Among subjects who had an increase of their plasma selenium levels, cognitive decline was greater in subjects with the smallest selenium increase. There was no association between short-term (2-year) selenium change and cognitive changes.

**Conclusion:** Selenium status decreases with age, and may contribute to declines in neuropsychological functions among aging people.

The increase in life expectancy and the exponential increase in the number of elderly people give new importance to the development of preventive strategies to reduce or delay the onset of cognitive impairment, a major component of age-related diseases. One leading cause of cognitive impairment, is an increase in brain oxidative stress.<sup>1,2</sup> Selenium, an antioxidant and a main constituent of brain selenoproteins, may be particularly important for the maintenance of brain functions.<sup>3</sup> Seleno-glutathione peroxidase (GSH-Px) constitutes an important line of defense against free radicals acting against hydrogen peroxide and lipid peroxidation.<sup>4</sup> However, the most important selenoprotein for cerebral functions is probably selenoprotein P.<sup>5</sup> This protein is synthesized at the cerebral level and protects the brain against oxidative damage, particularly peroxynitrite.<sup>6</sup> Furthermore, selenium status decreases with old age.<sup>7-10</sup> Therefore, marginal or deficient selenium status may be a risk factor for a decline of cognitive functions. Selenium and cognition changes could also both reflect the ageing process.

Only one study has investigated the relationship between longitudinal cognitive decline and baseline selenium level;<sup>11</sup> the highest declines in cognitive functions were associated with the lowest plasma selenium concentrations at baseline. Limited data are available from selenium supplementation studies in the elderly.<sup>12,13</sup> In the EPESE cohort (n=2082), Gray et al.<sup>13</sup> showed that subjects who currently used antioxidant supplements (vitamins C, E, A and selenium or zinc) had a lower risk of cognitive decline than non-users. A randomized, double blind, placebo controlled trial on 86 subjects showed that subjects supplemented in trace elements (including selenium) and vitamins for 1 year had an improvement in the 6 different cognitive test assessments.<sup>12</sup> However, it is impossible to isolate the specific effect of selenium in these two studies with multi-antioxidant supplementation

The present study considered selenium change during follow-up, rather than just considering baseline selenium level. We investigated the relationships between short-term selenium changes (the

first 2-year) and long-term selenium changes (9-year) with cognitive changes during the 9-year follow-up of the EVA study (“Etude du Vieillissement Artériel”).

## METHODS

### Subjects cohorts

The EVA study is a nine-year longitudinal study with 6 waves of follow-up.<sup>14</sup> During the first two-years (1991-1993; EVA0), 1389 volunteers (574 men and 815 women) born between 1922 and 1932 and residing in the town of Nantes (Western France) were recruited from electoral rolls, and to a lesser extent, via information campaigns. The subsequent follow-up waves were EVA2 (1993-1995), EVA3 (1995-1997), EVA4 (1997-1999), and EVA5 (1999-2000); the sixth and last follow-up of the EVA study (EVA6) was conducted between June 2000 and December 2001. The numbers of subjects who completed a general questionnaire and a cognitive evaluation at each wave were 1272 at EVA2, 1188 at EVA3, 1042 at EVA4, 792 at EVA 5 and 702 at EVA6. The study protocol was approved by the Ethical Committee of the University Center Hospital of Kremlin-Bicêtre, Paris. Signed informed consent was obtained from all participants at enrollment.

### Data collection

#### *Questionnaire and general medical examination*

The general questionnaire at baseline allowed us to obtain information on socio-demographic factors such as sex, age, educational achievement (no school or primary school/ high school or university), and consumption habits such as smoking (current/ former/ non-smokers) and alcohol consumption ( $\geq 20$  mL/  $<20$  mL per day). Alcohol intake was determined from the subject's estimated average amount of alcoholic beverages ingested weekly and expressed in ml alcohol /day. In addition, height and weight were measured and body mass index (BMI) was calculated ( $\text{kg}/\text{m}^2$ ).

Two independent measures of systolic and diastolic blood pressure were made with a digital electronic tensiometer after a 10-minute rest.

#### *Cognitive evaluation*

At each wave, trained neuropsychologists evaluated cognition with a neuropsychological battery of tests, including assessment of a range of cognitive domains and a global test, the Mini Mental Status Examination (MMSE).<sup>15</sup> Visual attention was assessed with the Trail Making Test part B (TMTB).<sup>16</sup> The Digit Symbol Substitution (DSS) from the Wechsler Adult Intelligence Scale-Revised measured sustained attention and logical reasoning.<sup>17</sup> Psychomotor speed was evaluated with the Finger Tapping Test (FTT).

#### *Biological parameters*

At EVA0, EVA2 and EVA6, selenium was determined in serum, using electrothermal atomic absorption spectrometry (Perkin Elmer 5100 ZT, Norwalk, CT, USA) as previously described.<sup>18</sup> A selenium electrode-less discharge lamp and a Zeeman longitudinal background correction were used. Serum was diluted in a solution containing 0.1 M nitric acid and 0.2 % (w/v) Triton X 100. 10 µl of this dilution and 5 µl of matrix modifier were introduced onto the platform of a pyrolytic graphite furnace. Concentration was obtained using an addition calibration. Seronorm<sup>®</sup> trace element serum was used as internal quality control (Sero<sup>®</sup>, Billingstad, Norway). We considered the long-term change of plasma selenium by the difference between the plasma selenium measured at the 9-year follow up and at baseline (n=751). We also assessed the short-term plasma selenium variability by the difference between the 2-year measurement and baseline (n=1064). Total plasma cholesterol and plasma glucose level were also measured using routine methods.

At each wave of follow-up, a new general questionnaire was completed by participants; clinical examination, neuropsychological tests and blood sampling were also repeated. We thus updated all variables during the study except for alcohol consumption, height and weight, which were obtained only at EVA0, EVA2 and EVA3.

## Statistical Methods

The characteristics of subjects at inclusion were described in 2 groups: those who did and did not complete the follow-up. To test the differences between these two groups, Chi square test and the Student T test were used. Percentile distribution and means with SD were described for both cognitive and selenium change variables. Classical linear regressions were used to assess the association between 9-year selenium change and cognitive change, and the association between 2-year selenium change and cognitive changes during the 2, 4, 6 and 9 years of follow-up. Analyses were controlling for age, sex, education and selenium level at baseline.

To simultaneously take into account cognitive changes at each wave of the study and the within-subject correlation of measurements, we used mixed models (MIXED procedure in SAS; SAS Institute, Cary, NC) to analyze associations between cognitive and selenium changes. We also dichotomized cognitive decline by using two cut-offs. For each subject and at each wave, we calculated the cognitive score difference between that wave and baseline. Cut-off points corresponded to the 25<sup>th</sup> and 10<sup>th</sup> percentile of the distributions of the mean of these differences. For MMSE, the cognitive decline variable was first defined by a loss of 2 points and then by a loss of 3 points (25<sup>th</sup> and 10<sup>th</sup> percentiles, respectively). The same cut-offs were used for all cognitive continuous variables and corresponded to differences of -3 and -6 points for DSS, +2 and -7 taps for FTT and a time difference of 7.8 and 32.4 seconds for TMTB. To analyze these dichotomous cognitive variables, we performed a mixed logistic model with Gaussian random effect (NLMIXED procedure in SAS).<sup>19</sup>

Selenium changes were analyzed as continuous variables. Analyses were, first, adjusted for time and selenium level at baseline. Time was an explanatory variable and considered to be a combination of follow-up duration and age at inclusion; this combination allowed us to test whether

the effect of time is more pronounced in older subjects, by introducing an interaction term between these two co-variables. Secondly, analyses were adjusted for other potential confounding factors (which could be fixed or updated at each wave of the study) associated with cognition or selenium level, such as socio-demographic factors, consumption habits, but also health factors or indicators such as: BMI (in kg/m<sup>2</sup>), hypertension (systolic or diastolic blood pressure  $\geq 140$  or  $\geq 90$  mm Hg respectively, or use of hypertensive drugs or report of hypertension medical history), diabetes (plasma glucose level  $\geq 7.80$  mmol/L or use of anti-diabetic drugs or report of diabetes medical history), dyslipidemia (total cholesterol  $\geq 6.2$  mmol/L or use of lipid-lowering drugs or report of dyslipidemia medical history), and history of cardiovascular diseases (self-reported history of myocardial infarction, angina pectoris, stroke or use of vascular drugs).

Results of mixed linear models are expressed by linear regression coefficient ( $\beta$ ) with their 95% confidence interval (CI). Results of mixed logistic models are expressed by odds ratio (OR) with their 95% (CI). All statistical analyses were performed using SAS software version 9.1 (SAS Institute, Inc. Cary, North Carolina).

## RESULTS

Mean ( $\pm$ SD) age was 65.0 years ( $\pm 3.0$  years) for both genders. The other main characteristics of the study population at baseline have been previously described.<sup>14</sup> Characteristics of the 702 subjects who completed the 9-year follow-up, were compared to the 687 who did not (including 101 deaths) and are reported in Table 1. Subjects who did not complete the whole study were more likely to be men, current or former smokers, and persons with hypertension, a history of cardiovascular disease or a higher BMI (Table1). We also showed that cognitive performances at baseline were lower in subjects who did not complete the whole follow-up. While plasma selenium level was associated with mortality,<sup>20</sup> there was no association between loss-to-follow-up and plasma selenium level (Table1).

### *Classical linear regression analyses*

In the EVA study population, baseline mean plasma selenium level ( $\pm$  standard deviation) was 1.09  $\mu\text{mol/L}$  ( $\pm 0.20 \mu\text{mol/L}$ ). We observed a decrease of plasma selenium over the entire follow-up period. Means of selenium declined  $-0.055$  ( $\text{SD}\pm 0.20$ )  $\mu\text{mol/L}$  at 2 years and  $-0.096$  ( $\text{SD}\pm 0.21$ )  $\mu\text{mol/L}$  at the 9-year follow-up (Table 2). We noted an increase in cognitive performances means (Table 2). To assess the short- and long-term association between selenium and cognitive changes, we performed linear regression models between 2-year and 9-year selenium change and cognitive changes during 2, 4, 6 and 9-year follow-up (Table 3). Associations were adjusted for age, sex, education and selenium level at baseline. We showed that 2-year selenium change was not associated with cognitive change during 2, 4, 6 or 9-year follow-up, for any of the cognitive tests. Nine-year selenium change was associated with 9-year cognitive change for MMSE, but not for the other cognitive tests.

### *Mixed Models*

#### Factors associated with cognitive change and selenium change

To take into account all cognitive changes during the follow-up and the within-subject variability, we performed mixed linear models. We used these models mainly to determine which factors were associated with cognitive change by restricting analyses to MMSE test. Cognitive change was associated with time of follow-up ( $\beta=0.09$ ; 95% CI = 0.07 to 0.11), but not with age at inclusion. The interaction term between time of follow-up and age at inclusion was not significant, suggesting that the effect of time on cognitive decline was no more pronounced in older subjects than in younger ones for the narrow age range studied (60-70 years at baseline). Diabetes and hypertension were modestly associated with a higher decrease of cognitive performances ( $\beta=0.31$  [0.004 to 0.62] and  $\beta=0.14$  [-0.02 to 0.30], respectively). Sex, education, tobacco status, alcohol

consumption, BMI, history of cardiovascular diseases, dyslipidemia and baseline plasma selenium level were not associated with cognitive performances change during the follow-up (data not shown). During the follow-up, occurrence of cardiovascular events, as well as obesity, were associated with greater declines in plasma selenium (data not shown). No association was found between plasma selenium and other factors.

#### Association between 2-year selenium change and 9-year cognitive change

Using crude mixed linear models, modelling cognitive change (2, 4, 6, 8 and 9-year) by the first 2-year selenium change, there was no association with any of the four cognitive tests (Table 4). These results were confirmed in the multivariate models; (for the MMSE,  $\beta=0.01$  (-0.52 to 0.50); for DSS,  $\beta= -0.27$  ( -1.6 to 1.1); for TMTB,  $\beta= -8.4$  (-20.7 to 3.9); for FTT,  $\beta= -2.8$  (-6.7 to 1.1).

To understand the relationship between cognitive decline and 2-year selenium change, we used a mixed logistic model with Gaussian random effects (Table 6). In these models, cognitive decline was considered as a dichotomous variable. After controlling for time and plasma selenium level at baseline, the short-term plasma selenium decrease was only weakly associated with cognitive decline. These results were not changed after controlling for potential confounding factors (Table 6).

#### Association between selenium change and cognitive change during the 9 years of follow-up

For these analyses, we considered cognitive and selenium variables simultaneously measured at inclusion, 2 years and 9 years. In these mixed linear models, cognitive performances (as continuous variables) were related to selenium levels and time of follow-up. Interaction terms between selenium and time of follow-up expressed the change of selenium according to cognitive change during follow-up (Table 5). In the crude analyses, selenium changes were associated with DSS and FTT but not with TMTB or MMSE. Analyses adjusted for time, sex, education, diabetes, hypertension, dyslipidemia and history of cardiovascular diseases gave similar results (Table 5).

However, for these models, to be applicable distributions of cognitive variables should be Gaussian; this condition was met for DSS and FTT but not for MMSE and TMTB. In sensitivity analysis, we applied the same models limited to subjects who had all three selenium measurements and cognitive evaluation (inclusion, 2 and 9-year). Results were quite similar, although with lower power because analyses were carried out on 570 subjects instead of the initial 1371.

Associations between selenium change and cognitive decline were studied by using a mixed logistic model with Gaussian random effect (Table 6). After controlling for time, sex, education, plasma selenium level at baseline, diabetes, hypertension, dyslipidemia and history of cardiovascular diseases, we observed that probability of cognitive decline increased with the decrease of plasma selenium change over time. Among subjects who had a decrease of their plasma selenium levels, the higher the decrease of plasma selenium, the higher probability of cognitive decline. Among subjects who had an increase of their plasma selenium levels, the probability of cognitive decline was higher in subjects with the smallest selenium increase. Plasma selenium change was associated with MMSE decline at the 2-points cut-off (OR=2.31; CI =1.12 to 4.77) but not at the 3-points cut-off (1.41; 0.52 to 3.83)). We observed an association for the DSS at the 25<sup>th</sup> cut-off (2.60; 1.22 to 5.57)), and for TMTB at the 10<sup>th</sup> percentile (3.18 (1.14 to 8.88)). For FTT, decline increased when selenium decreased for the two cut-offs we considered (for the 10<sup>th</sup> percentile, 2.40 [1.21 to 4.77] and for the 25<sup>th</sup> percentile, 4.33 [1.60 to 11.72]).

## DISCUSSION

The EVA study is a longitudinal study of cognitive and vascular aging in an elderly general population. The study provided plasma selenium measurements at 3 points in time, and assessments of cognitive functioning at 6 points in time. Declines in selenium were associated with cognitive decline as measured by four neuropsychological tests: MMSE, DSS, FTT and TMTB, after

controlling for potential confounders. There were weaker associations between short-term selenium change and cognitive changes during the follow-up.

The EVA cohort is a free-living community dwelling population with normal neuropsychological test results at inclusion and a selenium status similar to that recently reported in French adults.<sup>21</sup> All co-variables for every model were updated at each waves of the study, except BMI and alcohol consumption for which we only took into account the baseline data. Alcohol consumption use may be associated with both cognitive decline and changes in selenium. However, correlations between alcohol consumption of the first three waves were very strong and we made the assumption regarding these correlations and the change in tobacco status (which is linked to alcohol behavior) that, in the EVA cohort, alcohol consumption remained constant during the follow-up.<sup>22</sup>

In epidemiologic studies of cognitive decline, biological status is generally considered as a static exposure associated with health changes. In this study, we extended our approach to dynamic exposure, considering selenium changes during a time period rather than as a single measurement. However, this approach requires appropriate statistical analyses. Classical linear regressions are well suited to study selenium change and cognitive change between two given moments. For our study, however, this method is too restrictive when considering our initial objective, the wealth and complexity of our data and sample selection. Mixed models allow us to simultaneously take into account measurements of the whole follow-up, and results are thus more powerful because sample selection is not as strong. We performed mixed linear or logistic models considering cognitive change as continuous or categorical. As cognitive variables are not strictly normally distributed, conditions for computing mixed linear models are not optimal. The associations between selenium and cognitive change remained strong when cognitive change variables were dichotomized.

The various statistical models applied to our data offer both advantages and limits. However we think the mixed logistic model is best suited to study long-term cognitive decline assessed by neuropsychological tests in a healthy elderly population.

The study's obvious main limit is sample selection throughout follow-up. Subjects who did not complete the whole follow-up had the lowest cognitive performances at inclusion, and we could hypothesize that these subjects also had the highest cognitive decline during the follow-up. However, they did not differ with regard to selenium level at baseline or plasma selenium decrease for the first two years. Moreover, with analyses limited to subjects who had the three plasma selenium measurements and complete cognitive assessment (n=570), results were quite similar even though power was reduced. Finally, a relationship between plasma selenium and cognition change is found in subjects with weak cognitive decline. Sample selection may limit interpretation of our results, but should not affect the relationship itself.

In previous works conducted on the same cohort at baseline<sup>14</sup> and after the four-year follow-up,<sup>11</sup> we showed that, at baseline, plasma selenium and cognitive functions were positively associated. Baseline selenium plasma was lower in the subjects who develop cognitive decline as measured by 4-year decline of 3 points in MMSE scores. We therefore hypothesize that plasma selenium may be one of the factors associated with cognitive decline. This hypothesis is supported by the role of selenium in redox reactions and in thyroid hormone synthesis, both being involved in cognitive impairment.<sup>23,24</sup> In addition, the brain contains large amounts of selenium and represents a target organ with respect to selenium supply and retention.<sup>5</sup> So far, genes for at least 25 selenoproteins<sup>25</sup> have been identified in the human genome and most of them are expressed in the brain (although their specific roles for normal brain functions and neurological diseases have not been completely elucidated).

We found an association between plasma selenium change and 9-year cognitive decline only with 9-year selenium change, not 2-year change. These results are in agreement with the effect of antioxidant supplementation observed in some long-term studies<sup>13,26</sup> and the lack of effect after a 6-month period in mild Alzheimer disease's patients.<sup>27</sup> Nevertheless, these intervention studies were conducted with multi-vitamin and mineral supplements, thus making it impossible to identify a specific selenium effect. It is also possible that health changes, including poor cognitive function, may lead to dietary or behavioral changes that affect selenium levels.

Taken as a whole, our results suggest that plasma selenium decrease is associated with cognitive decline. The real importance of selenium in brain and the capacity of brain to manage selenium depletion is just beginning to be explored.<sup>5</sup> Molecular biology has recently contributed to the recognition of selenium and selenium -dependent enzymes as modulators of brain function. Our results are in agreement with this approach even if a recent experimental work on knock-out mice suggest that plasma selenium cannot reflect brain selenium status due to the maintenance of brain selenoprotein P synthesis.<sup>28</sup>

Our results, together with information on involvement of selenoproteins in brain functions, support possible relationships between selenium status and neuropsychological functions in aging people. In this context, the preventive effect of selenium supplementation at a nutritional level needs to be evaluated with large-scale studies. This dynamic approach could shed new lights on the potential benefits of supplementation.

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Table 1: Baseline characteristics\* of subjects according to 9-year follow-up status†

	Attendance at 9-year follow-up	
	Yes	No
	%	%
Sex (women)	62.1	55.2
High school	50.8	46.7
Smoking status		
Current	8.3	9.0
Former	30.5	35.5
Alcohol consumption ( $\geq 20$ ml per day)	28.8	30.1
Diabetes	4.9	6.0
Hypertension	46.5	52.8
Cardiovascular diseases	9.70	12.7
Dyslipidemia	47.1	45.3
MMSE score $\leq 25^{\text{th}}$ percentile	17.7	25.1
DSS Score $\leq 25^{\text{th}}$ percentile	20.3	27.5
FTT Score $\leq 25^{\text{th}}$ percentile	22.7	26.7
TMTB Score $\geq 75^{\text{th}}$ percentile	20.8	30.2
Age (year); mean	65.0	65.0
BMI (kg/m <sup>2</sup> ); mean	24.8	25.6
Baseline selenium level ( $\mu\text{mol/L}$ ); mean	1.10	1.09
2-year selenium decrease ( $\mu\text{mol/L}$ ); mean	0.049	0.063

\* Expressed as percentage unless otherwise indicated

† Number of subjects for whom information was available on specific baseline variables: among those followed up at 9 years, n=677-702 (except the 2 selenium variables, n=607); among those not followed up, 653-687 (except baseline selenium, n= 684 and 2-year decrease, n= 457)

Table 2: Means and percentile distributions of selenium and cognitive change from baseline

	2-year change							9-year change						
	No.	Mean $\pm$ SD	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	No.	Mean $\pm$ SD	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>
	Percentile of distribution							Percentile of distribution						
MMSE (points)	1235	-0.52 $\pm$ 2.25	-3	-2	0	1	2	681	0.09 $\pm$ 2.3	-2	-1	0	1	3
DSS (points)	1228	1.19 $\pm$ 6.32	-6	-2	1	5	8	650	0.06 $\pm$ 7.3	-9	-4	1	5	9
TMT (seconds)	1188	-12.7 $\pm$ 64.48	43	12	-10	-41	-91	628	-17.1 $\pm$ 50.9	37	9.5	-12	-39	-88
FTT (taps)	1220	11.03 $\pm$ 17.80	-9	0	10	21	32	631	5.6 $\pm$ 19.3	-17	-6	5	16	29
Selenium ( $\mu$ mol/L)	1064	-0.055 $\pm$ 0.22	-0.33	-0.20	-0.05	0.09	0.21	751	-0.10 $\pm$ 0.21	-0.35	-0.22	-0.09	0.02	0.17

Table 3: Linear regressions models for the association between 2, 4, 6 and 9-year cognitive change and 2 and 9-year selenium change. Results are expressed as linear regression coefficient  $\beta$  with 95% confidence intervals.

Cognitive change from Baseline												
Selenium Change												
2-year												
	2 years			4 years			6 years			9 years		
	No.	$\beta$	(95% CI)									
2-year												
MMSE	1034	-0.12	(-0.28 to 0.04)	975	-0.04	(-0.18 to 0.10)	867	-0.028	(-0.18 to 0.12)	583	0.2	(-0.02 to 0.42)
DSS	1027	-0.02	(-0.45 to 0.41)	968	-0.004	(-0.44 to 0.43)	859	-0.061	(-0.55 to 0.43)	555	-0.05	(-0.70 to 0.60)
TMTB	995	-2.41	(-7.04 to 2.22)	937	-1.9	(-5.58 to 1.78)	832	-1.92	(-5.51 to 1.67)	541	-2.18	(-6.71 to 2.35)
FTT	1021	-0.49	(-1.69 to 0.71)	963	-0.91	(-2.13 to 0.31)	853	-0.7	(-1.95 to 0.55)	546	0.74	(-1.02 to 2.50)
9-year												
MMSE										570	0.38	(0.14 to 0.62)
DSS										542	0.53	(-0.20 to 1.26)
TMTB										524	-3.2	(-8.61 to 2.21)
FTT										533	1.6	(-0.40 to 3.60)

Table 4: Association between selenium change during the first 2 years and cognitive change up to 9 years of follow-up: results of mixed linear models

	Crude association*		Adjusted association†	
	$\beta$	(95% CI)	$\beta$	(95% CI)
Decrease in MMSE score	-0.023	(-0.47 to 0.43)	0.0067	(-0.52 to 0.54)
Decrease in DSS score	-0.033	(-1.39 to 1.32)	-0.27	(-1.64 to 1.10)
Delay in TMTB time	-8.60	(-20.69 to 3.49)	-8.39	(-20.68 to 3.90)
Decrease in FTT score	-1.76	(-5.52 to 2.00)	-2.82	(-6.70 to 1.06)

For these analyses, we used the plasma selenium measurements at inclusion and at 2-year and the cognitive evaluations assessed at inclusion, 2, 4, 6 and 9 years.

\* Associations adjusted for time and baseline plasma selenium level

† Association adjusted for time, sex, education, baseline plasma selenium level, diabetes, dyslipidemia, hypertension and history of cardiovascular diseases.

Table 5: Associations between plasma selenium changes and cognitive changes during the 9 years of follow-up: Results of mixed linear models.

	Crude association*		Adjusted association <sup>†</sup>		Sensitivity Analysis <sup>‡</sup>	
	$\beta$	(95% CI)	$\beta$	(95% CI)	$\beta$	(95% CI)
Decrease in MMSE score	-0.008	(-0.12 ; 0.12)	0.0037	(-0.12 ; 0.13)	0.041	(-0.09 ; 0.17)
Decrease in DSS score	0.476	(0.12 ; 0.83)	0.38	(0.03 ; 0.73)	0.34	(-0.03 ; 0.71)
Delay in TMTB time	-0.4	(-2.87 ; 2.07)	-0.197	(-2.65 ; 2.25)	-0.24	(-2.98 ; 2.50)
Decrease in FTT score	1.25	(0.35 ; 2.15)	1.18	(0.28 ; 2.08)	0.83	(-0.17 ; 1.83)

For these analyses, we used the plasma selenium measurements and cognitive evaluation assessments at inclusion, 2 years and 9 years

\* Associations adjusted for time and baseline plasma selenium level

<sup>†</sup>Association adjusted for time, sex, education, baseline plasma selenium level, diabetes, dyslipidemia, hypertension and history of cardiovascular diseases.

<sup>‡</sup>Sensitivity Analysis carried on the 570 subjects who had all three plasma selenium measurements and cognitive evaluations.

Table 6: Associations between 2-year or 9-year plasma selenium change decrease and 9 year cognitive decline. Results of mixed logistic models expressed by Odds Ratios and 95% confidence intervals of cognitive decline according to the decrease of selenium change\*

	Cut-off level			
		< 25 <sup>th</sup>		< 10 <sup>th</sup>
	OR <sup>†</sup>	(95% CI)	OR <sup>†</sup>	(95% CI)
Selenium decrease				
2-year				
MMSE	1.07	0.59 ; 1.95	1.04	0.49 ; 2.21
DSS	1.56	0.74 ; 3.28	1.51	0.59 ; 3.87
TMTB	1.32	0.64 ; 2.73	1.35	0.51 ; 3.54
FTT	0.91	0.47 ; 1.74	0.69	0.30 ; 1.59
9-year				
MMSE	2.31	1.12 ; 4.77	1.41	0.52 ; 3.83
DSS	2.60	1.22 ; 5.57	2.22	0.78 ; 6.33
TMTB	1.97	0.95 ; 4.11	3.18	1.14 ; 8.88
FTT	2.40	1.21 ; 4.77	4.33	1.60 ; 11.72

\*For each subject and at each wave, the cognitive score differences between the wave i and baseline were calculated. Cut-off points < 25<sup>th</sup> of the distributions of the mean of these differences corresponded to a difference of -2 points for MMSE, -3 points for DSS, +2 taps for FTT and 7.8 seconds for TMTB. Cut-off points < 10<sup>th</sup> of the distributions of the mean of these differences corresponded to a difference of -3 points for MMSE, -6 points for DSS, -7 taps for FTT and 32.4 seconds for TMTB

†Adjusted on time, sex, education, plasma selenium level at baseline, diabetes, hypertension, dyslipidemia and history of cardiovascular diseases