Systematic Review

Copper and iron in Alzheimer's disease: a systematic review and its dietary implications

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Abstract

Fe and Cu could represent dietary risk factors for Alzheimer's disease (AD), which has become a global health concern. To establish the relationship between diets high in Cu and Fe and cognitive decline or AD, we have conducted a systematic review of the literature (up to January 2011). We identified two meta-analyses, two systematic reviews, eleven placebo-controlled trials, five observational studies, forty-five case–control studies, thirty autopsy and five uncontrolled studies, and one case report. There were eleven interventional trials that tried to either supplement or deplete Fe and Cu, but none of them provided clear evidence of a beneficial effect on cognitive performance in patients with AD. The prospective studies revealed an association between a diet simultaneously high in SFA and Cu and cognitive decline. Case–control and autopsy studies showed elevated Fe levels in the brains of AD patients, whereas the evidence was less consistent for Cu. In most of the studies, Cu concentrations were unchanged in the cerebrospinal fluid and the brain but increased in the serum. In conclusion, the existing data suggest that diets excessive in Fe or Cu, together with a high intake of SFA, should be avoided in the elderly who are not at risk of anaemia. Basic studies and, building on this, clinical investigations are needed to further elucidate in which dietary patterns and in which patient groups an Fe- and Cu-rich diet might foster the risk of developing AD.

Key words: Alzheimer's disease: Copper: Iron

Fe and Cu are essential to human life[1]. Their chemical properties as transition metals make them crucial for a plethora of important biological processes such as oxidative metabolism, electron transport in mitochondria and cellular immune response[2,3]. On the other hand, both metals catalyse the Fenton and the Haber–Weiss reactions, producing reactive oxygen species[4], which can foster the pathological paths of neurodegenerative disorders[5] and have been implicated in age-associated diseases[6–9]. In the brain, they are found at high concentrations[10,11] that increase with age[12–15], and increasing evidence suggests that the neuronal homeostases of Cu, Fe and Zn are altered in Alzheimer's disease (AD)[16]. Although Zn has multiple physiological roles in AD including the aggregation and degradation of the amyloid β protein (Aβ)[17], it is redox silent and distinct from Cu and Fe in its chemical properties, its age- and tissue-specific concentration

dynamics and its nutritional status in the elderly. Therefore, the present review focuses on Cu and Fe only.

AD is a progressive brain disease that symptomatically leads to an impairment of memory and diverse cognitive functions[18]. It accounts for up to 75 % of the 35·6 million dementia cases, which are estimated to have occurred globally in 2010[19]. Its prevalence is forecasted to quadruple by 2050, when it is anticipated that one in eighty-five persons will be living with AD[20].

Whereas familial AD is inherited in an autosomal dominant manner, a variety of risk factors influence the sporadic late-onset AD that accounts for the vast majority of all cases[21]. Thus, a number of genetic risk factors have been identified, such as mutations in genes of the apoE, the amyloid β precursor protein (APP) or the presenilin 1 and 2, which participate in the cleavage of APP[22]. In addition, there is increasing

evidence of the important roles of metals in the molecular biology of AD[16] and lifestyle parameters – in particular diet – as protective factors[23–25].

The characteristic neuropathological features of AD are intracellular neurofibrillary tangles (brain regions: entorhinal cortex, hippocampus, amygdala, limbic system and isocortex) and extracellular plaques laden with the Aβ (brain region: isocortex)[26]. The amyloid hypothesis suggests that the amyloid precursor protein is processed in neurons into Aβ, which in turn triggers a cascade of events inducing oxidative stress, neuronal dysfunction, impaired plasticity and neurogenesis, and finally leading to apoptosis[27]. In this process, it has been hypothesised that Aβ passes through the membrane and aggregates to amyloid plaques[28] in the presence of high concentrations of Cu, Fe and Zn[29]. Fe and Cu may also mediate the toxicity of Aβ by hypermetallating the peptide, leading to increased oxidative stress. Despite the involvement of both metals in the molecular pathology of AD, their homeostatic deviances in patients with AD are still a matter of debate.

In the light of the importance of Cu and Fe for the development of AD[30,31], we conducted a systematic review to address the clinical relationship between Cu and Fe and AD and to discuss the dietary implications.

Method

We searched for studies dealing with Fe and/or Cu and AD. We used terms related to AD (e.g. dementia, cognitive decline and cognitive impairment) and Fe or Cu. We searched the following databases from their start date to January 2011: Medline; Embase; Cochrane Central Register of Controlled Trials; Cochrane Database of Systematic Reviews; Biosis; Science Citation Index; Publisher Database of Kluwer, Karger, Springer, Thieme, Krause & Paschernegg; Toxibo; Clinicaltrials.gov and the ALOIS register by the Cochrane Dementia and Cognitive Improvement Group. We also searched key authors' names and reference lists in the most recent and most cited published research and review articles.

No selection criteria were applied concerning the research design or the type of work; thus all papers written in English that dealt with Fe/Cu and AD in humans were integrated.

Ethics statement

The studies included in the present review were conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the respective ethics committee (e.g. institutional review board of Rush University Medical Center[32]). Written (or verbal) informed consent was obtained from all subjects/patients. For animal studies, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the respective ethics committee.

Results

In total, we integrated 101 studies in the present review. There are two meta-analyses[33,34], two systematic reviews[35,36], eleven randomised controlled trials[37–49], two prospective studies[32,50], three cross-sectional studies[51–53], forty-five case–control studies[54–98], thirty autopsy studies[65,98–126], five uncontrolled studies[127–131] and one case study[132]. A total of two studies were published twice[37,38,46,47], one study was both a case–control and an autopsy study[98].

Randomised-controlled trials

Supplementation of metals. There is only one study in which Fe or Cu was supplemented as a single component in patients with AD. Kessler et al.[37,38] conducted a prospective, randomised, double-blind and placebo-controlled phase II clinical trial with patients suffering from mild AD. They received either 8 mg Cu daily (n 18) or placebo (n 17) as an add-on to donepezil over 12 months. The treatment showed no benefits to cognitive function measured by the Mini Mental State Examination (MMSE) test and the AD assessment scale-cognitive subscale (ADAS-cog)[37]. Nevertheless, the Cu treatment was associated with a stabilising effect on the cerebrospinal fluid (CSF) levels of $A\beta_{42}$[37], whose reduction is used as a biomarker for AD[133]. $CSF-A\beta_{42}$ declined by only 10 % in the Cu group compared with 30 % in the placebo group[38].

In the Age-Related Eye Disease Study, 2166 participants aged 65 years or older, whose cognitive status had not been determined, received daily antioxidants, Zn and Cu, antioxidants plus Zn and Cu or placebo for an average of almost 7 years, following which they completed a cognitive battery[39]. Compared with the placebo treatment, supplementation of antioxidants with or without Zn and Cu did not show an effect on cognitive performance[39]. However, since a cognitive test at baseline was lacking, the results represent only a cross-sectional comparison of treatment groups.

Further studies aimed to identify the effects of supplementation of multiple nutritional factors (including Cu and/or Fe) on cognitive function in the elderly or on the development of AD[40–44]. The outcomes of these studies, however, do not permit deciphering the effects that derive from single components.

Depletion of metals. Contrary to the supplementation of metals, four randomised, placebo-controlled studies[45–49] and one uncontrolled study[134] tested different chelators in AD based on the laboratory findings that the formation of amyloid plaques and Aβ neurotoxicity rely on interaction with Cu, Fe and Zn.

There have been two Cochrane systematic reviews[35,36] that dealt with the literature concerning the chelating agent clioquinol (PBT-1, iodochlorhydroxyquin) and that concluded an absence of evidence of a beneficial effect. Both the reviews refer to the study of Ritchie et al.[45], a randomised, double-blind phase II study in which eighteen donepezil-treated patients with AD received placebo or clioquinol for 36 weeks. No significant difference in cognition between the groups

could be determined as measured on the ADAS-cog[45]. Initially, PBT-1 had been tested in twenty patients with mild-to-moderate AD, who had received 20 or 80 mg clioquinol for 21 d[134]. Slight cognitive improvements were reported only in the high-dose group, and there was no placebo-treated control group[134].

PBT-2, a second-generation 8-OH quinoline metal protein-attenuating compound, has been tested in a randomised, double-blind study with seventy-eight patients with AD, who were under treatment with acetylcholinesterase inhibitors[45]. The patients received 50 mg PBT-2, 250 mg PBT-2 or placebo per day for 12 weeks. There was no improvement in MMSE or ADAS-cog scores, though CSF-Aβ_{42} level decreased in a PBT-2-dose-dependent manner[46]. In the 250 mg PBT-2 group, two tests on executive function showed improvement over placebo. The primary outcome of the study (safety and tolerability) was met[46,47].

The efficacy of the Cu chelating agent D-penicillamine was tested in a randomised, double-blind and placebo-controlled study with thirty-four AD patients over 6 months[48]. Although the extent of oxidative stress decreased, no significant differences in change of cognitive functioning between the intervention group and the placebo group could be determined[48].

In an earlier randomised, double-blind and placebo-controlled study[49], desferrioxamine (DFO), a siderophore (Greek: Fe carrier) with a chelating function and an affinity for Cu^{2+}, Zn^{2+}, Fe^{3+} and Al^{3+}[135], had been tested in patients with moderate AD. A total of forty-eight patients received DFO once daily, five times a week for 24 months, placebo or no treatment. Activities of daily living were assessed and video-monitored, revealing a treatment-associated reduction in the rate of decline of daily living skills[49]. The no-treatment group was reported to deteriorate twice as rapidly as the DFO-treated group.

Prospective studies

In the study conducted by Morris et al.[32], in a community-based prospective setting, 3718 elderly participants were assessed for cognitive function via a home interview, which included four cognitive tests. Daily diet was measured by a FFQ at 3-year intervals for 6 years. It has been reported that dietary intakes of Cu and Fe were not associated with cognitive function after adjustment for confounders. However, a diet high in Cu combined with a high dietary intake of saturated fats was associated with a much faster rate of cognitive decline, while this interaction was not apparent with intakes of Fe or Zn or cholesterol. Those participants in the highest fifth of Cu intake (derived from high Cu doses in vitamin supplements), combined with a diet high in saturated fats, lost cognition at a rate of 19 years in a period of 6 years. Thus, the cognitive decline was three times higher than expected[32]. In contrast, Cu intake was not associated with cognitive change when the diet was not high in saturated fats.

This is corroborated by an observational study on eighty-one subjects with mild-to-moderate AD, clinically followed after 1 year[50]. Consistent with the findings by Morris et al. (2006),

hyperlipidaemic patients with higher levels of Cu were more prone to greater cognitive decline. In addition, free serum Cu levels at baseline were associated with a more severe cognitive decline in MMSE scores over time[50]. This association was independent of lipid serum levels.

Cross-sectional studies

A cross-sectional study with 800 community-dwelling Australians found no significant association between serum ferritin and cognitive function, measured by the Cambridge Cognitive Test[51]. In participants with (n 51) or without (n 749) dementia, serum ferritin was not related to cognitive function at either point of measurement[51].

In a Spanish study with 260 non-institutionalised elderly, Ortega et al.[52] reported that better cognitive function measured by MMSE was associated with greater dietary intake of Fe and other nutrients including vitamin C, Zn and thiamine, while it became worse when the amount of energy supplied by fats and cholesterol increased.

The Rancho Bernardo study found in a sample of 1451 participants that both low and high plasma Fe concentrations were associated with lower performance in certain cognitive tests including total and long-term recall[53]. In women, Cu and Fe concentrations (n 849) inversely correlated with scores in the long- and short-term recall of the Buschke and Fuld Selective Reminding Test and with performance on the Blessed Information-Memory-Concentration Test[53].

Autopsy studies

Studies analysing post-mortem brain tissue samples reveal consistently increased levels of Fe in the brains of AD patients compared with controls[99,102,103,105–110,124]. Particularly, Fe was found at elevated levels in the hippocampus, the amygdala and in parts of the cortex. There is, moreover, a change in the protein level involved in the Fe homeostasis: ferritin, an intracellular Fe-storage-protein, is increased in microglia[111] and senile plaques[112] with a fivefold increase in the cortical ratio of its protein subunits, the heavy and the light chain[123]. The latter was disease and brain-region specific and may provide evidence of a dysfunction of the Fe homeostasis on the level of gene expression. Additionally, transferrin (TF) is found in senile plaques at increased concentration[104], and the Fe regulatory protein 2, a cytoplasmic mRNA binding protein, was strongly increased in the AD brain and associated with intraneuronal lesions, including neurofibrillary tangles, senile plaque neurites and neuropil threads[113]. Only a few studies report unchanged or decreased Fe levels[114–116,125], but they either did not use short post-mortem interval tissue specimens from well-characterised AD brains[114–116] or analysed the pituitary gland, which is a predictor of environmental Hg exposure, but less relevant for the association between AD and Fe[125].

In the senile plaques of AD patients, Fe and Cu as well as Zn were found at highly elevated levels[100,101,121]. In the plaque-free neuropil of patients with AD, Cu and Fe concentrations

were approximately two and four times higher, respectively, than in the neuropil of healthy brains[121].

With respect to Cu, autopsy studies reported decreased[99,116,117] or unchanged levels[98,99,114,115,118,136] in the hippocampus, cerebellum, cortex or amygdala in patients with AD compared with controls (Table 1). Loeffler et al.[119] found that brain Cu levels tend to increase with age, whereas a relative increase in AD brain tissue was significant only for the frontal cortex and in comparison with young controls, but not in other brain areas nor compared with age-matched controls. In contrast, a relative increase in ceruloplasmin, a Cu-carrying protein that is involved in Fe metabolism, was reported in the CSF, hippocampus, entorhinal cortex, frontal cortex and putamen of AD patients[65,119]. The Cu attributable to ceruloplasmin, however, was calculated to account for <1% of the regional brain Cu and may therefore represent a compensatory effect due to oxidative stress rather than a surrogate for brain Cu levels[119]. Also, other research groups found relatively decreased[126] or unchanged[98,120] levels of ceruloplasmin.

Case–control studies

Apart from post-mortem analysis, ferritin Fe can also be measured non-invasively with MRI in living brains. MRI studies revealed an increased level of Fe in the brains of patients with AD, particularly in the three basal ganglia regions, putamen, caudate and globus pallidus[54,56–59]. In patients with an early onset of AD, ferritin Fe was found to be particularly elevated in the basal ganglia[57]. Another non-invasive method, phase imaging, enables differentiation of the severity of Fe deposition in different brain regions. A case–control study applying phase imaging found higher levels of Fe deposits in the hippocampus of AD patients than in controls[60]. In addition, the phase values correlated with MMSE and the duration of disease in patients with AD[60].

Reports on systemic levels of Fe in subjects with AD are inconsistent; two studies found decreased plasma Fe levels[61,62], whereas others found unchanged concentrations in CSF and serum compared with controls[63,64,93]. The CSF level of TF has been reported to be normal in patients with AD[65], whereas the CSF ferritin level was increased[66]. Another study revealed that the ratio of ceruloplasmin:TF is increased in AD patients and correlated positively with

peroxide levels and negatively with serum Fe concentrations[97].

A number of further case–control studies investigated an association of certain genes that are involved in Fe homeostasis and the risk of developing AD. Mutations in the haemochromatosis (HFE) gene (e.g. C282Y and H63D), which cause an autosomal recessive disorder that is associated with a deregulation of the Fe metabolism, haemochromatosis[137], have been reported to be associated with an increased risk of developing AD[67–69]. Sampietro et al.[69], for example, compared the HFE genotypes of 107 patients with late-onset AD with that of ninety-nine healthy controls, and found that the frequency of the HEF-H63D mutation was highest in the patients with an early time of disease onset. This finding, however, is not undisputed[70–72], and a recent meta-analysis of eight[4] studies failed to find an association between haemochromatosis genotypes and AD (mild cognitive impairment)[34].

A second Fe-related genotype of current interest is subtype C2 of TF (TF C2), a plasma glycoprotein transporting Fe[138]. TF C2 was demonstrated to occur at an increased frequency in patients with AD[73,127,128]. The study by Zambenedetti et al.[73] involved 132 patients with a diagnosis of probable AD, and two age-matched control subjects for each patient. The report displayed that the risk of AD increases more than five times in apoE ε4/ε4 carriers and roughly 1·5 times ($P=0.07$) in TF C2 carriers with a significant interaction between the two alleles. Moreover, comparative genotyping of healthy controls and patients with AD or mild cognitive impairment revealed that epistatic interaction between the TF C2 gene and the C282Y mutation in HFE leads to an increased risk of AD, which is exacerbated in carriers of apoE ε4/ε4[89,90].

Conversely, some studies found no association between TF C2 and a higher risk of AD[85–88]. For instance, in a study with 221 AD patients and 167 controls from a Basque region, an association between apoE ε4 allele and increased risk of AD combined with a young age at onset was found, but no association between TF C2 or HFE mutation and disease susceptibility[87]. Another group reported that homozygosity of the TF C1 allele, but not TF C2, has a role as a potential risk factor[91].

In relation to Cu, a number of recent studies reported increased serum Cu[74–80,82,83,95,96], plasma Cu[61,62,94] or

Table 1. Alterations of copper or iron in post-mortem brain tissue samples of patients with Alzheimer's disease compared with samples of healthy controls

Increased concentration	Decreased concentration	Unchanged concentration
	Cu	
Hippocampus, entorhinal cortex, frontal cortex, putamen[102,119] and senile plaques[121]	Hippocampus, amygdala[99], basal ganglia[116] and cortex[117]	Hippocampus, cerebellum, cortex[98,99,118], amygdala[99,118] and hippocampus[114]
	Fe	
Senile plaques[121], amygdala, piriform cortex, olfactory system[109], hippocampus/amygdala[99,124], frontal cortex[102], grey motor cortex[103,124], temporal cortex[105], hippocampus[106], frontal/parietal/temporal lobe[107], putamen/thalamus/globus pallidus/area occipitalis[108] and cortex[116]	Basal ganglia[116] and hippocampus[114,115]	Pituitary gland[125]

CSF[61] levels of Cu in patients with AD or mild cognitive impairment[93] compared with healthy controls. In these studies, total Cu[78,80,94,139–141], ceruloplasmin[140,141] as well as 'free' Cu (ceruloplasmin non-bound Cu)[78,93,140,142,143] have been found at increased rates. The free Cu fraction correlated positively with CSF biomarkers of AD including Aβ and hyperphosphorylated τ[143] and inversely with MMSE[143]. On the other hand, some researchers found unchanged total Cu serum[61,63,81,82,96,144] and CSF[63,64,76] concentrations, while only one Turkish study reported relatively decreased Cu serum levels in AD subjects[62]. A recent meta-analysis has summarised the aforementioned case studies and reported evidence of increased Cu levels in serum and no difference between patients with AD and controls in CSF Cu levels, whereas data regarding the plasma levels did not allow conclusions to be drawn[33].

We found two studies that revealed that carriers of the apoE ε4 allele, which has been described as a genetic risk factor for AD[145], demonstrate higher levels of circulating Cu than non-carriers[83,84]. In addition, the temporal α-1 electroencephalography activity is more strongly associated with free serum Cu in apoE ε4 carriers than in non-carriers[77], while the Cu, Zn superoxide dismutase activity in erythrocytes is not correlated with the apoE genotype in AD patients[55]. AD patients treated with D-penicillamine for 24 weeks had reduced superoxide dismutase activity in erythrocytes[55].

Further studies

Further studies of relevance encompass uncontrolled studies and case reports; two uncontrolled studies revealed an association between higher levels of free Cu in serum and worse performance in MMSE, both in AD patients (n 81)[50] and in healthy subjects (n 64)[131], respectively. Other studies with AD patients revealed an association between reduced total plasma Cu levels and worse cognitive performance (ADAS-cog scores)[130] or CSF biomarker levels characteristic for AD (Aβ$_{42}$ > 375 pg/ml, total τ < 445 pg/ml and phosphorylated τ < 61 pg/ml)[129]. In a case study with a pair of elderly monozygotic twins that were discordant for AD but with very similar habits and lifestyle, serum Cu and total peroxide levels were 44 % higher in the twin with AD[132].

Discussion

Randomised-controlled trials

In the placebo-controlled, randomised trials, there is no clear evidence that supplementation or depletion of Cu or Fe brings beneficial effects on the cognitive performance of patients with AD. All supplementation trials administered Cu as an add-on to normal medication or in combination with other nutrients, which would hamper drawing of any conclusion even if there was a beneficial effect. The depletion studies were small-sampled (n 20–78) and ran over short periods (3–24 months). The trial by Squitti et al.[48] was conducted over 24 weeks; this was not sufficient time to detect a cognitive decline in the placebo group. Implicitly, no final conclusion

is possible on the clinical benefit of D-penicillamine without a longer study period. This also applies to the PBT-2 trial over 12 weeks in which MMSE and ADAS-cog scores diverged for the placebo and intervention groups, but effects remained insignificant[46]. The early (1993) DFO trial included AD patients diagnosed by ADAS-cog and ran over 2 years. Unfortunately, the dropout rate for performing different cognitive tests (e.g. Wechsler Memory Scale) was too high to analyse anything but the video-recorded behaviour over time, which could be biased by the scoring procedure and behaviour changes induced by the method itself. Furthermore, it is worth noting that DFO is a charged molecule that is rapidly metabolised and does not easily pass the blood–brain barrier, which may limit its applicability; it also is unclear whether the beneficial effects of DFO relied on the chelation of Fe, Al or other metals. In summary, the current evidence arising from randomised controlled trials on the association between Cu or Fe and AD is inconclusive and awaits longer studies with larger samples.

Prospective studies

The paucity of clinical data regarding nutritional trials is countered by a longitudinal study by Morris et al.[32]. The study had a large sample (n 3718), a median follow-up of 5·5 years and deduced cognitive scores from four different tests, but may be limited since the mixed effect models were not adjusted for the apoE genotype that influences both lipid[146] and Cu[77] metabolism in AD. In addition, dietary Cu intake was calculated by multiplying the daily intake of food with the Cu content of foods, which depends on the soil and can vary regionally. However, it is unlikely that this analytical step heavily biased the results, as the findings of the study were strongest for supplementary Cu intake. While no association was found for cognitive decline and Cu and dietary cholesterol[32], a trial with the cholesterol-lowering drug atorvastatin found that AD patients treated with atorvastatin (n 32) over 1 year showed significant improvements in cognitive performance compared with placebo, and that the treatment was also associated with a reduction in circulating Cu levels in blood[147]. The second prospective study[50] was small-sampled (n 81), with a short follow-up and explored the predictability of cognitive decline in relationship with Cu levels. It provided no information on the size of the effect that simultaneous hyperlipidaemia and high Cu levels have on cognitive impairment or whether apoE influences the association. In summary, the prospective studies suggest that a diet that is rich in both Cu and saturated fats fosters cognitive decline in elderly subjects.

Cross-sectional studies

The findings by Ortega et al.[52] are based on comparing groups with MMSE scores ≥ 28 and < 28. The association of Fe intake and MMSE scores was not adjusted for age, which lowered the scores. Also, the conventional cut-off point for dementia is 24[148], so it is difficult to deduce what the findings mean for the association of dietary Fe with AD. In contrast,

another study found systemic Fe and Cu levels to be associated with scores of certain cognitive tests but measured neither MMSE scores nor the prevalence of AD[53]. The third cross-sectional study[51] did not find an association between dementia and serum ferritin levels. However, serum ferritin may not be a reliable proxy for brain Fe[149]. In summary, the current evidence of cross-sectional studies between an association of AD and Cu or Fe levels is inconclusive.

Autopsy studies

In general, the heterogeneous outcomes of the autopsy studies might have been influenced by several factors. First, there may be differences in the underlying tissue samples according to non-matched differences in the patients including age, sex and genetic or environmental factors such as exposure to toxic elements. Second, the small sample sizes of the studies (nine to twenty-one AD patients; ten to seventeen controls) come with increased variability. Third, it is possible that fixation of brain tissue samples with formalin[29,109,150] influences the results[151]. Fourth, the trace elements were measured by different methods such as inductively coupled MS[114], instrumental neutron activation analyses[99,116] or radiochemical neutron activation analysis[118]. Fifth, there are dissimilarities in the preparation of the post-mortem tissues. For instance, some studies used short-term post-mortem interval tissue specimens and samples from AD cases confirmed by histology[116,150], while a few did not[114], some measured Cu as μg Cu/g dry brain weight[99,117], others referred to wet weight[118,136]. Any conclusion reached should take these methodological heterogeneities into account. It may nonetheless be summarised that the autopsy studies almost consistently revealed that Fe is increased and that homeostatic Fe regulation is disrupted in the brains of patients with AD. The high levels of Cu, Fe and Zn in the amyloid plaques indicate the central roles of the metals in the formation of the central histological features of AD (for details, see Bush[16]). The situation with Cu levels in brains of AD patients is less clear. Most studies found no significant differences in Cu concentrations between AD patients and controls. Some of those that suggest otherwise compared AD patients with young controls[119] and used formalin fixation and no short-term post-mortem interval samples ($>48\,h$)[116]. However, definitive answers on whether Cu is unchanged in the brain during AD remain a matter of scientific debate.

Case–control studies

The presented case–control studies provide four lines of evidence. First, they confirm the findings of autopsy studies that Fe is increased in the brains of AD patients in comparison with controls by applying phase imaging or MRI. Second, seven case–control studies compared Fe, TF and ferritin levels in plasma, serum and CSF of patients with AD with controls. The studies' heterogeneity may arise from differences in the sample collection and preparation, in the methods used for Fe measurement, in the diagnosis of AD, in the characteristics of the study populations and in the limited number of studies. These all hinder drawing general conclusions on systemic

Fe levels in AD. Third, genes associated with Fe metabolism (HFE, TF C2) were analysed for an association with AD. For HFE, a meta-analysis negates such an association; for TF C2, the results are inconsistent and necessitate additional scrutiny. Fourth, case–control studies analysed alterations in Cu levels in plasma, serum and CSF of AD patients in comparison with controls. A current meta-analysis reported increased Cu levels in the serum of AD patients and unchanged CSF levels. The apoE ε4 genotype was associated with higher Cu levels than in non-carriers and altered brain activities, suggesting that the increased risk of apoE ε4 carriers for AD may partly be based on a role in the effects induced by the dyshomeostasis of Cu.

Further studies

Apart from general limitations that are associated with uncontrolled studies and case reports, the interesting study by Pajonk et al.[130] may be further limited by its short follow-up of 8 weeks, and the fact that the analysis excluded all patients in the highest tertile of plasma Cu levels. The results of further studies[129,131] highlight the current scientific debate on whether it is more appropriate to address total Cu or free Cu levels in the study of mental decline[131].

Summary

In conclusion, the current trials provide no conclusive evidence that depletion or supplementation of Cu or Fe is beneficial for AD; prospective studies found that a diet concurrently high in Cu and saturated fats may foster cognitive decline in age; Fe has been consistently found at elevated levels in the brains of AD sufferers by both autopsy and case–control studies. The specific outcomes for Cu are more conflicting; while evidence suggests that the systemic Cu level is increased in patients with AD, further research is needed to define the alterations of Cu in the brain during AD. Also, the relevance of certain genes including TF C2 or apoE awaits further investigation.

Molecular basis

Is the prospective studies' finding biologically plausible? The molecular effects of dietary Cu found in cells and animals are complex and understood only in part. They appear to depend on whether only Cu or Cu and further nutritional factors are experimentally altered.

In the case of a single elevation in Cu levels, the amyloidogenic pathway is inhibited[152,153]. One possible explanation for this is a Cu-induced change in APP processing. APP is an integral membrane protein with two Cu binding domains[154], which is cut by endonucleases[27] into lipid rafts, membrane microdomains enriched in cholesterol and sphingolipids[155,156]. When Cu binds to APP, a conformational change is induced that is thought to affect the clustering of APP in the cell membrane, which, in turn, alters the rate at which APP is processed[157]. In consequence, the concentration of Aβ decreases, which would be in line with the

findings of some clinical studies[38,129,130], but in contradiction to others[74,76]. Moreover, the processing of the APP depends on the flotillin-2-related endocytosis of APP[158,159], which depends on cholesterol[159] and Cu[155]. Increased Cu levels attenuate Aβ synthesis via the inhibition of APP endocytosis[155].

On the other hand, Cu overload has been reported to result in the overexpression of APP[160], lipid peroxidation, generation of the reactive aldehyde 4-hydrox-2-nonenal and oxidative stress[161], thus aggravating the vicious circle of AD pathogenesis. A comprehensive understanding of how Cu is involved in AD awaits further research. However, if – in addition to increased Cu intake – further nutritional parameters such as intake of cholesterol or SFA are experimentally altered, the molecular effects appear to be distinct. Hence, the addition of trace amounts (0·12 parts per million) of Cu in distilled water to cholesterol-fed rabbits can induce a reduction in cognitive abilities and increased levels of amyloid plaques[162].

In vitro experiments have shown that Aβ can form cation channels in the lipid bilayer membrane[163]. Enrichment of cholesterol reduces membrane fluidity and results in the destabilisation of the Aβ channels and an exclusion of Aβ in a metal- and pH-dependent manner[164]. In the light of the aforementioned pathogenic effects of Cu overload, additional presence of Cu may further foster neurotoxicity via soluble Aβ and lipid oxidation. Furthermore, hypercholesterolaemia is thought to simultaneously heighten the brain levels of Aβ and Fe[165], which exacerbates oxidative stress[31]. Hence, the membrane integrity and stability of Aβ in the membrane are delicately balanced, and even small changes in the concentrations of metals, cholesterol or saturated fats may be able to influence the pathogenesis of AD. This conclusion might be limited since brain Fe and Cu homeostases depend on a multitude of molecular players and processes (see for reviews, Hung *et al.*[30]; Zecca *et al.*[31]) that have not all been taken into account in the present review. With regard to clinical evidence, the cholesterol-promoting effect of a high dietary intake ratio of SFA:PUFA should be kept in mind[166–168]. Fig. 1 provides an integration of the effects of Cu and cholesterol into the pathology of AD.

However, we are missing a lot of information that could help us see the complete picture. For instance, the complex homeostases of brain Fe and Cu and the relationship of systemic to brain levels are only understood incompletely. Also, the relevance of apoE in the interplay of Fe, Cu and lipid metabolism during AD is unclear. Supplementary experiments in mural AD models at different life stages could give answers to questions of whether the temporal pattern of dietary combinations are relevant to the development of AD and could lay the ground for additional clinical trials.

Practical implications

In a recent prospective study with elderly people, Gu *et al.*[169] identified a dietary pattern that was strongly associated with a lower risk of developing AD. It included higher intakes of salad dressing, nuts, fish, tomatoes, poultry, cruciferous vegetables, fruits, and dark and green leafy vegetables and
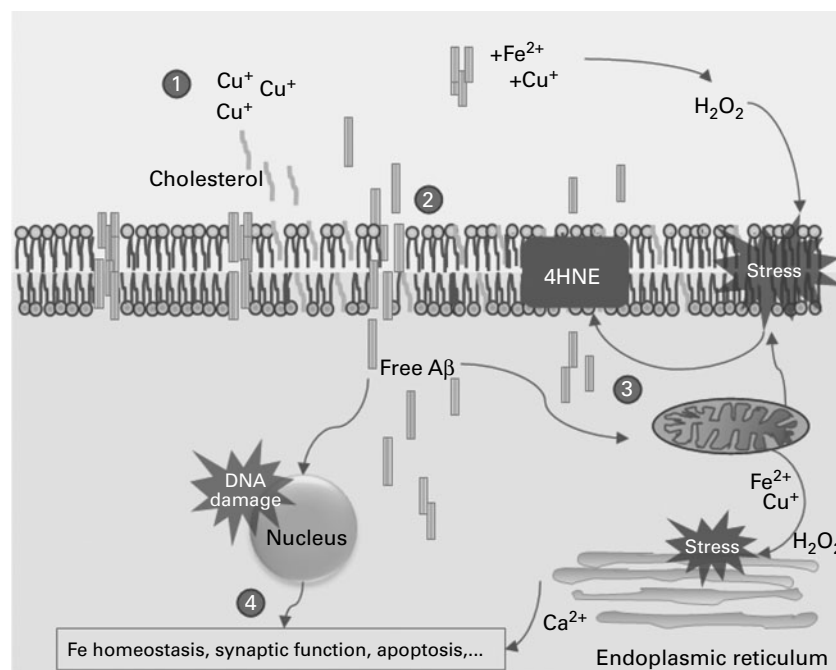

Fig. 1. Effects of cholesterol and Cu on the amyloid β pathology. Considering, in step (1), that cholesterol and Cu levels rise, cholesterol will be integrated into the membrane whose fluidity, in turn, is reduced. The membrane-bound Aβ protein dissociates (2)[164] to form extracellular amyloid plaques at which Fe^{2+} and Cu^+ generate H_2O_2, which results in lipid peroxidation and the subsequent generation of 4-hydroxynonenal (4HNE), a neurotoxic aldehyde. In the cell, the free Aβ exhibits diverse pathogenic mechanisms (3) including mitochondrial oxidative stress, decreased production of ATP, production of H_2O_2 in the mitochondria, the Fe- and Cu-catalysed generation of the hydroxyl radical, that induces oxidative stress in the endoplasmic reticulum[27]. Finally (4), the cholesterol-enriched diet can lead to apoptosis, DNA damage, blood–brain barrier disruption, as well as dysregulation at the level of Fe regulatory proteins[165].

a lower intake of high-fat dairy products, red meat and butter[169]. The identified dietary pattern contains few SFA and is likely to be associated with a low intake of Fe[170,171]. The same could be true for a Mediterranean diet (high intake of vegetables, legumes, fruits, fish, nuts and cereals, but a low intake of saturated lipids, meat and poultry, and a moderate intake of ethanol[172]), which has been reported to be associated with a reduced risk of AD[24]. Against the clear clinical evidence of elevated Fe levels in AD, it is tempting to assume that the benefits of a Mediterranean diet on AD do not exclusively rely on a distinct lipid intake but also on a lower level of Fe intake. In contrast, the foods with the highest contents of Cu (beef liver, oysters, molluscs, certain nuts, almonds and cocoa[173,174]) are neither typical nor atypical for a Mediterranean diet. Its benefits for AD[24] therefore are probably not based on differences in the intake of Cu.

Although circulating Cu relates to the nutritional status of Cu[175], the origin of the free Cu fraction is still under discussion, potentially relying on inflammation[176] or an increased efflux from cortical cells[16]. The alteration of systemic and brain Cu level in AD therefore depends on diet to a degree that is not yet known. However, it is startling that the participants on the highest quintile of Cu intake in the Morris Study took Cu in vitamin or mineral supplements[32]. This gives significance to the dietary intake of Cu in the elderly regardless of the current status of understanding.

In this light, it is of note that dietary intakes of different metals and their physiological levels in the body are not independent of each other and other factors. Thus, an elevation in Fe levels can be secondary to a high-Cu diet as Cu facilitates Fe intake[177], and hypercholesterolaemia might be induced by misbalancing dietary metal intake[97]. Moreover, the inverse correlation of the blood ceruloplasmin:TF ratio[97] may also suggest that brain Fe accumulation is related to systemic alterations, which in turn depend on diet.

Therefore, the practical implications could be to avoid Cu-containing supplements and a high intake of SFA or excessive diet of Fe. Fe deficiency in developed countries is most common in certain subgroups of the population including toddlers and women of childbearing age[178]. Data from the nutritional survey 2007–2008, National Health and Nutrition Examination Survey III, showed that the average nutritional intake of Cu is between 1·3 and 1·6 mg/d (1·1–1·3 mg/d) for adult men (women) in different age cohorts[179], whereas the dietary reference intake is 0·9 mg/d of Cu for adults[180]. For Fe, the dietary reference intake recommended by the National Academy of Sciences is 8 (15–18) mg/d for adults (women aged 14–50 years)[180], while the actual intake is 15·6–18·1 mg/d for adult men and 12·6–13·2 mg/d for adult women at different ages[179]. That means that the intake of Cu and Fe is up to 100 % higher than recommended. All efforts to reduce Fe or Cu levels should target the metals' physiological windows in order to avoid deficiency but achieve levels that are below the threshold levels critical for AD. What these thresholds look like with regard to AD is speculative with respect

to the heterogeneity of different case–control studies. For instance, some studies found total Cu serum levels of >13 µg/l for the controls[79,80], while others reported levels of <10 µg/l both, for controls and AD patients[63,77]. Also, AD is characterised by multiple aetiology and therapeutic ranges of metal reduction may not be generalised without considering further (dietary) parameters.

Despite the eschewal of high dietary intakes of Fe and Cu, various alternative approaches to lowering metal levels in the brain are under investigation. Several molecules acting as chelating agents are currently being developed[181–183], while some nutritional constituents have also been examined in relation to their metal-chelating activities.

The green tea polyphenol (−)-epigallocatechin-3 gallate is being discussed as entailing protective effects on the progression of AD by different mechanisms, including an Fe-chelating function[184]. Curcumin, a polyphenolic diketone responsible for the yellow colour of turmeric, is also suggested to exert a protective effect against AD by binding Fe^{2+} and Cu^{2+} though the binding affinity for Zn^{2+} is small[185,186]. There are more chelating agents being discussed for the treatment of AD. For instance, ethylenediaminetetraacetic acid has been reported to induce improvement in patients with AD[182]. Pharmaceutical compounds that exert their anti-inflammatory effects by interaction with Cu-, Fe- or Zn-dependent proteins have also been found to lower the risk for developing AD[187], and a number of other molecules that act as chelating agents are currently being developed[188]. An alternative approach of reducing the level of stored Fe in patients with AD has been hypothesised recently with the use of calibrated phlebotomy that could reduce stored Fe without causing anaemia[189]. All practical implications, however, should be taken cautiously in light of the limited clinical evidence and the multiple causality of AD.

Conclusion

In conclusion, the present systematic review suggests that a diet rich in Cu and Fe might aggravate the detrimental effects of a high intake of cholesterol and SFA on the risk of developing AD. The association is biologically plausible. However, since the relationship between dietary metal and fat intake and dementia is clinically not well examined, additional studies are necessary to further address which nutritional patterns best fit to certain risk groups in the population.

Acknowledgements

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