Review

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The Relationship of Omega 3 Polyunsaturated Fatty Acids in Red Blood Cell Membranes with Cognitive Function and Brain Structure: A Review Focussed on Alzheimer's Disease

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Abstract

Significant research attention has focussed on the identification of nutraceutical agents for the prevention of cognitive decline as a natural means of cognitive preservation in the elderly. There is some evidence for a reduction of brain omega 3 polyunsaturated fatty acids (n-3 PUFAs) in normal aging and in Alzheimer's disease. n-3 PUFAs exhibit anti-inflammatory and anti-amyloidogenic properties as well as being able to reduce tau phosphorylation. Many observational studies have demonstrated a link between n-3 PUFAs and cognitive aging, and some, but not all, randomized controlled trials have demonstrated a benefit of n-3 PUFA supplementation on cognition, particularly in those subjects with mild cognitive impairment. The identification of a biomarker that reflects n-3 PUFA intake over time and consequent tissue levels is required. In this narrative review we discuss the evidence associating red blood cell membrane n-3 PUFAs with cognitive function and structural brain changes associated with Alzheimer's disease.

Key words: Docosahexaenoic acid, omega 3 polyunsaturated fatty acids, cognitive decline, Alzheimer's disease, red blood cell.

Introduction

Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disorder of aging characterised by progressive memory loss, cognitive impairment and the inability to carry out functional activities of daily living (1). AD is characterised pathologically by the presence of cerebral β-amyloid (Aβ), neurofibrillary tangles composed of hyper-phosphorylated tau and neurodegeneration (2). The presence of the apolipoprotein E (ApoE) ε4 allele is the main genetic risk factor associated with sporadic disease, which is the predominant form of AD (3). Other factors that have been reported to influence the onset of AD include diet as well as physical and mental activity (4, 5).

n-3 PUFAs

Omega 3 polyunsaturated fatty acids (n-3 PUFAs) are dietary factors that have received significant research attention in relation to their beneficial effects on cognitive decline. The main n-3 PUFAs used in the body are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) and are mostly obtained from the consumption of oily fish or through dietary supplementation (6). DHA and EPA can also be synthesised to a limited extent from α-linolenic acid (ALA) obtained from plant oils (6). Docasapentaenoic acid (DPA) is one of the less extensively studied n-3 PUFAs, which nonetheless plays a role in influencing health outcomes that are responsive to DHA and EPA (7, 8).

DHA is the major fatty acid in neuronal membranes (30 %) and is enriched in synaptosomal membranes (9). DHA is involved in multiple inter-related brain functions including cell membrane fluidity, signal transduction and neurotransmission (10–12). DHA is thought to be the main n-3 PUFA involved in cerebral metabolism and as a consequence has been most extensively studied. The major facilitator superfamily domain-containing protein 2a (Mfsd2a) has recently been identified as the major transporter for the uptake of DHA (in the lysophosphatidylcholine form) into the brain (13). EPA also crosses the blood brain barrier (BBB), although through an as yet unidentified mechanism (14) and is present in brain tissue, albeit at considerably lower concentrations (15). EPA and DHA exhibit overlapping and unique biological roles (15, 16) and EPA serves as a precursor to DHA in the biosynthetic pathway.

n-3 PUFAs and AD

Cerebral DHA levels are known to be deficient in AD specifically in brain regions associated with disease (17, 18) and DHA is decreased in the brain in normal human aging (19). There are a number of possible explanations which could account for the reduction in cerebral DHA

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including insufficient dietary intake, reduced BBB transit, increased neuronal death or genetic variability in delta-5 desaturase (FADS1) and delta-6 desaturase (FADS2); enzymes involved in the rate limiting steps of DHA and EPA synthesis (20). Increased levels of plasma fatty acid binding proteins, which are known to increase with age (21, 22), could also account for reduced cerebral DHA and/or increased oxidative damage to fatty acids caused by free radicals associated with age and inflammation could lead to diminished cerebral levels (23–25).

Many observational studies have demonstrated a link between n-3 PUFAs and cognitive aging related to AD (26). Some randomised controlled trials (RCTs) have also demonstrated a benefit of n-3 PUFA supplementation, particularly in terms of immediate recall, attention and processing speed in patients with mild cognitive impairment (MCI), but not in those with AD or in healthy subjects (27). MCI if present in conjunction with cerebral Aβ represents the prodromal stage of AD and offers a window of opportunity for therapeutic intervention. Identifying elderly patients at risk of cognitive decline is important to enable timely treatment before AD pathology becomes irreversible; as such n-3 PUFAs might offer a potential well tolerated, inexpensive treatment in the early stages of AD.

There is increasing evidence to suggest that n-3 PUFAs play a role in the disease mechanisms associated with AD. n-3 PUFAs have been shown to promote long term potentiation (LTP), a mechanism underpinning functional plasticity the basis of learning and memory, in cell culture and animal models (11, 28). There is also evidence to suggest that DHA confers neuro-protection in part through the direct inhibition of tau phosphorylation (29, 30). Moreover, DHA and EPA have been shown to alter amyloid precursor protein (APP) processing in favour of reduced Aβ production (31–35).

In terms of inflammation, n-3 PUFAs are known to displace omega 6 polyunsaturated fatty acids (n-6 PUFAs) from cell membranes resulting in the production of more benign eicosanoids that possess less potent inflammatory and thrombotic effects and are less efficacious vasoconstrictors (6, 36). Thus, changing DHA/EPA concentrations in cell membranes might serve to ameliorate cerebral inflammation, which is known to fuel AD pathology (37). In addition, increased membrane n-3 PUFA content might reduce the incidence of stroke, which is associated with an increased incidence of AD (38, 39) and vascular dementia (40). n-3 PUFAs also specifically suppress the expression of pro-inflammatory cytokines and promote microglial phagocytosis of Aβ and increase neurotrophin production (41, 42). n-3 PUFAs skew macrophage/microglial polarisation towards an M2 anti-inflammatory phenotype indicative of tissue repair (41, 43) and provide the building blocks for the production of E and D series resolvins, protectins and maresins - collectively known as specialized pro-resolving mediators (SPMs) (44). These mediators are involved in the resolution and termination of the inflammatory response, which was originally thought to be a passive process. Diminished levels of SPMs are found in the hippocampus (31) and in the entorhinal cortex in AD (45), which is in accordance with the chronic pro-inflammatory micro-environment associated with AD brain (46). Interestingly, SPMs have been shown to promote microglial phagocytosis of Aβ and reduce M1 microglial cell surface marker expression in addition to possessing neuroprotective properties (45). Thus, n-3 PUFA dietary supplementation might serve to reduce cerebral inflammation both directly and indirectly through the production of SPMs thereby limiting bystander damage to neurons and subsequent neurodegeneration. Furthermore, pro-inflammatory microglial activation has been purported to provide the link between Aβ plaques and hyper-phosphorylated tau (37, 47), therefore promoting microglial M2 anti-inflammatory activity and phagocytosis of Aβ through the increased consumption of n-3 PUFAs might serve to ameliorate tau pathology, the best clinical correlate of neurodegeneration (48, 49), hence curtailing AD-related symptoms.

Rationale for this narrative review

A number of systematic reviews and meta-analyses have addressed the effects of n-3 PUFA supplementation on measures of cognitive aging associated with AD and MCI (27, 50–52). Furthermore, clinical signs of AD and MCI have frequently been correlated with plasma DHA and/or EPA levels (9, 53, 54). However, plasma levels of fatty acids (free, cholesteryl esters or phospholipid bound) reflect recent dietary intake over a time frame of a few days and therefore do not represent a true picture of steady-state n-3 PUFA levels (55). RBC fatty acid concentration might represent a more reliable measurement of dietary habits and nutritional status; considering that fatty acids are stable in RBC membranes for up to 3 months corresponding to the lifespan of a RBC (55). RBC fatty acid concentrations have also been shown to reflect tissue concentrations (56). Thus, in this narrative review we aim to present the literature whereby RBC n-3 PUFA levels have been investigated in association with cognitive function and brain structure related to AD. A systematic review was out of the scope of this study.

Methods

In April, 2016, a search without time date span limitation was performed in Pubmed. A search strategy was implemented using key words in an 'AND' combination to identify studies pertaining to RBC n-3 PUFA (red blood cell, erythrocyte, omega 3 or PUFA) and cognitive function and brain structure related to AD (cogniti*, atrophy, dementia, 'mild cognitive impairment', Alzheimer*). Titles were subjected to screening followed

Table 1. Summary of the main findings of the studies relating RBC n-3 PUFAs to parameters associated with cognitive function and brain structure in relation to AD

Study design	Study Population	RBC n-3 PUFA metric	Main results
Heude et al., 2003: Longitudinal observational study with a 4 y follow up (EVA) (57).	n =246; age 63-74y ($\bar{x}$ = 68.9y ± 3.7); 58.7 % female	Total saturated fatty acids (SFA), stearic acid, palmitic acid, total monounsaturated fatty acids, oleic acid, total PUFAs, total n-6 PUFAs, arachidonic acid, linoleic acid, total n-3 PUFAs, DHA, EPA, n-3 : n-6 fatty acids, DHA:AA.	Higher stearic acid and total n-6 PUFAs was higher in the decline group as measured using the French version of the MMSE; decline classed as a < 2 point reduction in MMSE score. DHA and EPA levels were lower in the decline group and total n-6 PUFA was higher in the decline group. The ratios of n-3: n-6 PUFAs and DHA: AA were lower in the decline group. Lower n-3 PUFAs and higher stearic acid and n-6 PUFAs were associated with a higher risk of cognitive decline.
Wang et al., 2008: Cross sectional observational study (58).	n = 46; age 74.25y ± 10.1; 54.25 % female	DHA, EPA and α-linolenic acid	RBC DHA, lutein, beta- carotene, and LDL cholesterol correlated with MMSE score. A lower MMSE score was associated with lower lutein, beta carotene and RBC DHA levels and a higher LDL-cholesterol level.
Chiu et al., 2008: RCT to assess the effects of n-3 PUFA supplementation (DHA + EPA) compared to placebo on cognitive function in older adults with MCI or AD (59).	n = 46; age 70.1-81.1y; 55.85 % female	ALA, EPA, DHA, total n-3, total n-6, AA, LA	Daily EPA (720mg) and DHA (1080 mg) treatment showed better improvement on the Clinician's Interview-Based Impression of Change Scale (CIBIC-plus) than those in the placebo group over a 24 week follow up. There was no significant difference in the cognitive portion of the ADAS-Cog between these 2 groups. However, the treatment group showed significant improvement in ADAS-cog compared to the placebo group in participants with MCI. Higher proportions of EPA on RBC membranes were associated with better cognitive outcome.
Whalley et al., 2004: nested case-control design (60).	n = 120; age ≈ 64y, 49 % female	Total n-3 PUFAs, total n-6 PUFAs, total n-9 PUFAs, cis linoleic acid, AA, EPA, DPA and DHA	RBC n-3 PUFA content was higher in fish-oil supplement users than in nonusers, but cognitive function did not differ significantly between groups. Total RBC n-3 PUFA and the ratio of DHA to AA was associated with better cognitive function in late life before and after adjustment for childhood IQ.
Chiu et al., 2012: Cross sectional observational study (61).	n = 132; age 67.8 ± 6.6y; 72.7 % female	ALA, DPA, DHA, EPA, total n-3 PUFAs, AA, total n-6 PUFAs, SFAs, ratios of AA/EPA and AA/DHA.	Higher EPA and total n-3 PUFA concentrations and a lower ratio of AA to EPA in RBC membranes were associated with a higher cognitive composite score independent of age and sex but no longer significant after adjustment for education in older people with previous recurrent depression.
Milte et al., 2011: Cross-sectional study (62).	n = 79; age 65+ y; 38 % female	DHA, EPA, DPA, AA and total n-3 PUFA and n-6 PUFA	MCI group reported higher average scores on the Geriatric Depression Scale (GDS) and lower RBC EPA. Lower levels of EPA and higher n-6 PUFA were associated with poorer cognitive performance [Verbal paired Associates Score (VPA), Rey Auditory Verbal Learning Test (RAVLT), Excluded letter Fluency (ELF)].
Andrieu et al., 2016: RCT to assess the effects of multidomain intervention (comprising of nutritional counselling, physical exercise counselling and cognitive stimulation) and n-3 PUFA supplementation (DHA + EPA) and a combination of the two on cognitive function in older adults with memory complaints (63).	n=1525; age 75.3y ± 4.4; 64.1 % female	Omega 3 index	The Multidomain Alzheimer Preventive Trial was a 3 year, multicentre, randomised, placebo controlled superiority trial with 4 parallel groups. Non-demented subjects aged 70 years and older with memory complaints were randomly assigned in a 1:1:1:1 ratio to combined intervention (i.e. multidomain intervention plus n-3 PUFA capsules (two capsules a day, 800mg DHA + 225mg EPA), multidomain intervention plus placebo capsules, n-3 PUFA capsules alone or placebo. In the intention-to-treat population (n=1525), there was no significant difference in 3-year cognitive decline between any of the three intervention groups and the placebo capsules group.
Lukaschek et al., 2016: Cross sectional observational study (64).	n=720; age 77.6y ± 6.2; 50.4 % female	Omega-3 index	In sex- and age-adjusted logistic regression subjects with a low omega-3 index (< 5.7 %) were at a significantly higher risk for cognitive impairment. This association remained stable after further adjusting for educational, metabolic risk factors and affective disorders.
Whalley et al., 2008: Longitudinal observational study (65).	n = 120; age ≈ 64-68; 60 % female	Total n-3 PUFAs, total n-6 PUFAs, total n-9 PUFAs, cis linoleic acid, AA, EPA, DPA and DHA. Ratio n-3:n-6 PUFA.	Cognitive benefits were associated with higher total erythrocyte n–3 PUFA content, but were significant only in the absence of the APOE ε4 allele.
Pottala et al., 2014: Longitudinal observational study (WHIMS-MRI study) (66).	n = 1111; age 65-88y; 100 % female.	DHA and EPA and omega 3 index.	A 1 SD greater omega 3 index level was correlated with 2.1cm3 larger brain volume 8 years on. A 1 SD greater omega 3 index was correlated with a 50 mm3 greater hippocampal volume measured 8 years on.
Tan et al., 2012: Cross sectional observational study (dementia free Framingham study participants) (67).	n = 1575; age 67y ± 9; 54 % female	DHA and omega 3 index	Participants with RBC DHA levels in the lowest quartile (≤3.9 %) when compared to others had lower total brain volumes and greater white matter hyperintensity volumes. Participants with lower DHA (3.9 %) and omega 3 index (4.4 %) (Q1 vs Q2-4) also had lower scores on tests of visual memory and abstract thinking.
Ammann et al., 2013: Longitudinal observational study (secondary to RCT on HRT and cognitive decline). Median follow up 5.9y (68).	n = 2,157 ; age 65-80 y; 100 % female	DHA and EPA	No significant cross sectional cognitive differences were found between women in the high and low DHA and EPA tertiles at the time of the first cognitive battery (median 3 years) and in the rate of cognitive change over time (median follow up 5.9 years)
Danthiir et al., 2014: Cross sectional observational study (baseline data from a RCT) (69).	n = 390; age 65-90y; 53.6 % female	DHA, EPA and DPA	Higher current fish consumption was associated with worse performance on several cognitive speed constructs (inhibition, simple/choice reaction time, reasoning speed and memory scanning) and greater fish consumption in childhood predicted slower perceptual speed and simple choice reaction time.
Kröger et al., 2009: Longitudinal observational study (The Canadian Study of Health and Aging) (70).	n = 663; age ≥ 65y; 60.5 % female.	DHA, EPA and total n-3 PUFA (α linolenic acid, stearidonic acid, eicosatrienoic acid, eicosatetranoic acid, docosatrienoic acid, docosapentaenoic acid and docosahexaenoic acid)	No association was found between baseline RBC DHA, EPA and total n-3 PUFA and AD measured after a median follow up of 4.9 years.

References listed sequentially as mentioned in the main text.

by an assessment of relevant abstracts by one author. Articles had to meet the following inclusion criteria: (a) quantitative assessment of RBC n-3 PUFA levels reported in the article and (b) assessment of cognitive outcomes or brain structure. An updating literature search was performed in February 2017 to add recent key references. Only manuscripts written in English were included. Note, the definition of RBC 'total n-3 PUFAs' differs between studies, therefore the precise fatty acids measured in a specific study and designated as 'total n-3 PUFAs' are specified where relevant in parentheses in this review. Of note, the studies described here should be compared with caution considering the divergent roles that different n-3 PUFAs play in biology, particularly, DHA and EPA. The specific terms describing n-3 PUFAs should not be considered as interchangeable.

Results and discussion

Study characteristics

As a result of the initial and updating literature searches 14 articles were included in this review (Table 1) (57–70). Of the studies 12 were of observational design and 2 were RCTs. Four studies were performed in the USA, two studies were performed in France, Taiwan, Australia and Scotland and one study was undertaken in Germany and another Canada. Sample sizes varied between 46 to 2157 participants with subjects ranging from cognitively normal to demented with a diagnosis of AD.

Studies relating to RBC n-3 PUFAs and cognition

The Etude du Vieillissement Artérial (EVA) was the first study to relate fatty acid composition of RBC membranes with cognitive decline. This observational study consisted of 246 cognitively normal participants and demonstrated an inverse association between cognitive decline over a four year period and the ratio of n-3 to n-6 PUFAs in RBC membranes measured at baseline (57). It was suggested that higher levels of RBC n-6 PUFAs could reflect a deficiency in brain levels of n-3 PUFAs, which in turn could affect cognition. Subsequent studies have also associated RBC PUFA with cognition. A cross-sectional study investigating nutritional biomarkers of AD comprising 46 subjects has shown that a reduction in RBC DHA correlates with a reduction in MMSE score (58). Higher RBC EPA has been associated with better ADAS-cog scores in a small RCT comprising 46 participants with either MCI or AD (59). A longitudinal observational analysis of 350 participants born in 1936 has reported that those who take fish oil supplements have significantly greater RBC n-3 PUFA levels and that total RBC n-3 PUFA (exact fatty acids not specified) and the ratio of DHA to AA were associated with better cognitive performance in late life before and after adjustment for childhood IQ (60). Higher RBC EPA levels as well as total RBC n-3 PUFAs (defined as the sum of ALA, EPA, DHA and DPA) were positively associated with cognitive composite scores in a cross sectional study of 132 participants formerly suffering from depression at risk of cognitive decline (61). This association was independent of age and sex but, was no longer significant after adjustment for education. This may reflect a wider association between cognitive function and healthy lifestyle related to higher education. Moreover, a cross-sectional study of 79 participants has reported that patients with MCI have lower RBC EPA and higher depressive scores (62).

We have recently completed a three year RCT known as the Multidomain Alzheimer Preventive Trial (MAPT), which was designed to assess the effects of DHA (800 mg) and EPA (to a maximum of 225mg), multidomain intervention (comprising of nutritional counselling, physical exercise counselling and cognitive training) and a combination of the two on alterations in cognitive function in frail subjects with memory complaints aged over seventy (4). In the main analysis of MAPT, no significant effects of the interventions were found on cognition after adjustment for multiple testing (63). Exploratory sub-group analysis showed that participants on n-3 PUFA supplementation with a low omega-3 index (DHA + EPA ≤ 4.83 %, representing the lowest quartile of omega 3 index distribution) at baseline showed a trend towards less cognitive decline over 36 months in comparison to subjects on placebo with low baseline omega-3 index. Furthermore, exploratory within group analysis of MAPT data has shown that participants in the placebo group with a low omega-3 index at baseline underwent significant cognitive decline over 36 months, whereas those in the placebo group with a higher omega-3 index (quartiles 2-4) remained stable. Consistent with our findings, results from the KORA (KOoperativen Gesundheitsforschung in der Region Augsburg)-Age study have shown a cross-sectional association between low omega 3 index (< 5.7 %) and cognitive impairment in an elderly population of 720 subjects with cognitive status ranging from cognitively normal to suspected dementia (64).

n-3 PUFAs and the role of ApoE ε4

A longitudinal observational study comprising 120 participants has demonstrated cognitive benefits associated with higher total RBC n-3 PUFAs (exact fatty acids not specified), but only in the absence of the ApoE ε4 allele (65). In this study cognitive performance at the age of around 64 and cognitive changes between approximately 64 to 68 years of age were related to RBC n-3 PUFA on recruitment and ApoE ε4 allele status. This report was an extension of the study by Whalley et al.,

2004, described above and used their original sample of patients born in 1936 (60). In accordance with these findings, some studies have shown that the protective cognitive effects of n-3 PUFAs are seen only in ApoE ε4 non-carriers (71,72) and that plasma DHA levels show little change in ApoE ε4 carriers despite supplementation (73). In contrast, a recent study has shown the opposite, demonstrating that n-3 PUFA consumption is related to slower cognitive decline in ApoE ε4 carriers (74). Thus, the effects of ApoE ε4 on n-3 PUFA status and cognition warrants further research investigation.

Studies relating to RBC n-3 PUFAs and structural brain changes

The Women's Health Initiative Memory Study (WHIMS) Magnetic Resonance Imaging (MRI) a longitudinal observational study comprising 1111 dementia-free participants demonstrated that a higher baseline RBC omega 3 index correlated with larger total brain volumes and hippocampal volume measured 8 years on (66). Furthermore, a recent cross-sectional analysis of 1575 dementia-free participants from the Framingham Offspring cohort has shown that subjects in the lowest quartile with regard to RBC DHA levels exhibit lower total brain volumes but, greater white matter hyperintensity volumes without any significant changes in hippocampal volume (67). Participants in the lowest quartile of the Framingham Offspring cohort with regard to RBC DHA and omega 3 index also had lower scores in tests of visual memory, executive function and abstract thinking. Interestingly, the presence of increased white matter hyperintensities in the latter study is suggestive of dementia of a more vascular nature.

RBC n-3 PUFAs and cognition: the negative study findings

An observational analysis performed on 2157 cognitively-intact elderly women enrolled on an RCT designed to investigate the effects of hormone therapy on normal cognitive aging failed to show an association of baseline RBC DHA and EPA levels with cognitive change. Cognitive assessments were performed at a median of 3 years after randomization and then annually with a median follow up of 5.9 years (68). Another cross-sectional observational study of 390 cognitively normal older adults from the Older People, Omega 3 and Cognitive Health trial (EPOCH) found no evidence to support the hypothesis that higher RBC n-3 PUFA (exact fatty acids not specified) exert a beneficial effect on baseline cognitive performance (69). In fact, the results suggest a small negative effect of fish intake in childhood and in older age on older-age cognitive function that was tentatively hypothesised to be attributable to higher concentrations of the environmental neurotoxin methyl-mercury in the fish. Furthermore, the Canadian Study of Health and Ageing (CSHA), a longitudinal observational study comprising 663 participants demonstrated there to be no association between baseline total RBC n-3 PUFAs, (defined as the sum of RBC EPA, DHA, DPA, ALA, stearidonic acid, eicosatrienoic acid and eicosatetranoic acid), RBC DHA or RBC EPA and the incidence of AD measured after a median follow up period of 4.9 years (70). However, exposure to n-3 PUFAs assessed approximately 5 years before a diagnosis of dementia may not reflect a true representation of dietary habits. A stable and sustained regular intake of n-3 PUFAs is probably required to confer mental health benefits in the elderly.

Conclusion

Here we present evidence associating RBC n-3 PUFAs with cognitive function and brain structure associated with AD. The measurement of RBC n-3 PUFAs as a biomarker of n-3 PUFA status is advantageous considering its stability over time. There is also some evidence from RCTs to suggest that the administration of n-3 PUFAs (DHA and EPA) can prevent cognitive decline. Further studies aimed at clarifying the relationship between RBC n-3 PUFAs and cognitive impairment are required. Future research should aim to specifically explore the effects of n-3 PUFA supplementation on cognitive decline and brain structure in n-3 PUFA deficient elderly subjects. It might be that n-3 PUFA supplementation per se is not beneficial in the prevention of disease, but rather re-establishing homoeostatic levels in deficient elderly subjects is pivotal for the preservation of cognition. The mechanisms through which n-3 PUFAs operate, (neurodegenerative versus vascular and the involvement of inflammation) also deserves further research attention specifically as a function of ApoE ε4 status and FADS haplotype. The expression of such genetic variants could account for the differential responses to n-3 PUFA supplementation observed between studies. In fact, we are at present in the process of planning such a trial as an offspring study to MAPT in order to address some of these important as yet unanswered questions.

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