Dietary fatty acids and the aging brain

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Aging contributes to physiological decline and vulnerability to disease. In the brain, even with minimal neuronal loss, aging increases oxidative damage, inflammation, demyelination, impaired processing, and metabolic deficits, particularly during pathological brain aging. In this review, the possible role of docosahexaenoic acid (DHA) in the prevention of age-related disruption of brain function is discussed. High-fat diabetogenic diets, cholesterol, and the omega-6 fatty acid arachidonate and its prostaglandin metabolites have all been implicated in promoting the pathogenesis of Alzheimer's disease. Evidence presented here shows DHA acts to oppose this, exerting a plethora of pleiotropic activities to protect against the pathogenesis of Alzheimer's disease.

INTRODUCTION

"What's for dinner?" was one of the primeval questions the human brain evolved to answer. While researchers have learned a great deal about nutrients needed for optimum development, those needed for optimum aging remain elusive. The focus of this symposium, "Nutrition and the Aging Brain," addresses the difficult and important topic of what humans should be eating for optimum brain aging. Healthcare costs to care for the aged represent an ever-increasing problem for developed nations with aging populations. In the United States, the government projects Medicare deficits amounting to 52 trillion dollars will arise from an aging baby boomer population's chronic diseases of aging. This expense literally threatens to bankrupt the government and mandates healthcare reform legislation. The economic and medical problems of aging populations with exponentially rising healthcare costs are similar in many other developed and developing countries. There is no simple medical solution. A plethora of expensive new medications may provide better disease management and longer lives but, instead of reducing costs, would likely add to costs. Therefore, from the economic standpoint alone, there is a need for a fresh approach to the diseases of aging, including those disabling diseases that emerge with the aging brain, with the focus placed on cost-effective prevention measures.

NUTRITION AS A CRITICAL APPROACH TO PREVENTION OF AGE-RELATED MEMORY DEFICITS

Unlike infectious diseases, the problem of the chronic diseases of aging, including brain aging, seldom offers foreign pathogens to target. Diseases of aging are primarily the consequence of chronic imbalances or dysregulation of normal pathways with important functional roles, and the targets are normal gene products. Thus, the consequences of markedly stimulating or inhibiting single targets selected for important functional roles to control chronic diseases of aging in one or another tissue or cell type are likely to include adverse side-effects, such as alteration of the target's normal level of function in another cell or tissue where it is not dysregulated. From this perspective, the standard pharmacologist's strategy of finding the most potent and specific drug from a high throughput search of compound libraries for single targets may not be the best approach. Nutritional

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interventions are inherently pleiotropic and are key regulators of signal transduction pathways that have evolved to adjust and respond to the nutrient environment. One of the best examples of this is insulin signaling, which is under intensive study in relationship to aging because it appears to play a key role in regulating lifespan.[1] A crude nutritional intervention, caloric restriction, is generally accepted as one of the few tools available to modify rates of aging, including aging of the brain, and has been shown to attenuate brain aging in mice.[2] Thus, nutritional interventions are not only highly relevant to aging in general, they are also inherently more likely to have lower costs and a more favorable safety profile than novel drugs.

AGING OF THE BRAIN AND AGE-RELATED BRAIN DISEASES

Like aging of the whole organism, aging of the brain is inherently complex, multifaceted, and poorly understood. It is normally accompanied by oxidative damage, low levels of chronic inflammation, production of lipofuscin, and protein aggregate accumulation as well as myelin loss and very selective and modest neuron and synapse loss as occurs, for example, in the pars compacta of the substantia nigra. The most egregious phenotypes of normal aging are the reduced rates of information processing and axon transmission, possibly due to the vulnerability of oligodendrocytes and myelin to oxidative damage.[3] An overview at the level of gene expression is consistent with inflammation, oxidative damage, and reduced metabolism, possibly mitochondrial failure.[2]

In the presence of Alzheimer's disease (AD), a similar pattern of change is greatly exacerbated by accumulation of pathological protein aggregates in vulnerable regions. Pathological aggregates include those from β-amyloid (Aβ) protein, notably in extracellular plaques and vessels but also within neurons, as well as others made from the microtubule-associated protein (MAP) tau, which is normally seen as predominantly axonal and microtubule associated but which redistributes into paired helical filament aggregates in neuronal perikarya and neurites, chiefly dendritic.[4] Similarly, in Parkinson's disease and the Lewy body variant of AD, there is a selected regional accumulation of alpha-synuclein aggregates in perikarya (for example, Lewy bodies in the substantia nigra and cortex) and in neurites (Lewy neurites). Four age-related central nervous system diseases – AD, Parkinson's disease, Huntington's disease, and frontal temporal dementia – exhibit regional protein aggregate pathology associated with selective neurodegeneration that explains their clinical phenotypes.

The other prevalent age-related brain pathologies, stroke and vascular or multi-infarct dementia, involve primary vessel disease and are strongly associated with cardiovascular disease (CVD) risk factors that lead to large or small infarcts, respectively. The risk factors for CVD strongly overlap those for AD, and perhaps one-third or more dementia cases have mixed dementia with both types of pathology.[5,6] Thus, solely on the basis of the need to control CVD risk factors, there is very little doubt that diet and exercise measures put in place to preserve cardiovascular health are likely to prove critical preventive measures for overall healthy brain aging. One of the CVD dietary interventions, intake of the omega-3 (n-3) fatty acids, is not only relatively inexpensive but also has an excellent safety profile and has already been shown to reduce CVD mortality by 19–45%.[7] The focus of this review, however, will be the potential role of n-3 fatty acids in the prevention of the most prevalent and devastating form of pathological brain aging, AD.

ALZHEIMER'S DISEASE AND LIPID METABOLISM

Early-onset AD is strongly familial and occurs because of rare genetic mutations, but the vast majority of AD cases develop after age 65 and are late onset and sporadic, with aging as the dominant risk factor.[4] In fact, AD is so strongly related to age that incidence doubles every 5 years after age 65, resulting in a major public health problem in developed societies experiencing a demographic shift toward an aging population. While aging is the biggest single risk factor, AD is not an inevitable consequence of aging. The development of AD is subject to multiple genetic and environmental risk factors that determine age-dependent risk, presumably by modulating the pathogenesis. Because these genetic and environmental risk factors are not sufficiently powerful to act alone, the literature surrounding them is controversial and the evidence supporting them is frequently inconclusive.

Of the genetic risk factors, the E4 allele of the lipid transport protein apolipoprotein E (ApoE) is by far the single most potent and best established. Further, the clearest genetic protective factor for AD is the E2 allele of ApoE. ApoE, a lipid transport protein, is the single most potent known modifier of genetic AD risk, which raises the question of the role of brain lipids. The Aβ peptide implicated in initiating AD is hydrophobic and rides on ApoE and other lipid carriers, so ApoE genotype can influence Aβ. Even without Aβ pathology, however, ApoE4 genotype modulates other AD risk factors. For example, ApoE4 influences the response to head injury and compensatory sprouting as well as three other pathogenic influences: cholesterol metabolism, oxidative damage, and inflammation.[8]

Environmental factors that may impact AD risk include essential polyunsaturated fatty acids, which are substrates for pathways dysregulated in AD pathogenesis

such as lipid peroxidation and cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. The dysregulation of lipid metabolism pathways in the disease process is likely to interfere with learning and memory because lipid transport selectively involves regions with high synaptic plasticity dependent on lipid salvage pathways. Further, the best-studied protective environmental risk factors for AD include nonsteroidal anti-inflammatory drug inhibitors of COX enzymes that act on an omega-6 (n-6) lipid substrate, arachidonic acid (AA). Finally, increased consumption of statins, fish/n-3 fatty acids, and lipid-soluble antioxidants like vitamin E that prevent lipid peroxidation has been reported to reduce AD risk. Thus, circumstantial but indirect evidence strongly implicates lipid metabolism in AD pathogenesis.

MOLECULAR PATHOGENESIS OF ALZHEIMER'S DISEASE

AD is initiated by increased Aβ (Aβ1-42) protein accumulation derived from proteolytic cleavage of a larger Aβ precursor protein by two enzymes called β- and γ- secretase. Aβ1-42 is normally produced and cleared but has a marked propensity to aggregate and form neurotoxic oligomers, protofibrils, and fibrils.[4] These oligomers and fibrils of Aβ interact with metals and glia to cause oxidative damage and neuroinflammation. They interact with neurons, notably at synapses, to cause synaptic dysfunction and loss as well as activation of at least three kinases (JNK, GSK3β, and CDK5) that hyperphosphorylate tau protein, causing its release from microtubules (loss of normal function) and aggregation into possibly toxic soluble tau oligomers and intraneuronal neurofibrillary tangles. The precise pathways for tau toxicity remain unclear, but many reports argue tau pathology appears to be a better correlate of neurodegeneration and progression than Aβ plaques. Synapse loss, not neuron loss, is the best structural correlate of cognitive decline and is likely the proximate cause.[9,10] While the common belief is that neuron loss drives dementia, it is encouraging to note one can be cognitively intact with far fewer than the normal complement of neurons. This is nicely illustrated by the literature on hydrocephalus, which shows many instances of individuals with normal or even above-normal intelligence despite having grossly enlarged ventricles and very little brain.[11–13] These cases show retardation or dementia need not be the result of substantially fewer neurons, provided the remaining neurons are functionally normal and able to compensate. However, in AD many of the remaining neurons that should have increased compensatory sprouting and expanded dendritic arbor instead show a dying back of dendrites and a loss of dendritic spines.[14] Fixing this defect blocking compensatory synaptogenesis would be expected to produce major therapeutic benefits.

While Aβ and tau pathology develop over many decades in a prodromal period, it is the onset of synapse loss that drives cognitive decline. For example, in cross-sectional studies, selected regional synapse loss in CA1-related stratum lacunosum[15] and frontal cortex[16] is reported in the earliest stages (mild cognitive impairment; MCI) of clinically significant AD. Total synaptic loss has been indexed by synaptophysin loss, which continues throughout the clinical progression to severe dementia, but in some regions that are affected in the early stages of MCI onset, for example the superior temporal cortex and hippocampus, there is a much more dramatic, region-specific loss of drebrin, an actin-binding protein found in the dendritic spines of excitatory neurons.[17]

Consistent with an initiating role in synapse loss, Aβ1-42 oligomers bind and target excitatory synapses,[18] where they dysregulate the molecular Rac and Rho signaling pathways that control the dynamics of the actin cytoskeleton in dendritic spines. These pathways are implicated in synaptogenesis and in many of the known genetic causes of developmental cognitive impairment, such as the X-linked mental retardation syndromes.[19] More specifically, Aβ oligomers engage and disrupt a synaptogenic pathway (NMDA/FYN/Tiam1/RAC/PAK /LIMK1/COFILIN) dysregulated in AD.[20,21] Thus, the known genetic causes of developmental cognitive deficits and biochemical defects initiating AD converge at the nexus of an Aβ oligomer-mediated attack on plasticity at excitatory synapses involved in learning and memory. This raises the question of how best to safely intervene early (preclinically), before this destructive process takes place. Are safe and effective dietary interventions possible? There are several roles for dietary intervention.

INCREASED RISK OF ALHEIMER'S DISEASE FROM DIABETOGENIC DIETS HIGH IN CALORIES AND SATURATED FAT

Overeating and diets high in saturated fat and calories are epidemic and obvious causes of obesity and metabolic syndrome, which lead to insulin-resistant diabetes, a significant AD risk factor that doubles the total dementia risk.[22] Diabetes also doubles the specific AD risk.[23] Diabetes is an independent risk factor independent of the ApoE4 allele, which alone doubles or triples the AD risk,[24] but diabetes was reported to combine with ApoE4 to further raise risk to 4.5-fold over those lacking either factor.[25] Thus, a simple recommendation is to control those dietary risk factors involving overeating and high-fat/high-calorie diets that are established risk factors for type II diabetes, CVD, and dementia.

A second and more complicated possibility is to address the role of neurotrophic insulin effects and

insulin signaling in AD.[26] There is increasing evidence for a defect in insulin-like signaling that may limit glucose utilization, synaptic plasticity, and survival signaling. In fact, clinical trials with the insulin-sensitizing drugs peroxisome proliferator-activated receptor-gamma agonists have already shown some success in ApoE3 but not ApoE4 carriers.[26,27] As discussed below, the n-3 fatty acid docosahexaenoic acid (DHA) can ameliorate insulin-signaling defects in AD animal models.

ASSOCIATION BETWEEN OMEGA-6 FATTY ACID METABOLITES AND PATHOGENESIS OF ALZHEIMER'S DISEASE

Western diets are typically high in n-6 fatty acids, notably linoleic acid, which is an AA precursor, and comparatively low in the n-3 fatty acids, alpha linolenic acid, and the long-chain marine fatty acids, DHA, and eicosopentaenoic acid (EPA). N-6 and n-3 fatty acids compete for incorporation into the labile second position of brain phospholipids, so that high n-6, low n-3 intake ultimately leads to a preponderance of AA in brain phospholipids. Since AA is the substrate for COX and LOX enzymes, this creates a net proinflammatory environment that interacts directly with AD pathogenesis because Aβ aggregates directly activate glia.[28] Further, Aβ oligomers (Aβn) activate MAP kinases to phosphorylate and upregulate cytosolic phospholipase A2 (cPLA2) and AA, resulting in elevated phospho-cPLA2 and AA metabolites from COX and LOX in AD and in AD animal models.[29] This helps explain why markedly elevated dietary n-6 polyunsaturated fatty acid intake (safflower oil) exacerbated excitatory synaptic marker loss, notably N-methyl-D-aspartate (NMDA) receptor subunits, as well as PSD-95 and drebrin loss in amyloid precursor protein (APP) transgenic AD model mice.[30,31] While there is no epidemiological data to support specifically lowering overall n-6 polyunsaturated fatty acid intake, Mediterranean diets appear to reduce AD and other dementia risk[32,33] and have lower levels of added sugar, saturated fat, trans-fat (n-6) fatty acids, and linoleic acid, a lower glycemic index, and a lower ratio of (n-6):(n-3) fatty acids. A Mediterranean diet embodies most of the recipe for lowering dietary risk for CVD, type II diabetes, and dementia. A key ingredient is higher n-3 intake in relation to n-6 fatty acids.

OMEGA-3 FATTY ACIDS AND DHA

As discussed above, n-3 fatty acids exert pleiotropic effects on the cardiovascular and central nervous systems that may be protective against age-related cognitive decline caused by either vascular or Alzheimer's dementia or a mix of both. Low n-3 fatty acid intake is one of many overlapping risk factors for both CVD and AD that include type II diabetes, hypercholesterolemia, hypertension, hyperhomocysteinemia, dietary saturated fats, cholesterol, low intake of antioxidants, high alcohol consumption, low physical activity or sedentary lifestyle, the presence of atrial fibrillation, and atherosclerotic disease.[5] Although low n-3 fatty acid intake is only one of many risk factors, it is one of the easier to remedy, and increasing the dietary intake is remarkably effective. For example, recent meta-analysis indicates n-3 fatty acids from fish can provide a 36% reduction in an unambiguous endpoint, death from coronary artery disease.[34] The cardiovascular protective effects of n-3 fatty acids are backed by repeated positive clinical trial results that lead to practical recommendations for dietary supplementation,[7] but, unfortunately, clinical trials for dementia prevention are more difficult to conduct than those for CVD. Evidence of the benefit of fish oil is compelling because it indicates secondary cardiovascular events may be prevented in high-risk patients with a first event.

Unlike cardiovascular events, the onset of dementia is erratic and insidious. Nevertheless, already by 2005, a US Department of Health and Human Services-funded evidence-based meta-analysis of the literature on n-3 fatty acids and dementia was able to conclude there was sufficient evidence to warrant clinical trials for the treatment and prevention of AD.[35] Since that 2005 review, new studies have often shown a 40–50% reduced risk of dementia associated with high n-3 intake. Nine studies showed increased fish consumption associated with reduced AD risk, while eight of ten studies associated higher blood n-3 levels with reduced cognitive decline or dementia.[36] Some but not all reports indicated a lack of protection in ApoE4 carriers, which likely represent nearly half of all AD cases and might explain why risk reduction from n-3 has appeared to plateau around 50%. Plausible ad hoc explanations for an ApoE effect include the differential effects of ApoE isoform on DHA trafficking or the increased oxidative stress, including lipid peroxidation products, in ApoE4 carriers,[37,38] consistent with preclinical studies.[39]

Another nutrigenomic factor that may influence the response to supplements is the recently discovered defective alleles of the delta-5 desaturase and delta-6 desaturases responsible for converting short-chain dietary alpha linolenic acid into very-long-chain EPA and DHA.[40] Future n-3 intervention studies need to consider likely nutrigenomic factors regulating the response to dietary polyunsaturated fatty acid into their intent-to-treat analyses or they may risk belying real efficacy with predictable nonresponder populations.

The blood level analysis reports of protection from dementia include one from the prospective Framingham study that revealed high (upper quartile) plasma DHA

(and no other lipid) assessed approximately 10 years before ascertainment of cognitive status predicted reduced dementia and AD.[41] The protected group had daily DHA intake estimated to average approximately 180 mg/day, which is more than double the average US daily intake. One can make a strong mathematical case that the average dietary intake of alpha linolenic acid and conversion to DHA is sufficient to meet normal needs in healthy animals and humans as measured by the balance of synthesis and consumption.[42] However, the apparent protective effects of exogenous very-long-chain n-3, usually from marine sources, in epidemiology and animal models argue there is something missing from the equation in the context of chronic age-related diseases or trauma. This raises the question of how DHA mechanisms protect against dementia.

MECHANISMS OF DHA PROTECTION AGAINST AGE-RELATED DEMENTIA

A recent review referenced 12 neuroprotective or anti-AD effects of DHA reported in preclinical models for DHA.[36] Many of these are illustrated in Figure 1. The neuroprotective effects are all from preclinical models but may be relevant because supplementing parenteral feeding with fish oil improved survival and recovery from severe head injury in patients with brain trauma.[43]

Preclinical models have shown the following neuroprotective effects of DHA: 1) Anti-inflammatory. DHA reduces AA and metabolites via COX and LOX (prostaglandins, hydroxyeicosatetraenoic acids), which are increased by elevated cPLA2 activity as discussed above. Reduced AA was found in brains of DHA-fed mice in an AD model.[31,44,45] 2) Potentiation of insulin/trophic factor via the protein kinase Akt due to increased phosphatidylserine-required membrane docking.[46] 3) Increased synthesis of brain-derived neurotrophic factor, a major neuroprotective factor.[47] This and other effects of DHA can be enhanced by exercise.[48] 4) Antioxidant. Possible protective effects on cellular membranes and stimulation of increased antioxidant enzymes (catalase, glutathione peroxidase) have been shown.[49] 5) Anti-apoptotic/anti-inflammatory and other neuroprotective effects via metabolites. DHA is the precursor to neuroprotectin D1, which has multiple anti-apoptotic and other anti-AD activities.[50] 6) Promotion of neurogenesis and neurite outgrowth. Dietary DHA promotes neurogenesis, neurite outgrowth, and improved cognition,[51] and increasing DHA selectively with the *fat*-1 transgene confirms these effects can be seen in adults and are due to increased DHA.[52] A mechanism for how DHA promotes stem cell differentiation to neurons has been recently proposed.[53] 7) Increased expression of a glucose transporter in brain endothelial cells.[54] 8) Amelioration of impaired coupling between blood flow and glucose utilization in aged monkeys.[55] 9) As an integral membrane component esterified to phospholipids, DHA improves synaptic membrane fluidity[56] and lipid raft function.[57] 10)

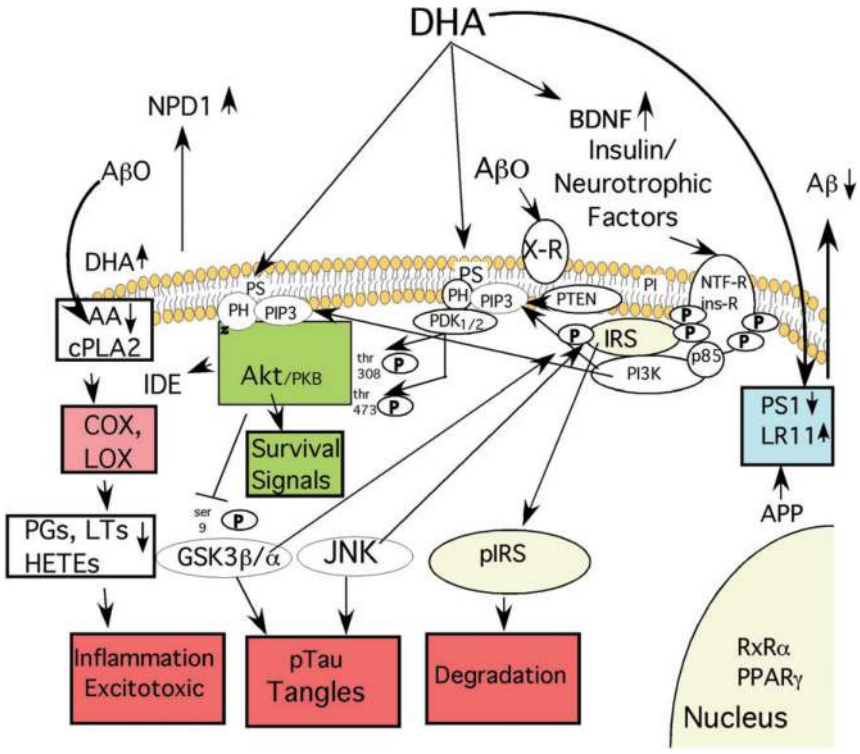

Figure 1 **Some of DHA's protective effects against Alzheimer's disease.**

Increased G-protein coupling, an effect best demonstrated in the retina.[58] 11) Activation of peroxisome proliferator-activated receptors and retinoid X receptor alpha receptors,[59,60] which may explain some metabolic and anti-inflammatory effects. However, whether retinoid X receptor alpha activation occurs with physiological DHA levels to promote neurogenesis is unclear.[61] 12) Remarkably effective protection against oligomer-induced synaptic marker loss in primary neurons in vitro.[62] One mechanism is the prevention of Aβ-induced phosphorylation of insulin receptor substrate (IRS) by tau kinases, notably JNK. Elevated phosphorylation of IRS-1 occurs in AD and AD animal models, which should result in uncoupling of insulin and neurotrophic factor signaling to the neuroprotective PI3-K/Akt pathway. This is one mechanism for insulin/trophic factor resistance and failed synaptic plasticity and compensatory sprouting responses to trophic factors. DHA has also been found to block Aβ oligomer-induced defects in a synapse-regulating pathway (NMDA/FYN/Tiam1/RAC/PAK /LIMK1/COFILIN) dysregulated in AD.[62] 13) Reduction of Aβ production and/or Aβ accumulation in vitro and in animal models in eight of nine studies. In the one study in which a reduction in Aβ was not found, dietary DHA failed to increase DHA or reduce AA levels.[36] DHA appears to reduce Aβ production by several proposed mechanisms, including alteration of APP and secretase mobility, reduction of expression of presenilin 1 and gamma secretase activity,[44] and induction of increased neuronal expression of the anti-amyloidogenic chaperone SorLa/LR11.[63] Omega-3 fatty acids may also increase expression of an Aβ-clearing transport protein, transthyretin,[64] or increase expression of insulin-degrading enzyme and clearance of Aβ.[65] 14) Limitation of tau kinases that promote tau pathology/neurofibrillary tangles, notably JNK and GSK3.[44,62]

DHA is remarkably pleiotropic, with many mechanisms for reducing Aβ production, tau kinases and tau pathology, neurodegenerative pathways, and neuron and synapse loss. However, DHA is very susceptible to lipid peroxidation to F4 isoprostanes, which are elevated in AD,[66,67] indicating insufficient protection and providing a very strong rationale for combining DHA with protective antioxidants like alpha lipoic acid or vitamin E. The combination of DHA with the polyphenolic antioxidant curcumin has also been advocated because the latter has additional anti-aging, anti-amyloid, and AD protective activities.[68] Efficacy for this combination appears to extend beyond Aβ-based models. Preliminary data from ongoing preclinical trials with the combination of curcumin and fish oil or curcumin and DHA in multiple AD models, including triple transgenics expressing mutant human tau[62] and pure wild-type human tau transgenics (SA Frautschy et al., unpublished data), suggest efficacy with this cocktail approach. The major obstacle for using curcumin (and related polyphenolics) as a supplement in humans is extremely poor bioavailability, chiefly from poor solubility, rapid glucuronidation, and high first-pass metabolism. Nevertheless, these obstacles to effective dosing in humans can be overcome with alternative lipidated formulations.[69] One such formulation is already under investigation in small clinical trials for AD and other diseases of aging as well as trials in mice for longevity.

COMPLETED CLINICAL TRIALS

Based on the encouraging epidemiology and preclinical data, researchers have proceeded with small clinical trials to test the utility of n-3 preparations in subjects with MCI or established AD at sites in Japan, Sweden, the Netherlands, the United States, and Taiwan as listed here: 1) Japan: 240 mg/day DHA + AA (3 months) improved memory and attention in amnestic MCI patients ($n = 12$) but not in AD patients ($n = 8$).[70] 2) Sweden: A trial with 4 g fish oil (1.7 g DHA for 6 months; $n = 174$) found no significant effect in patients with a mini-mental state examination (MMSE) score of <27. In this trial, 68% of the patients were ApoE4 positive, a genotype some epidemiology suggests responds poorly. However, in a subgroup of patients with MMSE scores of >27 there was improved delayed recall and attention, and cognitive function appeared stabilized with the fish oil ($n = 32$).[71] 3) Netherlands: The Memo trial tested 302 subjects (65+ years) who were considered normal if their MMSE score was >21 (a low cutoff for "normal"). Three groups were investigated: 1,800 versus 400 mg EPA + DHA versus placebo for 26 weeks. Increases in attention were observed. Surprisingly, this was especially pronounced in the E4-positive group and in the men, but there was no change in overall cognition.[72] 4) Netherlands: Scheltens reported the 12-week Souvenaid trial with a proprietary nutrient mix (containing DHA, uridine monophosphate, B vitamins, and antioxidants) in 212 mild AD (MMSE scores, 20–26).[73] Results showed improved delayed verbal recall, particularly in the mildest cases. Based on apparent success, a larger US-based follow-up Souvenaid trial is planned. 5) Taiwan: Chiu et al. reported the findings of a 6-month trial with MCI and AD patients in whom 1.8 g/day total n-3 appeared to result in significant improvement in Alzheimer Disease Assessment Scale-Cognitive scores compared to those in olive-oil-treated controls; however, the effect was only observed in the MCI patients, with no effect seen in the AD patients (NCT00628017).[74] 6) USA (Portland, Oregon): In the Oregon Health & Sciences University pilot study, in patients with MMSE scores of 15–26 ($n = 39$; fish oil [675 mg DHA and 975 EPA] ± 600 mg ALA versus placebo), fish oil plus ALA stabilized scores for MMSE and activities of daily living ($P = 0.02$).[75] 7) USA (Martek Bio-

sciences Corporation, Columbia, Maryland, United States): Yurko-Mauro et al. have completed MIDAS, a shorter trial in 465 subjects with pre-existing memory complaints randomized to receive 900 mg/day algal DHA for 6 months. The results, reported in July 2009 at the International Conference on Alzheimer's Disease in Vienna, show a significant improvement in the following primary outcome measures: paired associate learning, a visuospatial episodic memory test, and lowered heart rate.[76]

The results from these six clinical trials are not entirely consistent but generally suggest fish oil or DHA alone may have little or no benefit in cases of established AD with MMSE scores < 26 but may be useful for early intervention in subjects with mild memory complaints or minimal cognitive impairment. In addition, as reviewed elsewhere in this symposium by Dr R Wurtman, there is some evidence that, perhaps even in mild cases of established AD, a combination with other agents such as uridine monophosphate,[77] B vitamins, and/or antioxidants may also be especially protective. The observation that cognitive function appeared significantly stabilized or even improved in several trials encourages additional larger trials, particularly with those at the earliest stages of decline or even for primary prevention prior to any symptoms. In the 18-month duration NIH Alzheimer's Disease Cooperative Study, DHA showed reduced cognitive decline only in non APOE4 carriers. The great advantage of nutritional or exercise intervention programs lies in their potential for primary prevention.[78]

Several suggested mechanisms argue for more benefits with a preventative approach. For example, because nonsteroidal anti-inflammatory drugs have generally not shown robust effects in trials for established AD but may have worked at preclinical stages in the Alzheimer's Disease Anti-Inflammatory Prevention Trial, any n-3 nonsteroidal anti-inflammatory drug-like activity in reducing AA metabolites would likely be most useful for early intervention. Similarly, the most sensitive synaptic marker index of DHA depletion in APP transgenic mouse studies was drebrin, a dendritic spine marker in excitatory synapses.[30] Severe drebrin loss in hippocampus and superior temporal cortex has been reported in AD, and superior cortical drebrin loss occurs with the transition to MCI.[17] Like the accumulation of Aβ1-42, which begins to plateau by the emergence of clinical decline, drebrin loss is another DHA-sensitive endpoint best suited to an early intervention.

ONGOING TRIALS

To look at disease progression in established AD (MMSE < 26), J. Quinn (Portland, Oregon, United States) coordinated a multi-site Alzheimer Disease Cooperative Studies Consortium Trial with 402 mild-to-moderate AD subjects randomized to receive 2,000 mg algal DHA (Martek Biosciences Corporation) for 18 months.[78] In the Opal trial (n = 867) examining cognitive decline in subjects aged 70–79 years with MMSE > 24 and randomizing to placebo or 500 mg DHA + 200 mg EPA (n = 800) for 2 years results were inconclusive since there was no decline in either group.[79] B. Vellas (Toulouse, France) is leading a large (n = 1,200) trial for primary prevention of cognitive decline in frail elderly persons with MMSE > 24 using a multidomain intervention including exercise, nutrition, psychological counseling and n-3 (V0137), 800 mg DHA/day, over 3 years. The findings of these trials will provide more data about the effects of DHA or fish oil alone, but the best results are likely to come from nutrition-based cocktails.

CONCLUSION

The evidence from preclinical models indicates brain aging and dementia can be influenced by nutritional factors. The argument remains most compelling for avoiding high-saturated and trans-fat/high-calorie diets; these have previously been related to obesity, type II diabetes, and increased CVD risk, all of which also risk factors for age-related dementia. On the positive side, increasing intake of n-3 fatty acids, including marine long-chain n-3 and DHA in particular, appears to offer some protection against unhealthy brain aging that leads to dementia. Based on strong positive epidemiology and a wealth of pleiotropic protective activities observed in preclinical models with fish oil and its principal neuroprotective component, DHA, the marine n-3s as fish oil and algal DHA have already gone into pilot clinical trials. Results from six small clinical trials suggest possible protective effects in the earliest stages of cognitive impairment, but not in subjects with established AD. There is already some suggestion that nutritional cocktails including antioxidants, B vitamins, and other synergistic components may prove to exert stronger protective effects. These initial results encourage hope that n-3 may prove useful for prevention, but additional results from larger trials, particularly those with early intervention, will be required to prove efficacy. Some of these trials are already under way.

DHA reduces the production of the Aβ peptides from APP by increasing expression of the anti-amyloidogenic chaperone LR11, a protein that prevents APP from reaching the proteolytic secretases that cut Aβ from APP. DHA can also reduce expression of presenilin 1, a critical component of the gamma secretase that creates Aβ. Reducing Aβ production lowers levels of Aβ oligomers that act on candidate membrane receptors

(X-R) with multiple deleterious signal transduction effects. By incorporating into membrane phospholipids, DHA may also have multiple "fluidizing" effects on membrane structure, lipid raft, and protein-protein coupling discussed in the text. DHA incorporation will also competitively reduce membrane levels of AA, whose release from membrane phospholipids by phospholipase A2 generates free intracellular AA, which is a substrate for COX and LOX enzymes that produce various prostaglandin, Hete, and leukotriene products known to promote the elevated inflammation and excitotoxicity involved in AD pathogenesis. Aβ oligomers activate inflammation in glia and neuronal cPLA2, resulting in elevated AA and its COX and LOX products in AD and APP transgenic mice. DHA not only reduces these products, but DHA itself is transformed by LOX to a potent neuroprotective mediator, neuroprotectin D1, along with related mediators that have anti-inflammatory, neuroprotective, and other anti-AD activities. DHA can activate peroxisome proliferator-activated receptor and retinoid X receptor transcription factors. DHA has also been reported to be rate limiting for phosphatidylserine on the inner membrane leaflet that promotes docking with the PI3-K product PIP-3 and resultant activation of both Akt and upstream PDK, the critical effectors of the PI3-K/PDK/Akt survival signaling pathway stemming from activation of insulin and neurotrophic factor receptors. Signaling through this PI3-K pathway upregulates an Aβ protease insulin-degrading enzyme, has multiple pro-survival effects on apoptotic regulators ("survival signals") that protect neurons, and also inhibits a major tau kinase, GSK3β. Through this or other actions, DHA also reduces activity of another important tau kinase, JNK. GSK3β and JNK are known to hyperphosphorylate tau, resulting in neurofibrillary tangle formation, but they also target the critical insulin receptor substrates (IRS-1 and IRS-2). This causes IRS to uncouple from both insulin and neurotrophic factor receptors and to be rapidly degraded, resulting in a state of insulin/neurotrophin resistance. This is likely to contribute to synaptic loss and a dying back of neuritic arbor. Finally, DHA can increase production of brain-derived neurotrophic factor, which plays an important positive role in activity-dependent synaptic plasticity but is lost in AD. In summary, DHA has multiple pleiotropic activities predicted to slow AD pathogenesis at many levels.

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