

Omega-3 fatty acids, energy substrates, and brain function during aging

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Abstract

The maintenance of optimal cognitive function is a central feature of healthy aging. Impairment in brain glucose uptake is common in aging associated cognitive deterioration, but little is known of how this problem arises or whether it can be corrected or bypassed. Several aspects of the challenge to providing the brain with an adequate supply of fuel during aging seem to relate to omega-3 fatty acids. For instance, low intake of omega-3 fatty acids, especially docosahexaenoic acid (DHA), is becoming increasingly associated with several forms of cognitive decline in the elderly, particularly Alzheimer's disease. Brain DHA level seems to be an important regulator of brain glucose uptake, possibly by affecting the activity of some but not all the glucose transporters. DHA synthesis from either α -linolenic acid (ALA) or eicosapentaenoic acid (EPA) is very low in humans begging the question of whether these DHA precursors are likely to be helpful in maintaining cognition during aging. We speculate that ALA and EPA may well have useful supporting roles in maintaining brain function during aging but not by their conversion to DHA. ALA is an efficient ketogenic fatty acid, while EPA promotes fatty acid oxidation. By helping to produce ketone bodies, the effects of ALA and EPA could well be useful in strategies intended to use ketones to bypass problems of impaired glucose access to the brain during aging. Hence, it may be time to consider whether the main omega-3 fatty acids have distinct but complementary roles in brain function.

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1. Introduction

The proportion of elderly people in most developed countries is increasing and is expected to continue for at least 20–30 years. Healthcare costs increase significantly for the elderly, largely as a function of declining health and autonomy. Loss of memory and alterations in behaviour accompany declining brain function associated with aging, and are key symptoms of degenerative

brain diseases such as Alzheimer's disease and other forms of dementia.

The dementias are but one of several forms of chronic debilitating brain disorder. One estimate suggests that on a global basis, the burden of illness caused by the full spectrum of brain disorders now matches and may well surpass that of cardiovascular disease and cancer combined [1]. Hence, one of the imperatives of biomedical research over the next 20 years will be to better understand how to maintain optimal brain function and cognition in middle-aged and elderly adults.

Several nutrition-related factors heighten the risk of declining cognition, including insulin resistance, Type 2 diabetes and marked declines in body weight and total

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body fat. Given the increasing recognition of the important link between energy substrate supply and brain function, research is beginning into the role of nutrition and other lifestyle factors in at least some forms of cognitive decline associated with aging [2–6]. Our group's research strategy in this field is focussed on developing a better understanding of whether deteriorating access of energy substrates to the aging brain contributes to an increased risk of declining cognitive function. Indeed, we wonder if this deterioration may reach the point that it is appropriate to ask whether brain 'energy starvation' could be present in certain conditions like the dementias.

This paper reviews several aspects of the case that unfavourable nutritional status conspires to increase the risk of energy starvation in the aging brain. We also present evidence suggesting that the three main omega-3 fatty acids may have complementary yet distinct ways of helping maintaining optimal brain function by their actions on brain energy substrate supply.

2. Brain glucose uptake, positron emission tomography (PET), and brain function

The brain's principle fuel is glucose, which it consumes at about 25 $\mu\text{mol}/100\text{g}/\text{min}$ or about 100 g/d [7]. Ketone bodies (or simply ketones) are the brain's principle alternative energy substrate to glucose, especially during fasting or illness. However, glucose always supplies a minimum of 25–30% of the adult human brain's energy requirement, even during prolonged fasting or starvation [7].

Regional changes in brain glucose uptake are readily studied using PET, a minimally invasive technique that monitors the presence of positrons produced by a short-lived γ -radiation-emitting nuclide injected into the subject. For brain glucose uptake studies fluorine-18 is the preferred γ -emitting tracer and is incorporated into a glucose analogue— ^{18}F fluorodeoxyglucose. ^{18}F fluorodeoxyglucose is transported into tissues at the same rate as glucose itself but is not metabolized further, so it specifically represents glucose uptake unaffected by its subsequent metabolism.

PET studies have shown for over 20 years now that brain glucose uptake is impaired in Alzheimer's patients [8–10]. The impairment in glucose uptake is most affected in the temporal and parietal association cortices where it may be reduced by up to 20%. This effect is independent of and in addition to the usual age-associated decline in brain size and blood flow [11].

Until recently, it was unknown whether decreased brain glucose uptake is caused by or may contribute to the pathology of Alzheimer's disease [12]. Clearly, seriously damaged or dead neurons have low to negligible glucose uptake so a disease-driven decline in cognition can

potentially impair brain glucose utilization. However, long before any decline in brain function can be detected clinically, a mild but significant decrease in brain glucose uptake has recently been reported in individuals genetically susceptible to Alzheimer's disease [13,14]. This patchy 'pre-clinical' deterioration in glucose uptake occurs in the same areas of the temporal and parietal cortex where glucose uptake is most impaired in Alzheimer's disease. Hence, impaired brain glucose uptake now appears to be a potentially significant *contributing factor* to at least some types of declining brain function later in life [12–14].

3. Omega-3 fatty acids, aging, and brain function

One of the well-established deleterious effects of dietary deficiency of omega-3 fatty acids is on cognitive and behavioural development during infancy. Spurred by the interest in the role of omega-3 fatty acids in brain development, research has begun into the possible implications of low omega-3 fatty acid intake for brain function in the elderly. Thus far, the main observation is that the elderly consuming lower amounts of omega-3 fatty acids, particularly fish [15–23], have an elevated risk of Alzheimer's disease. However, this relationship is not always observed [24]. Post-mortem samples of Alzheimer's brain have decreased age-adjusted docosahexaenoic acid (DHA, 22:6 ω 3) content [25]. Collectively, these studies implicate lower brain DHA in the pathogenesis of Alzheimer's disease. Whether lower brain DHA in Alzheimer's disease is caused by lower DHA intake or by lower DHA synthesis (or by a combination of the two) remains to be determined.

While a relationship between low DHA intake and higher risk of Alzheimer's disease seems plausible based on studies of the role of omega-3 fatty acids in supporting normal brain function in infants and animals, there are as yet only preliminary and inconclusive reports of a therapeutic effect of omega-3 fatty acid supplementation on cognition or memory in elderly people without dementia [23]. Given the long time course before cognitive defects become clinically detectable and the variable rate of cognitive deterioration, intervention studies using DHA supplementation to prevent or treat cognitive decline will probably need to be large and lengthy if they are to be conclusive. It is also not yet clear whether the connection between low DHA and cognitive decline is causal (higher DHA intake protects against cognitive function) or is an effect of the disease process (neurodegeneration destroys brain DHA).

4. DHA and glucose metabolism

Rats made deficient in DHA by severe depletion of total omega-3 fatty acid intake have 80–90% lower

brain DHA, as well as 30–40% lower brain uptake of glucose and concomitantly lower cytochrome c oxidase activity in several brain regions [26,27]. Given the key role of glucose in brain function, suboptimal brain function in omega-3 fatty acid-deficient animals could be due at least in part to impaired brain glucose uptake linked to lower expression of the glucose transporter, GLUT 1, in blood vessels or astrocytes of the brain. Nevertheless, in these two studies [26,27], expression of GLUT 3 in neurons was unaffected, as was the concentration of GLUT protein and GLUT activity in brain microvessels or astrocytes. Hence, more work needs to be done to ascertain whether the defects in brain glucose transport that have been reported in DHA-deficient rats [26,27] can actually account for suboptimal brain function in rats made omega-3 fatty acid deficient. The relevance to cognitive decline associated with aging of this model of impaired glucose supply to the brain in omega-3-deficient rats also remains to be determined.

In several species including humans, glucose tolerance varies directly with the DHA content of skeletal muscle [28–31]. Insulin resistance and Type 2 diabetes develop in part due to less efficient uptake and processing of glucose by skeletal muscle, so these reports, particularly by Borkman et al. [28], make it plausible that low DHA status contributes to a heightened risk of insulin resistance in humans. Since insulin resistance is a common problem in the elderly and contributes significantly to the risk of Alzheimer's disease [2–6], we speculate that low omega-3 fatty acid intake, low tissue DHA (brain and elsewhere, especially muscle), impaired brain glucose uptake, insulin resistance and risk of Alzheimer's disease are all potentially linked.

5. Marked differences in ^{13}C -ALA and ^{13}C -DHA metabolism: preliminary data

Whether optimal health in adult humans necessitates intake of pre-formed DHA or whether sufficient DHA can be made endogenously remains controversial in several fields ranging from cardiovascular disease to immunology, neurology, and psychiatry. Biochemically, the complete 'desaturation-chain elongation' pathway converting the parent omega-3 fatty acid— α -linolenic acid (ALA; 18:3 ω 3)—to DHA exists in humans. However, in comparison to results from animal models or even human infants, adult humans have a very low capacity to convert ALA to DHA. This low capacity is clear in dietary supplementation studies with ALA, in which plasma DHA does not change significantly even in relation to high intakes of ALA (9–21 g/d for up to 6 weeks; reviewed by Cunnane [32]). The low capacity to convert ALA to DHA in humans is also apparent in stable isotope tracer studies, which generally show that

<0.1% of carbon- 13 (^{13}C)-ALA is found as ^{13}C -DHA in plasma for periods of up to 4 weeks after dosing with the tracer [33–35].

We have published a detailed assessment of the metabolism of ^{13}C -ALA in healthy young women [35] and, as have others [34], we concluded that part of the reason ^{13}C -ALA is so poorly converted to ^{13}C -DHA in humans is because it is readily β -oxidized. Despite inefficient conversion of ALA to DHA, dietary supplementation with ALA nevertheless reduces the risk of cardiovascular disease and cancer [37–39]. This suggests that not all the actions of ALA are necessarily dependent on conversion to DHA and that a more detailed assessment of both ALA and DHA metabolism in the elderly is warranted.

With the exception of one abstract suggesting ^{13}C -ALA conversion to ^{13}C -DHA is even lower in the elderly than in young adults [36], there are no full reports of the metabolism of ^{13}C -ALA in humans as they age. There are also no reports of the metabolism of ^{13}C -DHA given to humans of any age because this tracer has only recently become available. We have begun a comparison of the metabolism of ^{13}C -DHA and ^{13}C -ALA and will soon be extending it to the healthy elderly. Women in their mid-twenties consumed 50 mg of the tracer (^{13}C -DHA or ^{13}C -ALA) in yogurt at breakfast. Subsequent appearance of the tracer in breath $^{13}\text{CO}_2$ and in plasma total lipids was monitored over 8 days by isotope ratio mass spectrometry (Fig. 1). The small quantity of ^{13}C -DHA available to us at that time limited us to two subjects but there were six subjects in the ^{13}C -ALA group. Despite the preliminary nature of the ^{13}C -DHA part of this study, it appears that ^{13}C -DHA is much better retained in the plasma and is much less β -oxidized than ^{13}C -ALA, something that has long been assumed but we are now in a position to quantify and compare over different age strata. Results of this study should clarify whether the healthy elderly have altered synthesis or metabolism of DHA. It will then be useful to evaluate whether pathologies associated with aging that impact on cognition, e.g. insulin resistance, are associated with changes in DHA synthesis or metabolism.

6. Ketones: key alternative energy substrates to glucose

Ketones (β -hydroxybutyrate, acetoacetate and acetone) enter at several levels into the discussion of the link between omega-3 fatty acids, energy substrates, and brain function during aging. First and foremost, they are the principal alternative brain fuel to glucose. This is clear both from their ability to supply as much as 65–70% of the adult human brain's fuel needs during prolonged fasting [7,40] and from their key role in supplying both fuel and lipid substrates to the brain

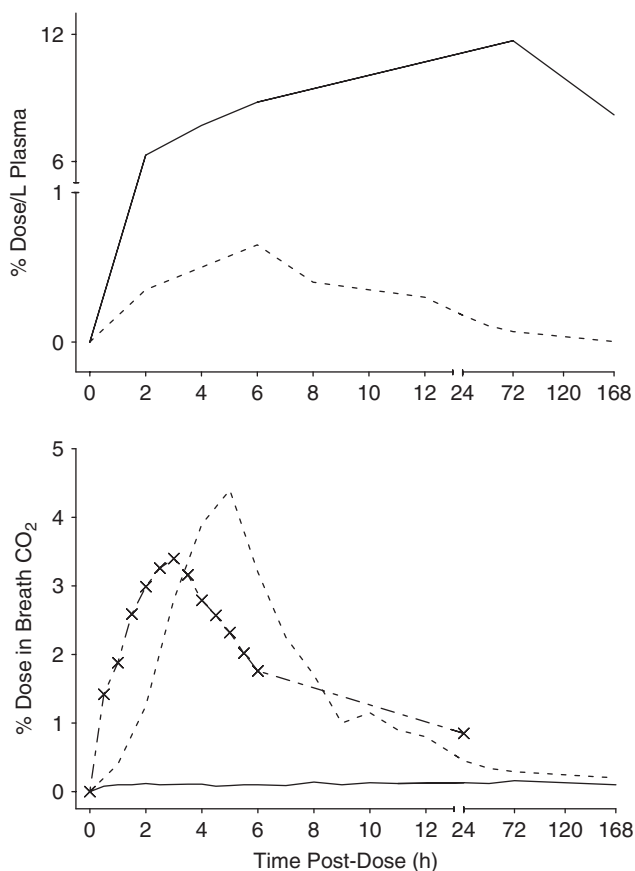


Fig. 1. Different metabolism of ¹³C-ALA and ¹³C-DHA in humans. Preliminary evidence for marked differences in the plasma levels (top) and β -oxidation (bottom) of ¹³C-docosahexaenoic acid (¹³C-DHA; solid line) compared to ¹³C- α -linolenic acid (¹³C-ALA; dotted line) in young healthy adults. Oxidation of ¹³C-glucose is also shown (X - - X bottom). For each tracer, healthy young women were given a 50 mg oral dose and follow-up was for 168 h (8 days). The β -oxidation data represent a cumulative oxidation of about 24% over 24 h for ¹³C-ALA, as compared to <5% for ¹³C-DHA, and about 37% for ¹³C-glucose. Data are based on $n = 6$ for ¹³C-ALA, $n = 6$ for ¹³C-glucose and $n = 2$ for ¹³C-DHA.

during foetal and neonatal development [41–45]. This role of ketones as crucial structural and fuel substrates for the developing brain is supported by the observation that healthy, well-fed infants are in a constant state of mild ketonemia [46]. Thus, mild ketonemia is physiologically important for normal human development and, unlike in adults, is not necessarily a sign of energy or insulin deficit.

Second, whether given by intravenous infusion or produced endogenously in response to intake of medium-chain triglycerides, ketones protect the brain in acute models of experimental stroke [47] and hypoglycaemia [48,49]. They are also associated with very short-term improvement in cognitive function in dementia patients [50]. Medium-chain triglycerides were chosen for Reger et al.'s study [50] because they readily access mitochondria where they are β -oxidized without

the need for transport via carnitine palmitoyl transferase (CPT), thereby becoming effective substrates for ketone production.

Most studies reporting the protective effects of ketones on the brain have been short term, but the efficacy of moderate ketonemia (2–5 mM) lasting over periods of 1–3 years in mitigating refractory epileptic seizures in children supports the view that mild-to-moderate ketonemia is not only beneficial to the brain but can be well-tolerated and effective in the long term [51,52]. The potentially beneficial effects of mild-to-moderate ketosis on the brain do not diminish the challenge of achieving and maintaining mild-to-moderate ketonemia, a condition almost incompatible with normal 'western' dietary habits that involve near-constant stimulation of insulin by dietary carbohydrate.

Medium-chain triglycerides are perhaps the most efficient way to produce mild ketonemia in humans but their gastrointestinal side effects in many individuals limit their utility on a large scale. Long-chain fatty acids are major fuels in the body but their β -oxidation is dependent on CPT, so they are less efficient ketogenic substrates than are medium-chain triglycerides. Among the most common long-chain fatty acids in the diet, one of the omega-3 fatty acids, ALA, is a 5–6-fold better substrate for CPT than is stearate and is a 3-fold better substrate than palmitate (Fig. 2) [53]. ALA is also more easily oxidized than linoleate or oleate [53]. In isolated rat hepatocytes, ALA is preferred by 2–3-fold over either linoleate or oleate as a substrate for ketogenesis [54]. Very high-fat diets enriched in ALA can lead to moderately higher ketonemia in rats than diets based on saturated fats [55]. Collectively, these studies suggest that ALA could be beneficial in producing mild ketonemia aimed at retaining or restoring cognitive function in the elderly. The use of ALA as a mildly ketogenic fatty acid would have the additional beneficial effect of producing a small trickle of DHA, which might

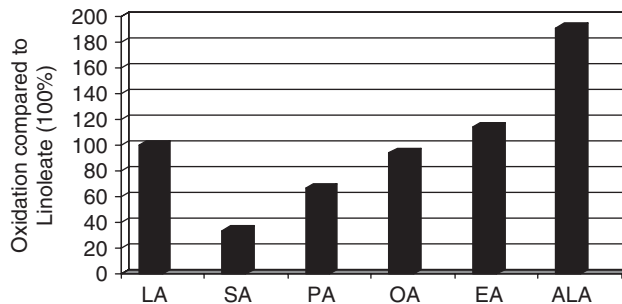


Fig. 2. Differential β -oxidation of long-chain fatty acids. These data, normalized to values for linoleic acid (LA; 100%), are summarized from a variety of models reported in the literature and are described in full elsewhere [53]. The comparison is normalized because of the different units used to express fatty acid oxidation in the various studies cited. SA—stearic acid, PA—palmitic acid, OA—oleic acid, EA—elaidic acid, and ALA— α -linolenic acid.

help the functionality of neurons now better supported by the additional brain fuel supply.

7. ^{11}C -acetoacetate: a new PET tracer

Strategies employing ketogenesis aimed at more efficiently supplying fuel to the aging brain would benefit from imaging methodology that provides a window on brain uptake of ketones. Although *in vivo* NMR spectroscopy may be applicable for this purpose, PET is probably the technique of choice, its utility having been clearly demonstrated by its widespread clinical use in the assessment of brain glucose uptake. The PET tracer needed for studies of brain ketone uptake is carbon-11, an isotope with a half-life of just 20.5 min. Blomqvist et al. [56,57] demonstrated the feasibility of making ^{11}C - β -hydroxybutyrate for human studies of brain ketone uptake in diabetes. In planning our own PET studies of brain ketone uptake, we have followed up the pioneering work of Blomqvist et al. [56,57] but have found it easier to make ^{11}C -acetoacetate, the methodology for which we will be publishing soon.

The key question that such a methodology could answer is whether brain uptake of ketones changes (overall or regionally) either in the healthy elderly with intact cognitive function or in those experiencing cognitive decline. In other words, is brain ketone transport susceptible to the pathology of aging in a manner analogous to the deterioration in brain glucose transport? At least in a single short-term experiment, cognitive function in individuals with Alzheimer's disease is modestly improved by mild ketonemia [50], which implicitly suggests that ketones must still be able to access the Alzheimer's brain or they would not be able to improve cognition. This supposition is testable using PET analysis of brain ketone uptake using either ^{11}C -acetoacetate or ^{11}C - β -hydroxybutyrate. Furthermore, since brain ketone uptake is mediated by a monocarboxylic acid transporter [58], PET methodology would be suitable to quantify the efficacy of nutritional or pharmacological strategies aimed at stimulating this transporter.

8. Insulin resistance: a key factor in brain starvation?

The problem of insulin resistance during aging is an implicit part of any discussion of strategies to improve energy substrate availability to the brain. Insulin resistance is common during aging and appears to be a major, if not the main, 'lifestyle' risk factor for cognitive decline during aging [2–6]. Insulin resistance is the precursor state to Type 2 diabetes mellitus and involves inefficient tissue uptake of glucose, which, in turn, leads to more insulin production, impaired insulin efficacy,

and insulin resistance. Skeletal muscle is the main site of glucose utilization in the body and so declining muscle mass in the elderly appears to be a factor potentially contributing to the increased risk of insulin resistance in the elderly.

Normally, i.e. in the absence of insulin resistance, low carbohydrate intake or total fasting decreases plasma insulin, which allows the liberation of free fatty acids from adipose tissue. When insulin is low, tissues capable of using fatty acids as energy substrates (skeletal muscle, heart) do so, while those dependent more on ketones produced by fatty acid β -oxidation (brain) have access to an increased supply produced primarily in the liver. When insulin rises after a carbohydrate meal, this immediately shuts off free fatty acid mobilization and glucose takes over again as the principle fuel.

In insulin resistance, transport of glucose into peripheral tissues is impaired, yet plasma free fatty acids are frequently elevated [3]. Elevated plasma free fatty acids should promote ketonemia but this works only when insulin is low, e.g. late in the post-prandial period in people with normal insulin sensitivity. However, the chronic hyperinsulinemia of insulin resistance blocks ketone production [59]. Hence, insulin resistance not only impairs tissue uptake of glucose but also impairs production of ketones, which are the main alternative fuels to glucose. It is unclear whether insulin resistance affects free fatty acid uptake but the brain is unable to meet its energy needs from fatty acid β -oxidation, so whether insulin resistance affects free fatty acid uptake elsewhere is somewhat irrelevant to the brain's fuel supply. Thus, when brain glucose uptake is impaired, inhibition of ketogenesis by insulin resistance [59] blocks at least some areas of the brain from getting sufficient amounts of either of the primary brain fuels (glucose and ketones). We hypothesize that this situation of impaired access of both the brain's major fuels leads to a heightened risk of brain starvation in insulin resistance.

Brain glucose uptake is widely considered to be independent of insulin so insulin resistance should not affect this process. Nevertheless, insulin resistance is a key factor in cognitive deterioration so, on the surface, these observations seem inconsistent. Insulin resistance could potentially impair cognition through mechanisms independent of brain glucose transport, so this topic still needs further research to clarify these uncertainties. We believe stable isotope and PET methodology are well suited to address how insulin resistance changes energy substrate metabolism and impacts on cognitive function during aging.

9. Eicosapentaenoic acid (EPA): a mediator of ketone production?

So far, this review into links between energy substrates and brain function during aging implicates two omega-3

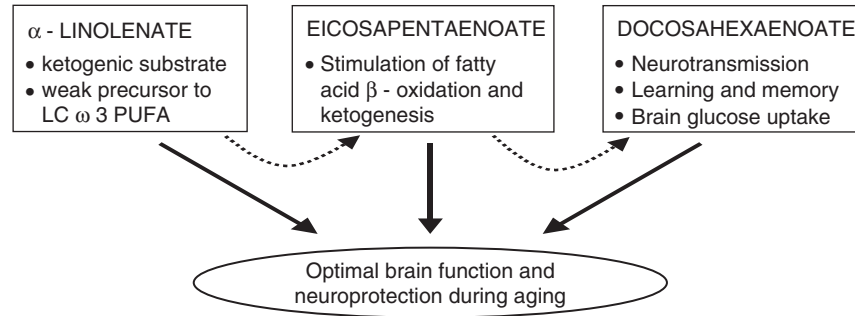


Fig. 3. Distinct yet complementary roles of omega-3 fatty acids in brain function during aging: a proposal. The dotted arrows indicate the weak level of conversion connecting α -linolenate to eicosapentaenoate and docosahexaenoate. The solid arrows indicate ways in which each of these omega-3 fatty acids is proposed to have potentially important effects on brain function distinct from their interconversion.

fatty acids—ALA and DHA—on several levels: (i) DHA seems important for normal brain glucose uptake and is commonly inadequately consumed by the elderly [15–23]. (ii) DHA also seems important for glucose uptake by skeletal muscle [28] so it may be an important mediator of body's overall ability to use glucose, i.e. it's insulin sensitivity. (iii) DHA is important for optimal neuronal function, a link that probably involves multiple mechanisms including effects on brain glucose uptake. (iv) ALA is a very poor precursor to DHA but nevertheless has a potentially important supporting role in brain function as an efficient and well-tolerated substrate for fatty acid oxidation and ketogenesis.

The intermediate omega-3 fatty acid, EPA (20:5 ω 3), may also be implicated in brain function during aging. However, the negligible changes in plasma DHA reported in studies with moderately high intakes of EPA [60,61] suggest any beneficial effect of EPA is unlikely to depend exclusively on DHA. Aside from its well-known role as a precursor to the three series eicosanoids and related peroxy-fatty acids [62,63], little is known about whether EPA has other functions in the body. However, we speculate that EPA may well facilitate fuel supply to the brain. Evidence is emerging to show that EPA is an activator of one or more of the classes of peroxisome proliferator activated receptors (PPARs) that promote long-chain fatty acid oxidation [64,65]. We hypothesize that as a PPAR activator, EPA could stimulate ketogenesis. If so, EPA would facilitate the effect of ALA as an efficient ketone substrate and both would help support the role of DHA as a key structural element in membrane phospholipids.

ALA and EPA both appear to be poor precursors to DHA in adult humans, so it seems imprudent to expect that sufficient DHA can be produced endogenously from either ALA or EPA to meet the brain's needs during aging. Hence, just as 30 years of research has gradually led to legislation of DHA in infant formula in many countries, it now seems appropriate to rigorously assess whether a dietary source of DHA is advisable in adults, especially the elderly.

10. Omega-3 fatty acids: distinct but complementary roles?

Because of the poor conversion of ALA and EPA to DHA yet beneficial effects of all three omega-3 fatty acids on different aspects of energy metabolism that impinge on the brain, brain function in adults should not be thought of as dependent on DHA alone. We suggest that the distinct roles of omega-3 fatty acids in energy metabolism and brain function are complementary and that optimal retention of cognitive function in the elderly probably depends on a blend of all three roles (Fig. 3). Whether therapeutic avenues exist in which impaired brain function can be corrected by one or more omega-3 fatty acids remains a tantalizing question.

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