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Zinc in Alzheimer's Disease: A Meta-Analysis of Serum, Plasma, and Cerebrospinal Fluid Studies

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Abstract. To evaluate whether zinc levels in serum, plasma, and cerebrospinal fluid are altered in Alzheimer's disease (AD), we performed meta-analyses of 27 studies on the topic published from 1983 to 2014. The subjects' sample obtained by merging studies was a pooled total of 777 AD subjects and 1,728 controls for serum zinc studies, 287 AD subjects and 166 controls for plasma zinc, and of 292 AD subjects and 179 controls for CSF zinc. The main result of this meta-analysis is the very high heterogeneity among the studies either in demographic terms or in methodological approaches. Although we considered these effects in our analyses, the heterogeneity persisted and it has to be taken into account in the interpretation of the results. Our meta-analysis indicated that serum zinc appears significantly decreased in AD patients compared with healthy controls, and this result is confirmed when serum and plasma studies were analyzed together. If we considered the age-matched studies, the meta-analysis carried out on only six studies showed no significant difference in zinc levels between AD and healthy controls (SMD = −0.55, 95% CI (−1.18; 0.09); $p = 0.094$; $I^2 = 91\%$). In the light of these findings, we speculated about the possibility that the decreases observed could indicate a possible dietary zinc deficiency and we suggested that the possible involvement of zinc alterations in AD may have an interplay with copper metabolism.

Keywords: Alzheimer's disease, cerebrospinal fluid, meta-analysis, plasma, serum, zinc

INTRODUCTION

In the last 20 years, the number of neurologic studies on metals has increased extraordinarily. Transition metals, such as copper and iron, are essential nutrients, which have important functions in neurobiology. They catalyze primary biological redox reactions [1], but if the redox state of cellular environment is altered, they disrupt neuronal architecture, promoting oxidative stress via Haber Weiss and Fenton chemistry [2]. In particular, considerable attention has been directed to the potential role of copper, iron, and zinc misbalances in the pathogenesis of Alzheimer's disease (AD) [3]. Regarding copper, numerous studies demonstrate that

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copper not bound to ceruloplasmin (Non-Cp-Cu) is a causative factor in AD. Meta-analyses in living patients show that copper and Non-Cp-Cu are increased in AD [4] and associate with the typical clinical deficits of the disease [5–8], and with amyloid-β (Aβ) and tau in cerebrospinal fluid (CSF) [9]. Non-Cp-Cu correlates with a worse prognosis of AD [10] and with the mild cognitive impairment (MCI) condition [4, 11], and a 6-year longitudinal study [12] demonstrates its predictive value in MCI conversion to full AD. Zinc is the second most abundant metal in our body after iron, is a co-factor in many proteins, enzymes, nucleic acid, carbohydrates, and lipids, as well as in essential biological functions, and is needed for DNA and RNA synthesis and stabilization. Metallothioneins (MTs) are proteins that bind zinc and copper and play an important role in zinc and copper absorption and storage.

Zinc is absorbed in the small intestine and is responsive to dietary intake (review in [13]). Most body zinc is stored in skeletal muscle, bone, liver, and the brain, while circulating zinc, at a concentration of 10–15 μmol/L (65–98 μg/dL), accounts for less than 1% of total zinc.

Zinc is necessary for brain development and physiology. In fact, neurological complaints associated with zinc deficiency, such as learning, memory, and emotional stability, relate to functions of brain structures normally rich in this metal, such as hippocampus, amygdala, and neocortex [2]. These are also the neocortical regions most prone to AD pathology. Zinc movement from the blood to the brain is mediated by the blood-brain barrier and blood/cerebrospinal barrier [14], along with MTs, whose expression are regulated by zinc, copper, and other metals [15], and which are active in trapping and storing metals. MTs are ubiquitous cysteine rich proteins, which have a high affinity for copper and zinc. Metallothionein-3 (growth inhibitory factor) seems to be exclusively expressed in the brain and is involved in neuronal damage repair through its neuro-inhibitory activity, sequestering zinc in synaptic vesicles [16], and it is down-regulated in AD [17].

In the last decade, growing evidence has unveiled that zinc is released together with glutamate and copper into the synaptic cleft during synaptic excitation of diverse types of synapses. Specifically, zinc is released in the synaptic cleft by about half of the glutamatergic presynaptic terminals in the cerebral cortex [18]. It has been proposed that a main function of zinc released at the synapse is to quench glutamate firing, and that the lack of that quenching induces glutamatergic excitotoxicity, eventually leading to neuronal death [19]. The interaction of zinc with Aβ, the main constituent of amyloid plaques in AD brain, leads to an additional neuronal zinc deficiency at the glutamatergic synaptic level, further exacerbating these toxic processes. In the AD brain, hypermetallation (copper, iron) of the Aβ peptide has been postulated to be at the basis of redox cycles of oxidative stress and H_2O_2 production, Aβ oligomer formation and precipitation within plaques [20–22], resulting in synaptic loss and cognitive decline.

Literature on zinc levels in serum, plasma, and CSF of AD patients reports often conflicting results. Thus, we performed a meta-analysis of studies published comparing serum, plasma, and CSF zinc levels in AD patients to those in healthy controls in an attempt to evaluate whether this metal is altered in AD. We applied this statistical method combining the results of different studies on the role of zinc in AD, in order to estimate the 'true effect size' and increasing the power of the conclusions.

METHODS

Search strategy

To identify appropriate studies for meta-analysis, we entered the keywords "Alzheimer's disease", "zinc", "serum", "plasma", "CSF", "metals" or MeSH terms and their combinations in PubMed and selected studies from 1983 to 2013. The search strategy in PubMed was shown as an example: "Alzheimer Disease"[Mesh] AND ("zinc"[MeSH Terms] OR "zinc"[All Fields]) AND ("serum"[MeSH Terms] OR "serum"[All Fields]).

We also identified other studies by using "Scopus" and "Web of Knowledge" databases. Besides, reviewing the contents of this first selection, we also evaluated their reference lists to search for additional studies via Google Scholar. Additionally, we reviewed the citation lists from each article retrieved for the meta-analysis and from relevant review articles and without any language or time limitation.

We considered only papers showing comparative analyses of AD and healthy populations, presenting original results and published in peer-reviewed journals. In most studies, the severity of the AD patients 'cognitive decline had been assessed by a Mini-Mental State Examination (MMSE) [23]. The target of our meta-analysis was the comparison between AD patients and controls of the zinc weighted means reported in the selected papers. We followed the procedural steps indicated by Cochrane

(http://www.cochrane-handbook.org) and the guidelines for reporting of meta analyses of observational studies in epidemiology [24].

Our first selection consisted of 36 studies.

At this point, we further required that manuscripts explicitly present zinc mean and standard deviation values for both patient and control groups. From the first selection, two studies were dropped since they did not report healthy controls data [10, 25]. We also excluded five additional studies for the following reasons: the Huang's study [26] was not included because the control group consisted of 13 elderly individuals with MCI, and 6 healthy elderly participants; the Licastro et al. study [27] was excluded because information about the size of the healthy control group was missing; the Bomboi et al. [25] and Squitti et al. [10] studies did not report a healthy control group for comparison; and the Rulon's study [28] was excluded since zinc concentrations were measured in postmortem serum of AD and healthy controls. Moreover, we did not include the Haines's study [29] because they compared patients with cognitive impairment, which did not meeting the NINCDS-ADRDA criteria for AD. The study by Gronek and colleagues [30] was not included because the methodology was not reported; a very recent Gonzales-Dominguez et al. study [31] was excluded because the sample patients partially overlapped with subjects participating in their previous study already included in the meta-analysis [32]. Finally, we excluded the Bocca et al. [33] study because it was conducted partially on the same sample as the study by Alimonti and colleagues [34] and presented less information. At this point, our selection was reduced to 27 studies (25 in English, 1 in Turkish, and 1 in Russian).

Data extraction

Two independent reviewers extracted the following variables from each study: the name of the first author, year of publication, AD and control sample size, AD and control mean age and the correspondent standard deviation (SD), the percentage of women included in AD and control sample, MMSE scores, and the technique used. Also recorded were the mean zinc levels and the corresponding standard deviations, or, if they were not directly reported, they were estimating from median, range, and the sample size [35].

Statistical methods

To obtain the pooled estimate of the difference in zinc between AD and control group, a random effects model was applied, but when no significant heterogeneity was detected, a fixed effects model was applied. We used as summary statistic the standardized mean difference (SMD) calculated by the method of Hedges, which expresses the difference in mean between the two groups in each study relative to the variability observed in that study (so the effect is expressed in standard units rather than the original units of measurement). For interpreting Hedge's g effect size: 0.2 was a small clinical effect, 0.5 was a medium clinical effect, and 0.8 was large clinical effect. We evaluated heterogeneity via χ^2 and I^2 tests. The I^2 test describes the rate of variation across studies due to heterogeneity rather than chance, ranging from 0 (no heterogeneity) to 100 (maximal heterogeneity).

Because the effect size was heterogeneous across studies, sub-group and meta-regression analyses and sub-group analyses were applied to determine the possible effect of coded characteristic of studies (the technique used, differences in the mean age of AD and controls, differences in the number of women included in the AD and control groups). To verify the robustness of the conclusion of our meta-analysis, a sensitivity analysis was conducted.

Publication bias was investigated by using graphical tools as the funnel plot, a representation of standard error by SMD. Different statistic tests such as Begg and Mazumdar rank correlation and Egger's regression intercept were executed for testing the presence of publication bias. Temporal effect was also evaluated with a cumulative meta-analysis.

All results were reported with 95% confidence intervals (CI) and all p-values were two-tailed. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using STATA 10.1 (Stata Corp, College Station, TX, USA).

RESULTS

The studies included in our meta-analysis are listed in Table 1. We performed separate meta-analyses of the zinc data in serum (16 studies), plasma (5 studies), and CSF (6 studies), and one meta-analysis of the zinc data in serum and plasma pooled together (21 studies).

Quantitative synthesis

Zinc in serum

The meta-analysis of serum zinc consisted of 16 studies (Table 1) with a pooled total of 2,505 subjects: 777 AD and 1,728 controls. The study with the smallest AD sample size was that of Kapaki and colleagues [36]

Table 1
Studies included in the meta-analyses

References		AD Patients				Healthy Controls			
		N°	Gender (% Female)	Mean age (years)	MMSE	N°	Gender (% Female)	Mean age (years)	Method
Studies on serum									
Shore et al.	[44]	10	30	63.7 ± 8.3	–	10	70	61.9 ± 9.8	AAS
Jeandel et al.	[85]	55	72.7	81.7 ± 5.7	<25	24	79.2	76.4 ± 6.1	AAS
Molaschi et al.	[39]	31	100	77.2 ± 2.4	–	421	100	77.6 ± 2.3	AAS
Molina et al.	[42]	26	46.2	73.1 ± 8.2	13.2 ± 6	28	42.9	70.8 ± 7.3	AAS
Kapaki et al.	[36]	5	20	54.0 ± 2.0	–	28	35.7	44.5 ± 14.3	AAS
Gonzalez et al.	[86]	51	70.6	74.5 ± 2.3	–	40	45	70.3 ± 4	AAS
Maes et al.	[41]	15	80	78.4 ± 10.3	<16	13	53.8	78.3 ± 5.7	AAS
Ozcankaya et al.	[87]	27	29.6	72.3 ± 6.5	16.8 ± 13	25	36	64.4 ± 7.2	AAS
Sevym et al.	[43]	98	66.3	72.1 ± 6.7	–	76	59.2	70.3 ± 5.7	AAS
Alimonti et al.	[34]	53	67.9	74.5 ± 6.5	17 ± 6.5	124	34.7	44.8 ± 13	ICP MS
Dong et al.	[88]	18	50	80.3 ± 1.7	–	16	43.8	77.9 ± 1.7	ICP MS
Baum et al.	[40]	44	65.9	74.3 ± 8.7	–	41	48.8	79.1 ± 6.0	ICP MS
Brewer et al.	[89]	29	43	73.5 ± 7.8	24.4 ± 4	29	69	68.0 ± 6.0	AAS
Gonzales-Dominiguez et al.	[32]	30	60	80.9 ± 4.5	–	30	56.7	74 ± 5.7	ICP MS
Azhdarzadeh et al.	[38]	80	63	–	–	70	57	–	ICP MS
Rembach et al.	[37]	205	61.9	78.8 ± 8.6	18.9 ± 5	753	57.6	70.6 ± 7	ICP MS
Studies on plasma									
Basun et al.	[49]	24	70.8	73.0 ± 1.0	–	28	57.1	78.0 ± 3.0	Other
Mattiello et al.	[48]	21	71.4	75.0 ± 8.0	–	10	50	82.0 ± 2.0	ICP MS
Gerhardsson et al.	[46]	173	70.5	69.0 ± 8.5	16 ± 7.0	54	66.6	77.0 ± 8.5	ICP MS
Vural et al.	[47]	50	54	71.9 ± 6.8	–	50	52	65.1 ± 7.1	AAS
McIntosh et al.	[45]	19	57.9	77.0 ± 7.0	21 ± 4.0	24	58.3	73.0 ± 6.0	Other
Studies on CSF									
Hershey et al.	[50]	33	–	75.8 ± 1.8	–	20	–	38.5 ± 4.1	ICP MS
Sahu et al.	[52]	34	47.1	60.0 ± 10	–	34	26.5	matched	AAS
Molina et al.	[42]	26	46.2	73.1 ± 8.2	13.2 ± 6	28	42.9	70.8 ± 7.3	AAS
Kapaki et al.	[36]	5	20	54.0 ± 2.0	–	28	35.7	44.5 ± 14	AAS
Gerhardsson et al.	[46]	173	70.5	69.0 ± 8.5	16 ± 7.0	54	66.6	77.0 ± 8.5	ICP MS
Hozumi et al.	[51]	21	61.9	65.4 ± 13	–	15	60	48.4 ± 22	ICP MS

with five patients, while the study with the largest AD sample size was that from Rembach et al. [37], with 205 patients. The mean age in AD groups was >60 with the exception of the Kapaki et al. study [36], in which the mean age of the AD group was lower. The mean age information was missing for the Azhdarzadeh et al. [38] study. The percentage of AD women ranged from 20% [36] to 100% [39]. 13 studies were conducted on Caucasians, one on an Australian population [37], and two on Chinese populations [38, 40]. One of the latter studies, the Azhdarzadeh et al. [38] study, was conducted considering two different ethnic groups, one Chinese and the other one Iranian, and so we reanalyzed data including them and also considering them separately.

The results indicated that AD patients overall had lower levels of zinc than healthy controls [SMD = −0.46, 95% CI (−0.76; −0.16), $p = 0.003$] and, as showed in the forest plot (Fig. 1), the variation in direction and magnitudes of effect were highly variable and heterogeneous ($I^2 = 87.7\%$). Also when we separately considered the two populations of the Azhdarzadeh study [38], the SMD of zinc levels between AD and controls was significant [SMD = −0.51, 95% CI (−0.81; −0.21), $p = 0.001$].

The subgroup analysis, evaluating the method used for measuring zinc, suggested that the method was a possible source of heterogeneity. The heterogeneity in each subgroup was high too (Table 2).

The meta-regression analysis revealed no effect either for the difference between mean age of AD and control groups ($p = 0.711$), or for the difference in the percentage of women in the two groups ($p = 0.605$).

Cumulative meta-analysis suggested the exclusion of a temporal trend. Furthermore, the symmetry found in the funnel plot pointed out the absence of a significant publication bias. It was confirmed by the results of both Begg's and Egger's tests; Egger's intercept b was −0.41, 95% CI (−3.77; 2.95), $p = 0.799$ and Begg and Mazumdar's correlation test was not significant ($p = 0.300$).

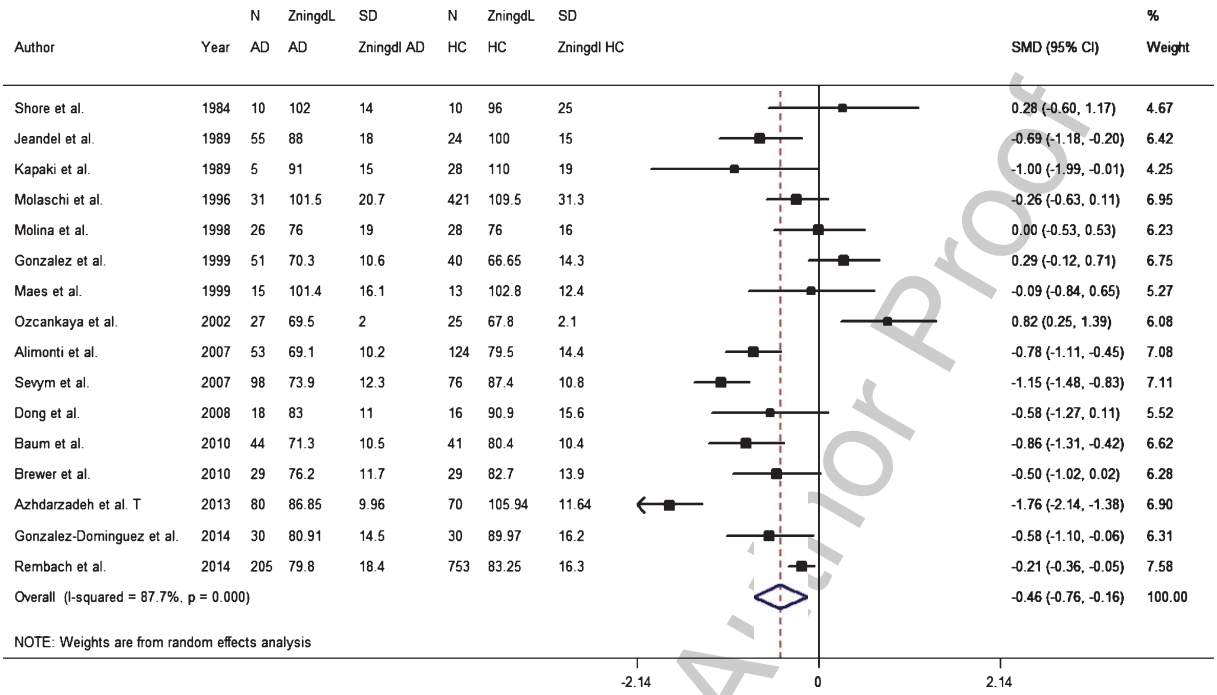

Fig. 1. Forest plot of meta-analysis of the standardized mean difference (SMD) of zinc levels in serum between AD subjects and controls. SMD (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the diamonds of the pooled SMD based on the random-effects model. Heterogeneity $\chi^2 = 121.98$ (d.f. = 15), $p < 0.001$. I^2 (variation in SMD attributable to heterogeneity) = 87.7%. Estimate of between-study variance $tau^2 = 0.31$; Test of SMD = 0: z = 2.97, $p = 0.003$. Dashed line represents the weighted mean value of all the studies analyzed. The solid line represents the zero of the difference (Δ) between AD and healthy controls.

Table 2
Meta-analysis of studies on serum. Pooled Standardized Mean Differences (SMD) with the corresponding 95% Confidence Intervals (CI) and p value, the I^2 statistic for overall, subgroup and sensitivity analyses

			n of studies	Pooled SMD (Random effects model, 95% CI)	*p*-value	I^2
Studies on serum	Overall		16	−0.46 (−0.76; −0.16)	0.003	87.7%
	Subgroup analysis	ICP MS	6	−0.79 (−1.31; −0.28)	0.002	91.9%
		AAS	10	−0.23 (−0.64;0.18)	0.268	84.2%
	Sensitivity analysis	Overall without Molaschi et al. [39] study	15	−0.47 (−0.80; −0.14)	0.005	88.4%
		Overall without Kapaki et al. [36] study	15	−0.43 (−0.74; −0.12)	0.006	88.4%
		Overall without Azhdarzadeh et al. [38] and Rembach et al. [37] studies	14	−0.38 (−0.68; −0.08)	0.014	80.4%

Sensitivity analysis was performed excluding the study by Molaschi and colleagues [39] because they considered only women unlike the others studies. Even excluding this study, the difference between AD patients and controls was maintained [SMD = −0.47, 95% CI (−0.80; −0.14); $p = 0.005$; $I^2 = 88.4\%$]. We also excluded the study of Kapaki et al. [36] as they analyzed younger AD patients in terms of mean age. The difference between AD patients and controls was maintained [SMD = −0.43, 95% CI (−0.74; −0.12); $p = 0.006$; $I^2 = 88.4\%$]. We also observed a significant result when excluding the studies of Azhdarzadeh et al. [38] and Rembach et al. [37], because they considered mixed population [SMD = −0.38, 95% CI (−0.68; −0.08); $p = 0.017$; $I^2 = 80.4\%$].

Analyzing the six approximately age-matched studies [38, 39, 41–44], we observed no significant difference in zinc levels between AD and healthy controls [SMD = −0.55, 95% CI (−1.18; 0.09); $p = 0.094$; $I^2 = 91\%$].

Zinc in plasma

Five studies on zinc in plasma were included in our meta-analysis (Table 1). The pooled sample size consisted of 453 subjects: 287 AD and 166 controls. The smallest AD sample size consisted of 19 patients [45] and the biggest was 173 patients [46]. Percentage of AD women ranged from 54% [47] to 71% [48]. The mean age in the patient groups was greater than 65 years. All these studies were performed on Caucasian populations. As depicted in the forest plot (Fig. 2), plasma zinc was not significantly different between AD and controls in three studies [46, 48, 49], but plasma zinc was lower in AD patients than in controls in the McIntosh and Vural studies [45, 47]. We observed across the studies a moderate but no significant heterogeneity ($I^2 = 36.4\%$; $p = 0.179$) so we applied a fixed effects model. The pooled analysis revealed no significant difference [SMD = −0.16, 95% CI (−0.36; 0.04); $p = 0.117$]. Meta regression analysis excluded any association with the difference in the percentage of women ($p = 0.502$) or with the difference in mean age ($p = 0.109$). Possible temporal trend was also excluded. No significant evidence of publication bias was observed [Egger's intercept = −0.64, 95% CI (−7.14;5.86), $t = 1.56$ and $p = 0.775$; Begg and Mazumdar's Test $p = 0.999$].

Zinc in serum and in plasma pooled together

We also performed a meta-analysis on 21 studies considering together serum and plasma zinc studies (Table 1). The pooled sample consisted of 1,064 AD patients and 1,894 healthy controls. The meta-analysis results showed zinc levels in AD patients significantly lower than those of controls [SMD = −0.39, 95% CI (−0.64; −0.15); $p = 0.002$) but there was high heterogeneity ($I^2 = 85.2\%$; Fig. 3]. The same result was observed when the two populations of the Azhdarzadeh et al. study [38] were considered separately [SMD = −0.43, 95% CI (−0.68; −0.19); $p < 0.001$]. We repeated the subgroup analysis, stratifying for method, and the forest plot revealed high heterogeneity between and within groups [AAS: number of studies = 11, SMD = −0.26, 95% CI (−0.69;0.13); $p = 0.157$; ICP MS: number of studies = 8, SMD = −0.58, 95% CI (−1.01;0.16) $p = 0.007$; Other: number of studies = 2, SMD = −0.28, 95% CI (−0.69;0.13); $p = 0.177$]. We performed the meta regression analysis, coding the method as two dummy explanatory variables so that all the information concerning the three levels was accounted for (Method 2 was 1 if method was ICP MS and 0 otherwise; Method 3 was 1 if method was Other and 0 otherwise). We observed that the differences among the three groups were not significant [$F(2,18) = 0.72$, $p = 0.502$].

No significant association was observed with the difference in the percentage of women ($p = 0.803$) as well as with the difference in mean age ($p = 0.363$). We applied a sensitivity analysis, as described in zinc session, excluding Molaschi's study [39], but we found no important change in the results [SMD = −0.40, 95% CI (−0.66; −0.14); $p = 0.002$; $I^2 = 85.9\%$] as well as with the exclusion of the Kapaki et al. stud [36] [SMD = −0.37, 95% CI (−0.62; −0.13); $p = 0.003$; $I^{2v} = 85.8\%$] or from that of Azhdarzadeh et al. [38] and Rembach et al. [37] studies [SMD = −0.49, 95% CI (−0.92; −0.05); $p = 0.029$; $I^2 = 93.4\%$; Table 3].

Considering only the seven studies with age-matched subjects, we observed no significant difference [SMD = −0.52, 95% CI (−1.08;0.05); $p = 0.075$; $I^2 = 89.7\%$].

The funnel plot of studies appeared symmetrical enough suggesting the absence of a significant publication bias. It was also supported by the Egger test [Egger's intercept = −0.17, 95% CI (−2.90;2.56), $p = 0.898$] and by the rank correlation test proposed by Begg and Mazumdar ($p = 0.291$). The results of cumulative analysis excluded a temporal effect.

Zinc in CSF

In the CSF meta-analysis, six studies were considered (Table 1). The total pool sample size was equal to 471 subjects: 292 AD patients and 179 healthy controls. The AD sample size ranged from 5 [36] to 173 [46]. The mean age in the patient groups was >60 years with the exception of the Kapaki et al. study in which the mean age of the AD group was lower. The percentage of women ranged from a minimum of 12% [36] to a maximum of 71% [46]. The pooled SMD showed no statistically significant difference in zinc levels between AD and controls [SMD = 0.06, 95% CI (−0.36; 0.48); $p = 0.776$; Fig. 4]. To investigate the high heterogeneity ($I^2 = 71.9\%$), subgroup analysis was reapplied and a moderate/high heterogeneity was observed within subgroups [ICP MS: SMD = 0.37, 95% CI (−0.13;0.87), $p = 0.142$, $I^2 = 65.2\%$; AAS: SMD = −0.35, 95% CI (−1.10;0.41), $p = 0.366$, $I^2 = 76.4\%$]. The meta-regression pointed out no significant association with gender ($p = 0.290$) or age ($p = 0.335$). No significant publication bias was found by Egger test [Egger's intercept = −0.37, 95% CI (−7.31;6.58), $t = -0.15$, $p = 0.891$] and by Begg and Mazumdar's rank correlation ($p = 0.851$). Sensitivity

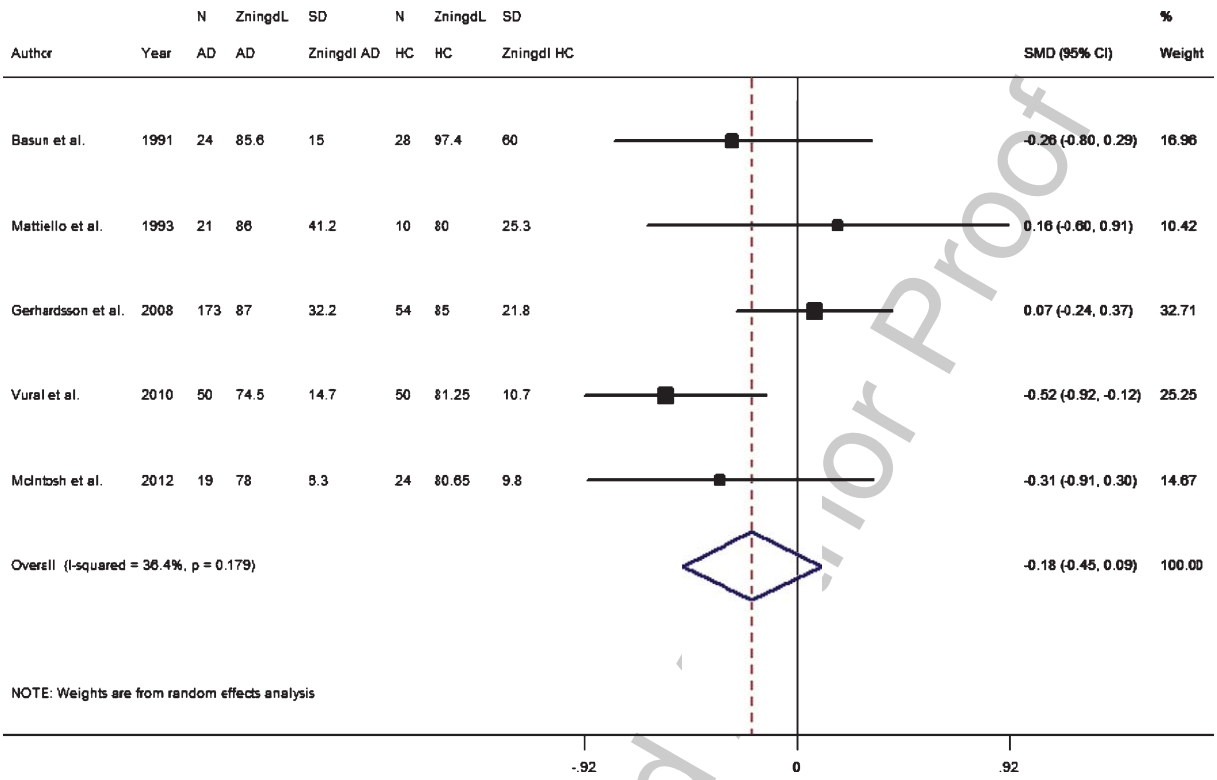

Fig. 2. Forest plot of meta-analysis of the standardized mean difference (SMD) of zinc levels in plasma between AD subjects and controls. SMDs (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the diamonds of the pooled SMD based on the fixed-effects model. Heterogeneity $\chi^2 = 6.29$ (d.f. = 4), $p = 0.179$, I^2 (variation in SMD attributable to heterogeneity) = 36.4%. Test of SMD = 0: z = 1.32, $p = 0.186$. Dashed line represents the weighted mean value of all the studies analyzed. The solid line represents the zero of the difference (Δ) between AD and healthy controls.

Table 3
Meta-analysis of studies on serum and plasma. Pooled Standardized Mean Differences (SMD) with the corresponding 95% Confidence Intervals (CI) and p value, the I^2 statistic for overall, subgroup and sensitivity analyses

			n of studies	Pooled SMD (Random effects model, 95% CI)	p-value	I^2
Studies on serum and plasma	Overall		21	−0.39 (−0.64; −0.15)	0.002	85.2%
	Subgroup analysis	ICP MS	8	−0.58 (−1.01; −0.16)	0.007	90.8%
		AAS	11	−0.26 (−0.69; 0.13)	0.157	82.7%
		Other	2	−0.28 (−0.69; 0.13)	0.177	0%
	Sensitivity analysis	Overall without Molaschi et al. [39] study	20	−0.40 (−0.66; −0.14)	0.002	85.9%
		Overall without Kapaki et al. [36] study	20	−0.37 (−0.62; −0.13)	0.003	85.8%
		Overall without Azhdarzadeh et al. [38] and Rembach et al. [37] studies	20	−0.33(−0.56; −0.09)	0.007	77.3%

analysis performed by exclusion of Kapaki et al. [36] study showed no important difference in results [SMD = 0.17, 95% CI (−0.23; 0.58); $p = 0.150$; $I^2 = 70.4\%$; Table 4].

DISCUSSION

The objective of our meta-analysis was to review different studies comparing circulating zinc levels in

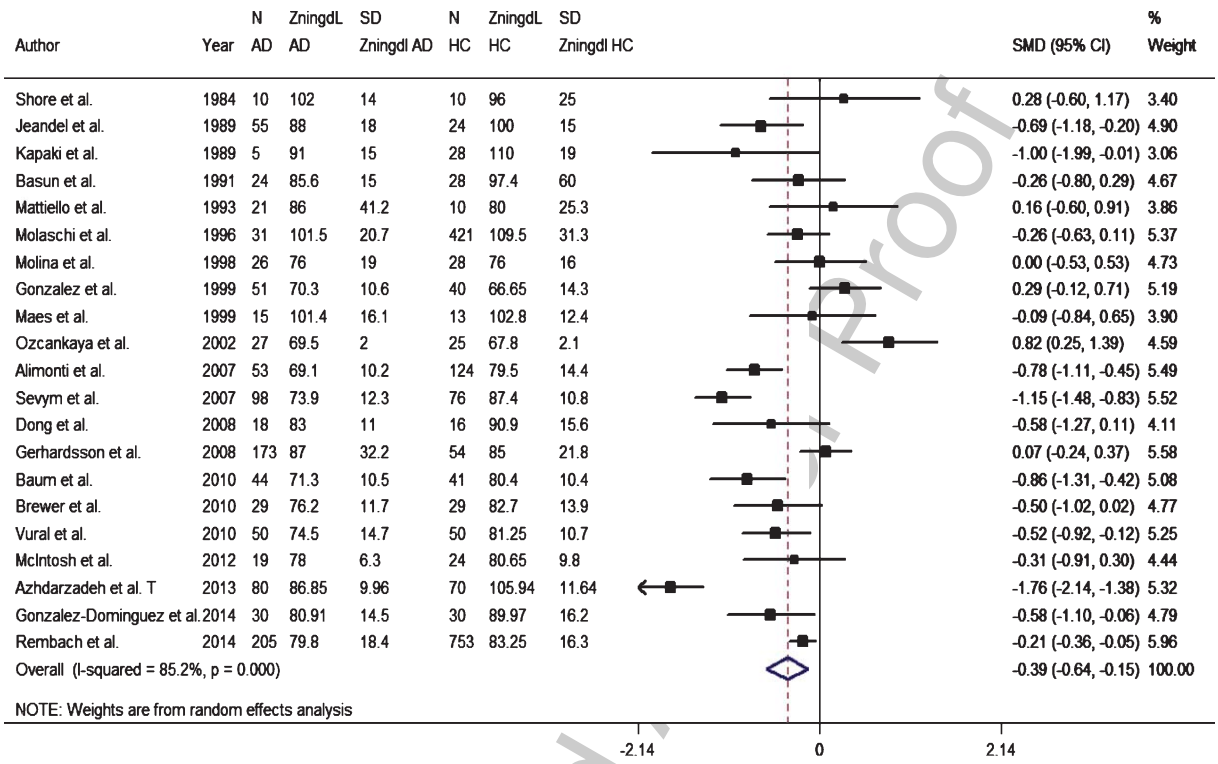

Fig. 3. Forest plot of meta-analysis of the standardized mean difference (SMD) of zinc levels in serum + plasma between AD subjects and controls. SMDs (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the diamonds of the pooled SMD based on the random-effects model Heterogeneity $\chi^2 = 134.93$ (d.f. = 20), $p < 0.001$. I^2 (variation in SMD attributable to heterogeneity) = 85.2%. Estimate of between-study variance $tau^2 = 0.255$; Test of SMD = 0: z = 3.17, $p = 0.002$. Dashed line represents the weighted mean value of all the studies analyzed. The solid line represents the zero of the difference (Δ) between AD and healthy controls.

Table 4
Meta-analysis of studies on CSF. Pooled standardized mean differences (SMD) with the corresponding 95% Confidence Intervals (CI) and p value, the I^2 statistic for overall, subgroup and sensitivity analyses

			n of studies	Pooled SMD (Random effects model, 95% CI)	p-value	I^2
Studies on CSF	Overall		6	0.06 (−0.36; 0.48)	0.776	71.9%
	Subgroup analysis	ICP MS	3	0.37 (−0.13; 0.87)	0.142	65.2%
		AAS	3	−0.35 (−1.10; 0.41)	0.366	76.4%
	Sensitivity analysis	Overall without Kapaki et al. [36] study	5	0.17 (−0.23; 0.58)	0.40	70.4%

AD patients to those in healthy controls and, by combining the results, to determine whether this metal is altered in AD.

The main result of this meta-analysis is the very high heterogeneity among the studies either in demographic terms (percentage of women, age) or in methodological approaches (methods of zinc analysis, study design). Although we considered these effects in our analyses, the heterogeneity persisted and it has to be taken into account in the interpretation of the results. Our meta-analysis indicates that serum zinc is significantly decreased in AD patients compared with healthy controls even though the high heterogeneity of the study analyzed recommends caution in the interpretation of the results. This result is also confirmed when we analyzed serum and plasma studies together.

The few studies conducted on plasma levels do not allow us to draw notable conclusions.

According to our meta-analysis, zinc levels in the CSF do not differ between AD patients and healthy individuals. However, the studies included showed some discrepancies: Hershey et al. [50] and Hozumi et al. [51] reported a zinc CSF-increase, but their healthy controls had a younger mean age in comparison

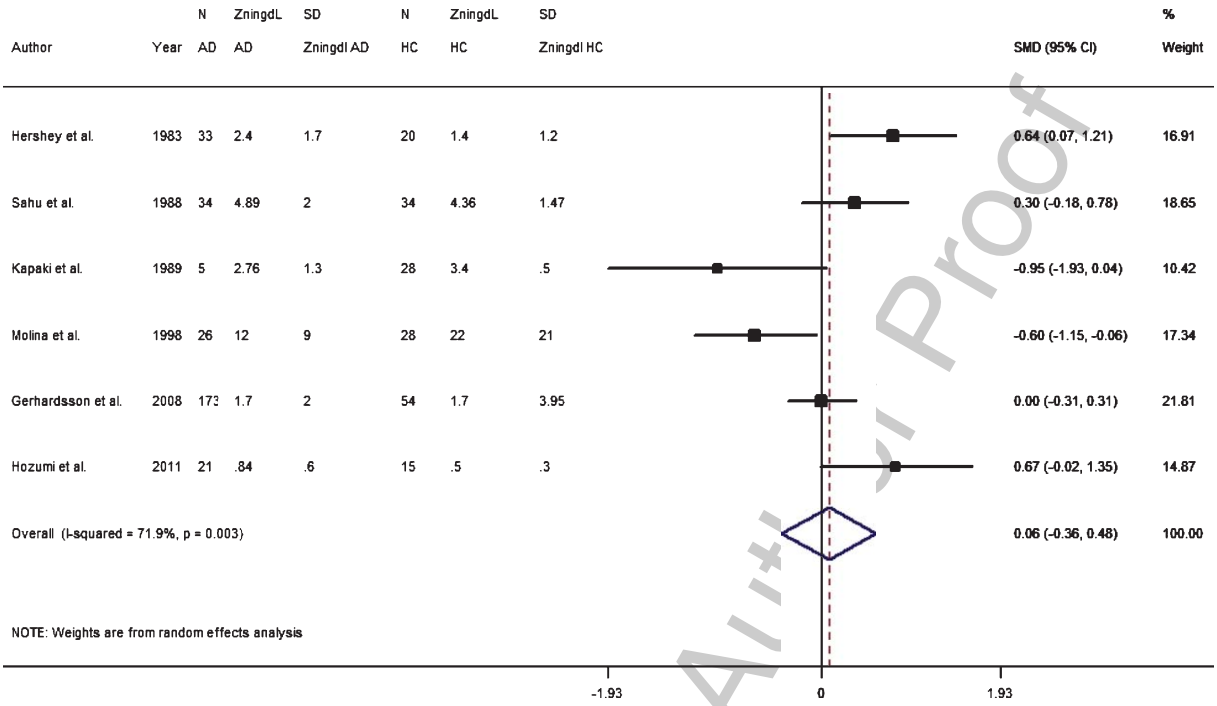

Fig. 4. Forest plot of meta-analysis of the standardized mean difference (SMD) of zinc levels in CSF between AD subjects and controls. SMDs (black squares) and 95% confidence intervals (bars) are given for each study. Also shown are the diamonds of the pooled SMD based on the random-effects model Heterogeneity $Chi^2 = 17.81$ (d.f. = 5), $p = 0.003$. I^2 (variation in SMD attributable to heterogeneity) = 71.9%. Estimate of between-study variance $tau^2 = 0.183$; Test of SMD = 0: z = 0.28, $p = 0.776$. Dashed line represents the weighted mean value of all the studies analyzed. The solid line represents the zero of the difference (Δ) between AD and healthy controls.

to the AD patients. This was particularly evident in the Hershey study, which reported a mean age quite distant from values of other studies. In contrast, two studies found a significant decrease of zinc CSF in AD patients [36, 42] and two others reported no difference [46, 52].

As depicted in the Results section, the high heterogeneity found among the studies is mostly explained on the basis of the method used for zinc measurements, zinc supplement intake (which was often not carefully controlled), and the age of the control individuals. Concerning the confounding effect of age, we applied meta-regression and aligned to the current guidelines suggested for meta-analyses [24], revealing a statistical effect of age on zinc circulating levels.

Our meta-analysis supports the hypothesis that lower levels of zinc are associated with AD. A growing body of evidence suggests that a deficiency, rather than an excess, of zinc leads to an increased risk for the development of neurological disorders and memory deficits [53]. Under normal brain physiology, the cellular zinc regulation results from the actions of different proteins involved in the uptake, excretion, and intracellular storage/trafficking of zinc. These proteins, including membranous transporters (ZnT and Zip) and MTs, which have been recently reported decreased in both aging and AD [53], can be an explanation for zinc deficiency in AD. However, the decreases that we have observed could also indicate a possible dietary zinc deficiency. In this respect, there have been various prospective studies, animal testing, or clinical trials investigating the effect of dietary zinc on cognitive function in elderly subjects [54–59].

Among these, Corona et al. [60] investigated the effect of dietary zinc supplementation in a transgenic mouse model of AD and found that controlling the brain zinc homeostasis was beneficial in delaying hippocampal-dependent memory deficits and strongly reducing both Aβ and tau pathology in the hippocampus in this mouse model. Concerning human studies, the Zenith study [61, 62], a multicenter prospective intervention study, reported a beneficial effect (but only at 3 months) of 15 and 30 mg/d of zinc supplementation for spatial working memory, and a detrimental effect of 15 mg/d for 'Matching to sample Visual search' test, even though the authors raised concern about subjects' compliance to the treatment [61]. The Zincage study [63], a prospective investigation carried out on a large sample of elderly healthy subjects of different

European countries, demonstrated that subjects' cognitive functions were decreased related to decreased zinc levels. Recently, Loef et al. [55] collected these and other studies in a systematic review to evaluate an association between zinc nutrition and AD, and reported that findings were not sufficient at that point to recommend a modification of dietary intake of zinc in AD. Our meta-analysis improves this knowledge, collecting scattered and heterogeneous results into one single piece of evidence, which demonstrates that AD patients have reduced levels of circulating zinc even though the high heterogeneity indicates that additional studied are needed. Zinc is a copper competitor in intestinal absorption, and the estimated 6-fold SD decrease in zinc levels that we have found is suggestive of systemic copper abnormalities. Thus, the finding of a serum zinc decrement in AD patients increases in meaning when it is interpreted as a further confirmation of the copper dysfunction extensively reported in AD [64]. Recently, this evidence has found support in meta-analyses and large population studies demonstrating increased levels of copper and Non-Cp copper in AD [4, 65, 66]. There is also a faster rate of cognitive decline associated with higher copper intake among persons with a median follow-up of 5.5 years whose diets were rich in saturated and trans fats [67]. Moreover, higher Non-Cp copper has been demonstrated to be associated with the MCI condition [11, 68] and with an increased rate of MCI conversion to full AD in a 6-year longitudinal study [12]. We do not know yet if the small percentage of zinc decrement in AD represents the other side of the coin of the copper dysfunction in AD, even though the two metals are certainly connected.

All these considerations support the idea that zinc supplementation can have the double potential to either re-establish the abnormal copper homeostasis typifying a percentage of AD patients [69], or to improve decreased levels of zinc. However, the role of zinc supplementation in AD needs further investigation, even though some studies have shown a delay of memory impairment [60] and others potentiation of cognitive abilities upon zinc supplementation [11, 55]. It might be possible that the loss of synaptic zinc through sequestration within plaques might lead to cognitive deficits [70] and to an impairment of neurogenesis. An interesting recent review suggests that a dietary supplement of zinc might be important at the very early stage of AD through the enhancement of hippocampal neurogenesis [71]. Recently it has been demonstrated that in the brain of Tg2576 mice treated with regimens of zinc acetate, there was a reduction in insoluble Aβ coinciding with a reduction in brain copper and interestingly no change in brain zinc, suggesting that blocking copper uptake can redistribute copper within the brain and reduce Aβ aggregation [72]. More precisely, zinc therapy is currently used for removing copper and preventing its reaccumulation in Wilson's disease, the paradigmatic disease of copper accumulation and toxicity. 100–150 mg/day of elemental zinc reduces copper absorption at the intestinal level, entrapping copper into enterocyte MT for excretion through the stools. This is usually used as a maintenance therapy for Wilson's disease patients [73]. Already around 2010, some [74–78] have emphasized the importance of a treatment based on the reduction of serum Non-Ceruloplasmin (Non-Cp) copper pool as a potential therapeutically beneficial approach for AD. In addition to the reviews on this topic appearing in that period [74–76, 78], one author [77] highlighted the relevance of the paradoxical effect of D-penacillamine, a chelating agent, which has been tested in AD [79]. D-penicillamine caused serious adverse events to AD patients, which determined the premature termination of that clinical trial [79]. In that review [77], the author discussed the D-penicillamine paradoxical effect in AD patients as suggestive of a causal link between Non-Cp copper pool and AD, resembling the paradoxical effect commonly observed in Wilson's disease people with neurological presentation [77, 80]. More specifically, 20% of Wilson's disease patients under chelating agents have paradoxical worsening of their neurological presentation [73]. A landmark study by one of the authors of the current manuscript [81] described a plausible mechanism of this paradoxical effect, temporally and causally linking an unfavorable course of neurological symptoms with an elevation in the serum Non-Cp copper pool, observed in Wilson's disease patients treated with the chelating agent trientine [81]. In this line, Hoogenraad [77] stressed the notion that the failure of the D-penicillamine clinical trial rather than showing the fallacy of the hypothesis on which this treatment was based on, namely that restoring the physiological copper homeostasis could be beneficial in terms of cognitive preservation, actually, confirmed it. On this basis, the review proposed a paradigm shift as, already discussed for Wilson's disease [80, 81], concerning the choice of zinc therapy to be preferred to copper-chelating agents to be used against AD [77]. This review [77] appeared just one year before the publication of the results of a pilot study by Brewer's group [75] addressing zinc therapy for AD, and reporting exiting results: a small 6-month clinical trial of zinc therapy reduced the serum Non-Cp copper pool in AD patients, elevated serum zinc levels,

and suggested improvement in three measures of cognition [19], confirming results of a very small study reporting that zinc aspartate given for 3 to 12 months improved memory, understanding and communication in 8 of the 10 AD patients treated [82]. However, high levels of zinc supplementation taken without medical supervision can cause side effects [83, 84]. Finally, a scientific consensus will be fully achieved only after a controlled clinical trial with zinc therapy evaluate benefit in reducing the progression of the cognitive decline, either through reducing copper absorption, or restoring normal neuronal zinc levels, or both.

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