ARTICLE

Brain copper may protect from cognitive decline and Alzheimer's disease pathology: a community-based study

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Copper is an essential micronutrient for brain health and dyshomeostasis of copper could have a pathophysiological role in Alzheimer's disease (AD), however, there are limited data from community-based samples. In this study, we investigate the association of brain copper (assessed using ICP-MS in four regions -inferior temporal, mid-frontal, anterior cingulate, and cerebellum) and dietary copper with cognitive decline and AD pathology burden (a quantitative summary of neurofibrillary tangles, diffuse and neuritic plaques in multiple brain regions) at autopsy examination among deceased participants ($N = 657$; age of death: 90.2(±6.2)years, 70% women, 25% *APOE*-ε4 carriers) in the Rush Memory and Aging Project. During annual visits, these participants completed cognitive assessments using a 19-test battery and dietary assessments (using a food frequency questionnaire). Regression, linear mixed-effects, and logistic models adjusted for age at death, sex, education, and *APOE*-ε4 status were used. Higher composite brain copper levels were associated with slower cognitive decline (β(SE) = 0.028(0.01), $p = 0.001$) and less global AD pathology (β(SE) = −0.069(0.02), $p = 0.0004$). Participants in the middle and highest tertile of dietary copper had slower cognitive decline (T2vs.T1: β = 0.038, $p = 0.0008$; T3vs.T1: β = 0.028, $p = 0.01$) than those in the lowest tertile. Dietary copper intake was not associated with brain copper levels or AD pathology. Associations of higher brain copper levels with slower cognitive decline and with less AD pathology support a role for copper dyshomeostasis in AD pathogenesis and suggest that lower brain copper may exacerbate or indicate disease severity. Dietary and brain copper are unrelated but dietary copper is associated with slower cognitive decline via an unknown mechanism.

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INTRODUCTION

Neuritic plaques and neurofibrillary tangles are the hallmarks of Alzheimer's disease (AD), a common progressive neurological disorder of aging. AD pathophysiology, including the deposition of these proteinopathies, has been associated with brain copper. For example, ionic copper has a strong affinity for amyloid-beta and promotes its aggregation [1–3]. Yet, a meta-analysis reported lower brain copper [4] and high systemic copper [5] among AD patients compared to those without dementia. Indeed, various AD animal models showed that reduced copper is associated with increased severity of plaques [6, 7].

Copper is an essential nutrient for the brain. It is the active site of various enzymes involved in cellular respiration, energy metabolism, neurotransmitter biosynthesis, iron metabolism, gene transcription, and antioxidant defense [8]. Its importance to brain health is demonstrated by the genetic diseases, Menkes disease and Wilson disease, where a decrease and increase (respectively) of brain copper causes neurodegeneration [9, 10].

Copper uptake is primarily from diet but other minor sources include drinking water and occupational and environmental pollutants. We previously reported that higher dietary copper in the presence of a high-fat diet is associated with faster cognitive decline [11]. Recent cross-sectional analysis using NHANES data also reported a non-linear association between copper intake and cognitive function [12]. But whether dietary copper is related to brain copper is not known, since the blood-brain barrier limits copper uptake into the brain. To our knowledge, no prior study has investigated the relationship of dietary copper intake and brain copper levels with longitudinal changes in cognitive function and AD pathology. In this study, using a large community-based sample of older adults who were followed up to a decade before death, we investigate the association of 1) brain copper levels and 2) dietary copper intake with a) longitudinal cognitive decline and b) AD neuropathology.

MATERIALS AND METHODS

The study was conducted among 657 autopsied participants of an ongoing longitudinal clinical-neuropathologic study, the Rush Memory and Aging Project (MAP) of older adults from around 40 retirement communities,

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subsidized housing, and individual homes around Chicagoland [13]. At enrollment, participants are without known dementia, and sign an informed consent (obtained according to the Declaration of Helsinki) for annual clinical visits during the follow-up, an Anatomic Gift Act for donation of brain, at the time of death and a repository consent to allow their resources to be shared. The study was approved by an Institutional Review Board of Rush University Medical Center. From the beginning of the MAP study in 1997 until 2017, 884 MAP participants died and had an autopsy, and 680 were consecutively analyzed for brain metal levels. Out of 680 participants, 664 had available neuropathological data, cognitive data, and brain copper data in all four regions. We excluded 7 participants with outlier values of brain copper in exactly one brain region, leaving 657 participants for analysis.

Brain copper levels

Brain copper levels were assessed in the brain tissue from the inferior temporal cortex, mid frontal cortex, anterior cingulate cortex (areas affected by AD), and the cerebellum (minimal AD pathology). The samples were cut into 50 ± 5 mg using a ceramic blade to avoid metal contamination. The samples were weighed and homogenized using Tris-buffered saline containing phosphatase- and protease- inhibitors and were stored at $-80\,^{\circ}C$ until analysis. The copper levels in each tissue sample were measured in triplicate using Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Agilent 7700). The concentrations determined from the standard curve were normalized to wet tissue weight. Averaging z-scored copper levels from all four regions gave composite copper levels.

Dietary copper intake

Dietary assessments in MAP started in 2004, when 83 MAP participants had already died, and 9 more had withdrawn from the study. Another 361 withdrew or died without completing an FFQ and 7 refused to fill out the FFQ. For 299, derivation of dietary data was in process. In our analytic sample of 657, 453 participants had dietary data available and of these 446 had longitudinal cognitive assessments. To investigate dietary copper association with cognitive decline we include cognitive data on 3588 visits for these 446 participants.

Dietary Intake of copper was assessed using a modified Harvard semi-quantitative food frequency questionnaire (FFQ) with 144 items validated for use in older Chicago community residents [14]. The copper content of each food was based on United States Department of Agriculture (USDA) food composition tables and supplementary nutrient databases. Daily copper intakes for each participant were computed by summing over the frequency-based nutrient content of all food items and dietary supplements. Various foods including nuts, whole grains, liver, seafood, sweet potato, green leafy, other vegetables, and fruits contributed to total copper intake. For this analysis, the mean daily intake of copper (from food and supplement) during the follow-up was considered.

Cognitive assessments

Annual cognitive assessments were done in the study, as described previously [15]. The global cognitive score was derived using a 19- test battery. Scores on five cognitive domains; i.e. episodic memory, semantic memory, perceptual speed, visuospatial ability, and working memory were obtained. In our analytic sample of 657, 621 participants had two or more complete cognitive assessments over up to 17 years of follow-up. During the mean follow-up of 6.7 ± 3.8 years we include cognitive data on the 4428 visits for these 621 participants.

Alzheimer's disease neuropathology

The study methods for brain autopsy and pathological evaluations have been described in detail previously [16]. Immunohistochemistry was used to assess the amyloid-beta (one of three antibodies as previously described [17]) and Phosphorylated tau-tangles (antibody: AT8, Innogenetics, San Ramon, CA, 1:1000) were assessed using from multiple brain regions, including entorhinal, mid frontal, inferior temporal, angular gyrus, calcarine, anterior cingulate, and superior frontal cortices and hippocampus as previously described [17]. A composite continuous summary measure of the total amyloid load and neurofibrillary tangle density per mm^2 was generated by the mean percent area of each region occupied by each pathology [18].

AD pathology markers including diffuse and neuritic amyloid plaques and neurofibrillary tangles were identified using modified Bielschowsky silver-staining from frontal, temporal, parietal, and entorhinal cortices, and hippocampus. The raw scores (the highest number in the 1 mm^2 area) were standardized in each region, then averaged across the regions to create three summary scores, which were then averaged to obtain the global AD pathology burden. We also analyzed semi-quantitative measures of AD pathology including Consortium to Establish a Registry for Alzheimer Disease (CERAD) for neuritic plaques severity [19], Braak stage for neurofibrillary tangle severity [20] and National Institute on Aging (NIA) -Reagan criteria [21] for defining pathologic AD with intermediate and high likelihood cases indicating a pathologic diagnosis.

Other covariates

Age at death in years was computed from the dates of birth and death. At enrollment sex, and education (in years), were self-reported. Apolipoprotein (APOE-ε4) genotyping was performed by Polymorphic DNA Technologies [22]. Clinical Alzheimer's dementia diagnosis was based on the criteria of the joint working group of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA) [23]. The mean frequency of participation in cognitive activities includes reading, writing letters, visiting a library, and playing chess or checkers, was constructed as a composite score described previously [24]. Physical activity (minutes per week) was derived for each clinical visit based on self-reported time spent over two weeks on five activities (walking for exercise, yard work, calisthenics, biking, and water exercise) [25]. Other dietary nutrients (saturated fats, vitamin E, lutein, and folate) were also assessed using the FFQ data as described previously [11, 26].

Statistical methods. Demographic and other characteristics of study participants were examined by tertile of composite copper level (combining all four brain regions inferior temporal, mid frontal, anterior cingulate, and cerebellum). The correlations between the copper levels within each brain region, overall brain copper, and dietary copper were assessed using Pearson correlation coefficients. Brain copper levels were compared between the groups: AD pathology among different groups (with and without clinical dementia diagnosis, dementia without AD pathology, and no dementia or AD pathology) using ANOVA. Baseline characteristics in Table 1 was compared using ANOVA or chi-square test. The relationship of brain copper (continuous variable and in tertiles) with AD-related outcomes was assessed by separate models using the composite copper level and the four region-specific copper levels. The composite scores for global AD pathology burden, amyloid beta load, and phosphorylated -tau tangles were square root transformed to fit the model assumption of linear regression models. We primarily studied three different associations: 1) Brain copper levels with cognition by regressing cognition score on brain copper levels and by employing linear mixed models during the years prior to death, (models controlled for age at death, sex, education, and APOE-ε4status); 2) Brain copper and AD pathology using a linear regression model (for global AD pathology burden, amyloid beta load, and phosphorylated -tau tangles) and ordinal logistic regression models (for Braak stage, NIA-Reagan, and CERAD) adjusted for age at death, sex, education, and APOE-ε4status; 3) Mean dietary copper (calorie-adjusted using the residual regression method [27] and assessed in tertiles) with cognitive decline using linear mixed model controlled for age, sex, education, late-life cognitive activities, physical activity, and APOE-ε4 status. The linear trend was also examined for all the models where observations within the tertile were assigned the median value in the corresponding tertile in regression models.

In secondary analyses to assess whether the association of brain copper with cognitive decline depends upon AD pathology score, AD pathology (NIA-Reagan) and an interaction between copper and AD pathology was added in the model. Additional examination done includes a) brain copper and cognitive decline models further controlled for other confounding factors, late-life cognitive activities, and physical activities, b) dietary copper and cognitive decline models further controlled for other calorie-adjusted nutrients: i) saturated fats, vitamin E, lutein, and folate, known for their relation to cognitive decline ii) dietary iron, zinc, and manganese. To investigate if dietary fat modifies the association of dietary copper on cognitive decline [11], we also tested a three-way interaction between dietary copper, saturated fat, and time in the mixed effect model.

The MAP data can be requested. Interested investigators may find additional information at https:// www.radc.rush.edu.

RESULTS

The mean age of death for the 657 study participants was 90.2 (±6.2) years, 70% were women, and 25% carried an *APOE*-ε4 allele.

Table 1. Characteristics of study population overall and by tertile of composite brain copper levels.

	Overall	Brain copper (all four regions)			The *P*-value for tertile comparisons
		Tertile 1	Tertile 2	Tertile 3	
N	657	217	223	217	
Composite copper z-score, Mean ±SD	0.000 ± 0.74	−0.73 ± 0.29	−0.09 ± 0.17	0.82 ± 0.60	
Copper (μg/g) [a]Median (IQR)	4.16 (3.6, 4.9)	3.21 (2.94, 3.58)	4.16 (3.95, 4.39)	5.22 (4.89, 5.85)	
Age at death, years	90.2 ± 6.2	90.7 ± 6.0	90.5 ± 6.5	89.4 ± 6.1	0.058[b]
Education, years	14.6 ± 2.9	14.5 ± 3.1	14.5 ± 2.7	14.8 ± 2.8	0.749[b]
Female%	70%	76%	70%	64%	0.029[c]
APOE-ε4 status, % present	25%	26%	24%	26%	0.807[c]
Pathological diagnosis of Alzheimer's disease, %	66%	69%	64%	64%	0.998[c]
Global AD pathology score	0.74	0.80	0.74	0.68	0.230[c]
Clinical diagnosis of Alzheimer's dementia[d], %	42%	49%	40%	38%	0.067[b]
Total Dietary copper, mg/day ($n = 453$)	2.3 ± 1.2	2.1 ± 1.0	2.4 ± 1.2	2.3 ± 1.3	0.520[c]

IQR Interquartile range.
[a]The median values expressed in μg/g are based on average values from four regions (inferior temporal, mid-frontal, anterior cingulate, and cerebellum). The tertile categorization was based on composite copper values- z scored of four areas combined.
Pathological diagnosis of Alzheimer's disease based on NIA-Regan score.
[b]Avona comparison.
[c]Nonparametric (*Kruskal –Wallis test* or chi-square test).
[d]Proximate to death.

The mean age of participants at the first cognitive assessment was 82.7 (±5.8) years and they had a mean follow-up of 6.7 ± 3.8 years prior to death. Out of 657 decedents, 279 (42%) had a clinical diagnosis of Alzheimer's dementia proximate to death. The copper levels in the inferior temporal, mid-frontal, and cerebellum were similar, whereas the anterior cingulate levels were slightly lower overall (Supplementary Table 1). The correlation of copper levels in different brain regions assessed was significant ($0.32 < r < 0.53$; $p < 0.0001$; Supplementary Fig. 1). The baseline characteristics of the study population as per the tertile of composite copper levels were similar, except there were fewer females ($p = 0.029$) in the higher tertile of copper levels than the lower tertile (Table 1). Overall composite copper levels were not significantly different by grouping participants based on the presence or absence of dementia diagnosis and AD pathology ((with and without clinical dementia diagnosis, dementia without AD pathology, and no dementia or AD pathology, ANOVA $p = 0.08$). There was no association between brain copper levels and mean dietary copper intake ($p > 0.05$).

Brain copper and cognitive decline

In the cross-sectional association between the composite brain copper levels and the global cognitive score proximate to death, we found no significant association ($N = 657$, β (SE) = 0.11 (0.07), $p = 0.09$). In the region-specific analysis, copper in the inferior temporal region (β (SE) = 0.11 (0.04), $p = 0.01$) but not in other regions (mid-frontal cortex: (β (SE) = 0.08 (0.04), $p = 0.07$); anterior cingulate (β (SE) = −0.004 (0.04), $p = 0.92$; cerebellum: (β (SE) = 0.005 (0.04), $p = 0.90$)) was positively associated with the cognition proximate to death.

In the longitudinal analysis, among those with at least two cognitive assessments during the follow-up years, a higher composite brain copper level was associated with a slower decline in global cognition, episodic memory, semantic memory, perceptual speed, and working memory (Table 2). Those in the highest tertile of the composite brain copper level declined more slowly by 0.030 unit per year for global cognition than the lowest tertile during the mean follow-up 6.7 ± 3.8 years (Table 2, Fig. 1). In region-specific analyses, copper in the inferior temporal region was associated with slower decline in global cognition (β (SE) = 0.015 (0.005), $p = 0.003$) as well as specific cognitive domains, including episodic memory, semantic memory, perceptual speed and working memory ($p < 0.05$, Supplementary Table 2). Higher copper in mid-frontal region was also associated with slower decline in global cognition and cognitive domain scores for episodic memory, semantic memory and working memory ($p < 0.05$, Supplementary Table 2). Higher copper levels in the anterior cingulate were also associated with slower decline in global cognition, episodic memory, and visuospatial ability (Supplementary Table 2). The copper levels in the cerebellum were not associated with cognitive decline.

When further controlled for other factors related to cognition including physical and cognitive activities, the composite brain copper level associations with slower decline in global cognition (p trend= 0.02), perceptual speed (p trend = 0.03), and working memory (p trend = 0.016) were retained, however associations with episodic memory (p trend = 0.054) and semantic memory (p trend = 0.058) were attenuated and no longer significant.

Brain copper and AD pathology

Composite brain copper was associated with lower global AD pathology burden (β(SE) = −0.069 (0.02), $p = 0.0004$; Fig. 2a), lower amyloid-beta load ((β(SE) = −0.132 (0.06), $p = 0.027$; Fig. 2b), and fewer phosphorylated tau-tangles (β(SE) = −0.289 (0.07), $p = 0.00003$; Fig. 2c). Tertile comparisons indicated a similar inverse relationship between brain copper and AD pathology (Table 2). When exploring individual brain regions, higher copper in the inferior temporal (β(SE) = −0.061 (0.014), $p = 0.00002$) and mid-frontal region (β(SE) = −0.046 (0.014), $p = 0.002$) was significantly associated with a lower global AD pathology score. Higher inferior temporal and mid frontal brain copper was also related to fewer phosphorylated tau tangles and a lower amyloid-beta load (Supplementary Table 3). Copper in the anterior cingulate had no association with any AD pathology score and copper in cerebellum region was inversely associated only with phosphorylated-tau tangles (Supplementary Table 3).

Table 2. Association of brain copper levels with cognitive decline (global cognition and different domains) among deceased Memory and Aging Project participants.

		Composite Brain copper levels (four regions)[a]				
	N	Continuous	Tertile comparison			
			T1	T2	T3	*P* -trend
Change in cognition, global and the domains						
Global Cognition β (SE, *p*-value)	621	0.028 (0.01,0.001)	Ref	0.021 (0.011,0.054)	0.030 (0.011,0.009)	0.009
Episodic memory β (SE, *p*-value)	619	0.027 (0.01, 0.006)	Ref	0.015 (0.012,0.216)	0.028 (0.013, 0.029)	0.03
Semantic memory β (SE, *p*-value)	609	0.035 (0.01, 0.0008)	Ref	0.023 (0.013,0.074)	0.030 (0.013, 0.025)	0.03
Visuospatial ability β (SE, *p*-value)	601	0.013 (0.01, 0.183)	Ref	0.010 (0.012,0.401)	0.007 (0.012, 0.559)	0.60
Perceptual speed β (SE, *p*-value)	600	0.024 (0.01, 0.007)	Ref	0.030 (0.011, 0.006)	0.028 (0.011, 0.015)	0.02
Working memory β (SE, *p*-value)	621	0.026 (0.01, 0.003)	Ref	0.017 (0.011, 0.111)	0.030 (0.011, 0.007)	0.007
Alzheimer's disease (AD) pathology outcome						
Global AD pathology, β (SE, *p*-value)	657	−0.069 (0.019,0.0004)	Ref	−0.118 (0.035,0.0008)	−0.095 (0.035,0.007)	0.006
Amyloid load, β (SE, *p*-value)	657	−0.132 (0.060,0.027)	Ref	−0.315 (0.11, 0.003)	−0.206 (0.11, 0.058)	0.092
Phosphorylated -tau tangles, β (SE, *p*-value)	657	−0.289 (0.068,0.00003)	Ref	−0.370 (0.12, 0.003)	−0.358 (0.12, 0.004)	0.001

[a]Composite brain copper levels: Brain copper in all four regions combined (inferior temporal, mid-frontal, anterior cingulate, and cerebellum**).**
Statistical models for cognitive outcomes were linear mixed-effects regression models; estimates are coefficients of the interaction of brain copper composite with time before death. The linear mixed-effect models were controlled for age at death, sex, education, APO-ε4 status, time and time * all covariates.
Statistical models for AD pathology outcomes were linear regression models; estimates are coefficients of brain copper composite. All the models were controlled for age at death, sex, education, and APO-ε4 status.

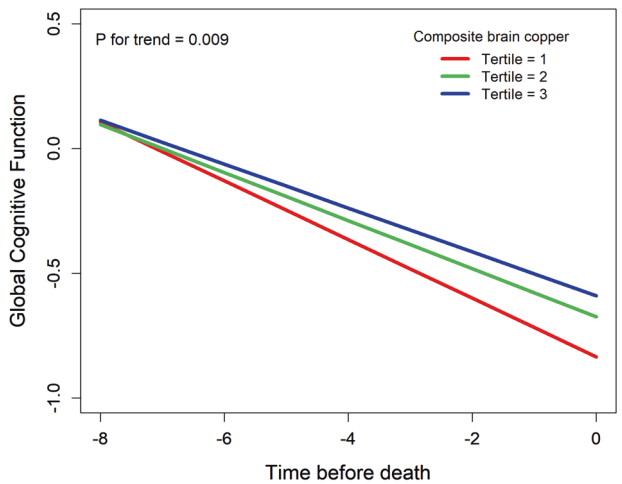

Fig. 1 Brain copper levels and cognitive decline. Association of composite brain copper levels with cognitive decline among 621 autopsied Memory and Aging Project participants. *Composite brain copper levels: Brain copper in all four regions combined (inferior temporal, mid-frontal, anterior cingulate, and cerebellum**)**. Linear mixed-effect model controlled for age at death, sex, education, and APO-ε4 status.

The composite brain copper level was also associated with reduced odds of Braak stage and NIA Reagan score (Supplementary Table 4). Participants in the highest and middle tertiles of composite brain copper levels compared to those in the lowest tertile had 40% (OR = 0.60, 95% CI (0.41, 0.88) and 38% (OR = 0.62, 95% CI (0.42, 0.90) lower odds of having an advanced Braak stage, respectively. For region-specific associations in separate models, higher copper within the inferior temporal and mid-frontal region was associated with lower odds of advanced Braak stage or NIA Reagan but not CERAD score. However, copper in the anterior cingulate and cerebellum region was inversely associated only with the Braak stage but not the NIA Reagan or CERAD scores (Supplementary Table 4).

To understand if the brain copper association with cognitive decline is independent of any association with AD pathology, we added global AD pathology to brain copper and cognitive decline models. The effect estimates were similar or slightly lower. Higher composite copper levels were associated with slower decline in global cognition (T3 vs. T1: (β(SE) = 0.028 (0.009), $p = 0.002$; p trend=0.008), episodic memory ((T3 vs. T1: (β(SE) = 0.023 (0.010), $p = 0.032$; p trend=0.026), semantic memory ((T3 vs. T1: (β(SE) = 0.040 (0.011), $p = 0.0003$; p trend=0.020), perceptual speed ((T3 vs. T1: (β(SE) = 0.027 (0.010), $p = 0.009$; p trend = 0.017) and working memory ((T3 vs. T1: (β(SE) = −0.023 (0.010), $p = 0.002$; p trend = 0.018). We further investigated any effect modification due to AD pathology and found no significant interactions between brain copper and AD pathology for cognitive decline (all p's for interaction >0.05); we did not further analyze these relationships.

Dietary copper, cognitive decline and AD pathology

In our analytic sample, the majority of participants (>99%) had an average copper intake (from foods and supplements) equal to or more than recommended dietary allowance of 0.9 mg/day. In our subgroup analysis of the analytical sample with complete dietary data and at least two cognitive assessments, we found those in the highest and middle tertile of mean dietary copper had a slower antemortem global cognitive decline (Table 3, $N = 446$ deceased participants). However, the effect estimate for the middle tertile was higher than that of the highest tertile (T2 vs. T1: β = 0.038, $p = 0.0008$; T3 vs. T1: T2: β = 0.028, $p = 0.01$) when controlled for potential confounding factors probably indicating some non-linear associations. Similar associations were found for various cognitive domains, including semantic memory, perceptual speed, visuospatial ability, and working memory (Table 3). Those in the highest tertile of dietary copper compared to those in the lowest, reported a slower decline in episodic memory. We also assessed this association in the overall MAP cohort with complete dietary data and at least two cognitive assessments ($N = 961$, including both deceased and alive) and found that, in this larger group, higher dietary copper intake was associated with slower decline in global cognition, episodic memory, visuospatial ability, and perceptual speed (Supplementary Table 5).

Further controlling these models for various dietary factors, including saturated fats and other nutrients (vitamin E, Lutein, folate) and other dietary metals (iron, manganese, and zinc), retained the associations of dietary copper with cognitive decline ($p < 0.05$, data not shown). We found no significant interaction between saturated fat and dietary copper for its effect on cognitive decline (p for interaction term = 0.614). There was no significant association between dietary copper and AD pathology in our analytic sample ($n = 453$, Supplementary Table 6) or overall autopsied participants with dietary copper intake (data not shown).

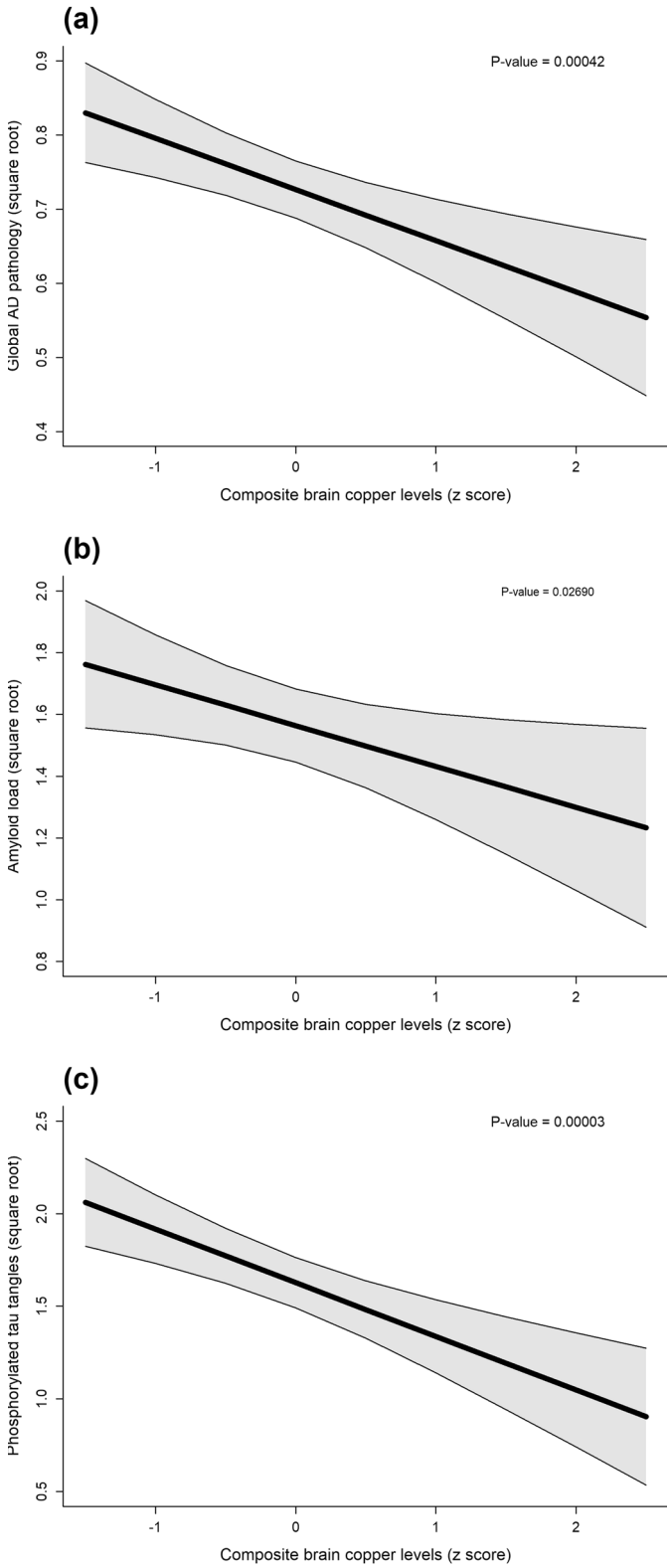

Fig. 2 Brain copper levels and AD pathology. Association of composite brain copper levels with **a** Global AD pathology **b** Amyloid load **c** Phosphorylated tau tangles Linear regression models controlled for age at death, sex, education, and APO-ε4 status.

DISCUSSION

Among decedents of a community-based clinical-neuropathologic study, higher brain copper levels were associated with slower cognitive decline during the decade before death and less AD pathology upon death. High brain copper levels in the inferior temporal and the mid-frontal cortex, areas particularly affected in AD, had the most robust inverse relationship with the AD outcomes (change in cognitive function and pathology). At the time of death, brain copper levels were not correlated with the mean dietary intake of copper over the years of follow-up. However, dietary copper was negatively associated with cognitive decline. To our knowledge, this is the first study investigating the role of brain and dietary copper in cognitive decline and AD pathology using a large community-based sample. These relations of brain copper with neuropathologic determinants and clinical outcomes of AD support the role of altered copper homeostasis in the disease process.

The inverse relation of brain copper levels with AD pathology and cognitive decline in this large community-based sample supports the current findings of lower brain copper in AD [28–30]. These results also support existing studies on the role of copper in AD pathophysiology. For example, in-vitro studies indicated that copper depletion promotes amyloid-beta production [31–33]. Animal studies report that elevation of intraneuronal copper (genetically [6], by pharmacological ionophores [34, 35] or via dietary supplements [36]) suppresses amyloid pathology. A phase two clinical trial of the metal-protein attenuating compound, PBT2, reported improvement in executive function [37] and its proposed mechanism of action was increased copper uptake (ionophore). A recent human study also reported elevated cerebrospinal fluid (CSF) levels of ceruloplasmin (a copper-binding protein that promotes iron export and lowers iron-induced oxidative stress) associated with faster cognitive decline and brain atrophy in those with amyloid-beta pathology [38]. Our study findings on copper in inferior temporal region, association with less global AD pathology, amyloid-beta, phosphorylated-tau tangles, and slower cognitive decline even when controlled for AD pathology, indicates that copper might play a protective role against disease severity and maintain cognitive resilience i.e., no cognitive impairment even in the presence of AD pathology via unknown pathways. To further understand the pathogenic role of copper dyshomeostasis in the AD process of the aging brain it is important to consider genetic, metabolic, and clinical factors [4].

The role of dietary copper in AD appears complex. While we found no association between dietary copper and brain copper, dietary copper was associated with slower cognitive decline. The lack of association between dietary and brain copper supports the previous findings of brain copper concentrations being independent of diet among those with and without neurodegenerative diseases [39]. We also could not explain variance in dietary copper interaction with saturated fat for cognition, as previously reported by our group in another cohort of older adults [11]. A recent cross-sectional analysis using NHANES data also reported a nonlinear association between copper intake and cognitive function with a threshold effect of copper intake on CERAD test, animal fluency test and Digital Symbol Substitute Test at around 0.8 and 1.4 mg/d [12]. In our study copper intake was associated with slower decline

Table 3. Association of mean dietary copper (calorie adjusted) with cognitive decline over years of follow-up ($n = 446$).

	[a]Dietary copper by tertile β (SE, *p* value)			*P* value for trend
	1	2	3	
Global Cognition	Reference	0.038 (0.01, 0.0008)	0.028 (0.01, 0.01)	0.04
Episodic memory	Reference	0.035 (0.01, 0.008)	0.032 (0.02, 0.02)	0.04
Sematic memory	Reference	0.057 (0.02, 0.006)	0.037 (0.02, 0.08)	0.15
Visuospatial ability	Reference	0.030 (0.01, 0.01)	0.013 (0.01, 0.30)	0.52
Perceptual speed	Reference	0.032 (0.01, 0.007)	0.018 (0.01, 0.12)	0.25
Working memory	Reference	0.022 (0.01, 0.05)	0.021 (0.01, 0.07)	0.11

[a]Calorie-adjusted dietary copper.
Linear mixed models adjusted for age, sex, education, late-life cognitive activities, physical activities, and APO-ε4 status.

in cognitive function during the years of follow-up, however we do not report any threshold effect with cognitive function at any single time point. The calorie-adjusted mean copper intake over the years of follow-up in our study obtained using a validated FFQ (captures participants' usual consumption of various food items and dietary supplements over the past year) was 2.3 ± 1.2 mg/day, which was higher than the mean intake reported using the two 24h diet recalls in the NHANES data (1.2 ± 0.7 mg/ day, not calorie-adjusted) [12]. However, the mean copper intake through food sources only in our overall study population was 1.4 ± 0.6 mg/day but 55% of our participants reported consuming multivitamins and dietary supplements containing copper. The difference in copper intake between our study and national dataset may be because our study group is majority non-Hispanic Whites, with average age of around 85 (±6) years at the time of first dietary assessment, whereas NHANES participants are much younger on average and more culturally diverse.

Could copper supplementation be therapeutic for AD? An animal study found dietary copper reduced amyloid-beta production in APP23 transgenic mice [36]. However, the phase 2 randomized control trial for oral copper supplementation of 12 months in AD patients reported no impact on CSF biomarkers [40] or cognitive decline [40]. Similarly, a copper and zinc supplementation trial among 2166 older adults with a mean of 6.9 years of follow-up reported no effect on cognition and incidence of cognitive impairment [41]. To further understand the role of dietary copper in AD, it may be critical to differentiate the role of copper from different sources, i.e., from foods vs. supplements, and to consider a possible peripheral target that impacts on brain status (e.g., endocrine, or immune systems).

Indeed, it is possible the amount or composition/source of copper may be critical in determining whether copper is beneficial or detrimental. Chronic low dose copper treatment ($CuCl_2$) in drinking water of rats [42], mice [43], and mice with AD-causing genetic mutations [44] causes memory impairment. Copper treatment to mice caused oxidative damage to DNA and proteomic analysis revealed loss of synaptic proteins and changed mitochondrial proteins in the hippocampus [45]. Changes to mitochondrial proteins were likewise observed in cortex of mice that were treated under the same regime. So, there is a risk that copper treatment may worsen disease outcomes.

This study has various strengths, including many brain autopsies analyzed from participants of a well-defined community cohort, copper level assessments and several brain pathologies examined in multiple brain regions, using standardized measures, nearly a decade of annual follow-up before death, a comprehensive battery of 19 cognitive tests, and dietary assessments on these participants. However, a primary limitation of the study is the cross-sectional analysis of brain copper levels and AD pathology, which prevents causal interpretation of the findings. In addition, the study has no data on the source of other potential environmental exposure to copper, including that from drinking water, and there is a potential for inaccurate assessment of dietary copper as the copper content of plant food depends on soil and may vary by region.

In conclusion, the relation between higher brain copper levels and slower cognitive decline, which is most robust in the regions vulnerable to AD, supports the role of copper homeostasis in maintaining brain health and possibly delaying the disease process. The inverse association of copper with AD neuropathology suggests these copper levels may reflect the severity of the disease or indicate its potentially beneficial effect on AD pathology. Dietary copper does not associate with brain copper levels, but it still was associated with slower cognitive decline. Further studies are required to understand and further validate copper as a potential therapeutic target for AD.

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AUTHOR CONTRIBUTIONS

PA, SA, AIB, DAB and JAS: conceptualization, PA, SA, SEL: data analysis PA: manuscript preparation, edits and revisions, SA, SA, KD, DAB, LLB, SEL, AIB, and JAS: manuscript review and editing, SA, AIB, and JAS: supervision, SA, DAB, AIB, and JAS: funding acquisition.

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COMPETING INTERESTS

AIB Holds equity: Alterity Biotechnology Ltd, Cogstate Ltd, Mesoblast Ltd, Collaborative Medicinal Development LLC. Paid consultant: Collaborative Medicinal Development Pty Ltd. Julie A Schneider and other authors report no competing interests.

ADDITIONAL INFORMATION

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