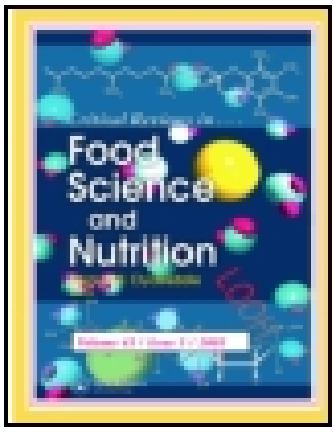

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Past and Present Insights on Alpha Linolenic Acid and the Omega-3 Fatty Acid Family

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PAST AND PRESENT INSIGHTS ON ALPHA LINOLENIC ACID AND THE OMEGA-3 FATTY ACID FAMILY

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ABSTRACT

Ŭ-linolenic acid (ALA) is the parent essential fatty acid of the omega-3 family. This family includes docosahexaenoic acid which has been conserved in neural signalling systems in the cephalopods, fish, amphibian, reptiles, birds, mammals, primates and humans. This extreme conservation, despite wide genomic changes over 500 million years, testifies to a uniqueness of this molecule in the brain and affirms the importance of the omega-3 fatty acids. Whilst docosahexaenoic acid (DHA) and its close precursor, eicosapentaenoic acids (EPA) have received much attention by the research community, ALA as the precursor for both, has been considered of little interest. There are many papers on ALA requirements in experimental animals. Unlike humans, rats and mice can readily convert ALA to EPA and DHA so it is unclear if the effect is solely due to the conversion products, or to ALA itself. The intrinsic role of ALA has yet to be defined. This paper will discuss both recent and historical findings related to this distinctive group of fatty acids, and will highlight the physiological significance of the omega-3 family.

Key words: omega-3 fatty acids, alpha-linolenic acid, docosahexaenoic acid, brain

Introduction:

The European Food Safety Authority recently confirmed a cause and effect relationship between the dietary intake of Ŭ-linolenic acid (ALA) and brain and nerve tissue development (EFSA, 2011). This decision comes after decades of research on omega-3 fatty acids and accumulation of sufficient evidence to support this claim. It is of great interest to review the early studies that were carried out to discover the physiological role of this fatty acid and also look at other potential functions of ALA in the diet. Whilst docosahexaenoic acid (DHA) and its close precursor, eicosapentaenoic acids (EPA) have received much attention by the research community, ALA as the precursor for both, has been considered of little interest. This paper focuses on ALA and helps to shed light on the importance of the single fatty acid in the omega-3 family that is considered to be essential.

Neurochemistry

Alpha-linolenic acid (18:3 n-3) is the parent of the omega-3 series of fatty acids. In small animals it is readily converted to EPA and DHA. The former is important in blood flow and control of inflammation whilst the latter is essential for neurogenesis, photoreception and synaptic signalling.

The structure of Ŭ-linolenic acid (ALA) is shown in Figure 1. ALA has 18 carbons with three methylene interrupted double bonds commencing at carbon 3 from the omega end (opposite to the carboxyl) of the molecule. It is converted by desaturation and chain elongation to a 22 carbon chain length fatty acids with six methylene interrupted double bonds- docosahexaenoic acid (DHA or C22:6 n-3). Brain cells have a very high membrane content of DHA. This

special structure provides the physical properties required for signalling in the brain and other cells which is not achieved if there are only 5 degrees of unsaturation (e.g. 22:5 n-3) (Bloom et al., 1999, Crawford et al., 1999, Jump, 2002, Eldho et al. 2003, Sobias et al. 2010).

To date, the merit given to ALA is largely due to its conversion to DHA. ALA is the essential fatty acid but DHA is the omega-3 fatty acid considered to play a crucial role in brain development. In different mammalian species brain size and number of cells varies but the DHA content of the brain cells do not (Crawford et al., 1976, Crawford et al., 1993). This fact exposes a high degree of evolutionary conservation, probably over 600 million years (Crawford et al., 2013). DHA is rapidly and selectively incorporated into brain cell membranes and is concentrated at synaptic signalling sites (Sinclair & Crawford 1972, Suzuki et al., 1997). Recently, it has been shown that addition of flaxseed (rich in ALA) to the diet of pregnant rats leads to a dramatic increase in brain weight and significantly higher concentrations of DHA in the brain (Lenzi Almeida et al., 2011).

Although DHA is synthesised from ALA, the process is strongly rate limited, particularly in humans. In 1972, Crawford and Sinclair first published evidence that DHA itself was an independent determinant of brain growth and evolution (Science, 2002, Leigh-Broadhurst et al., 2002). Deficiency of ALA has been show to cause fatty liver, loss of elastic tissue and severe behaviour pathology in Capuchin monkeys (Fiennes et al. 1973). Lamptey and Walker (1978) described cognitive loss in rats on an alpha-linolenic acid deficient diet.

Brain Function and omega-3 fatty acids

The balance between omega-6 and omega-3 fatty acids in our diets is important for health (Budowski & Crawford 1985) but has recently been eroded both by loss of omega-3 and increase in linoleic acid (omega-6). The balance used to be between 2 and 3 to 1 and is now from 10 to 20 to 1. The increased use of land based seed oils which contain large amounts of linoleic acid: the parent of the omega-6 fatty acids is considered to be one of the most significant dietary changes that has impacted this balance. The dramatic changes are also a consequence of the decline in the marine and fresh water foods which are the richest source of omega-3 and especially preformed DHA. Conversion of ALA to EPA and DHA also appears to be disrupted when the ratio of omega-6 to omega-3 is high. It has been shown in hepatoma cells, that the highest rate of formation of EPA and DHA occured when a 1:1 ratio existed between linoleic acid and ALA (Harnack et al., 2009). The disruption of the balance between omega-6 and omega-3 fatty acids is being linked to the rise in mental ill health (Hibbeln, 1998; Hibbeln et al., 2006a, 2006b,). Support for this connection comes from workers in Israel (Yehuda, et al., 2005). Moreover, the estimate of the burden of ill health in 30 European countries in 2010 revealed that brain disorders were much more costly than previously estimated and constituted a major health economic challenge for Europe costing at least ú798 billion per year (Olesen et al., 2012).

The bulk of the evidence to date on omega-3 fatty acids has focused on the role of DHA in the brain and EPA in blood flow. The significance of DHA to brain growth and function is now internationally recognised (FAO-WHO, 2010). There is very little ALA or EPA in the brain. The mechanism of action of DHA is often considered to provide the correct physical conditions for the receptors, transporters and other membrane proteins to function

properly. There is also new evidence that DHA acts as a precursor for powerful anti-oxidant neuroprotectins (Bazan et al., 2006; Farooqui, 2012). This explains how the brain is protected against oxidative stress. The self protective nature of DHA would mean that loss of DHA in the diet would not only contribute to problems of maintaining the brain but also to oxidative stress considered to be a contributor to the cause of Alzhemierôs disease (Lukiw et al., 2005, Bazan et al., 2011) and heart disease (Marchioli et al., 2005, FAO-WHO, 2010). Added to this body of evidence the relationship between attention deficit and hyperactivity disorders in children is now beginning to attract the attention of people testing the effects in trials in school children with some positive outcomes (Richardson, 2006, Sumich et al., 2009). Experimental evidence for omega-3 deficiency causing behavioural pathology was first seen in primates (Fiennes et al., 1973). There is also now good explanatory evidence that DHA stimulates the expression of over 100 genes important in brain growth and development (Kitajka et al., 2004). There is additional evidence that itôs unique six methylene interrupted cis-double bond sequence may be responsible for itôs mechanism of action in the signalling processes that are the key to the function of the brain (Bloom et al., 1999).

There is a wide variability in composition of the liver fatty acids amongst different species (Crawford et al., 1976). The variation is largely due to diet. Interestingly, those with diets richest in DHA have the largest brain sizes relative to body size. The variation vanishes when it comes to the brain composition itself, which is the same regardless of genes or diet. The difference between species is not the chemistry of the brain but the size to which it evolved. Note the brain contains no plant origin omega-3 or omega-6 fatty acids only the 20 and 22 carbon chain lengths with docosahexaenoic acid (DHA - 22:6 n-3) dominating the picture.

Other omega-3 fatty acids are not involved. In the developing rat brain, DHA was found to be preferentially selected over all other fatty acids at rates of at least ten times higher than ALA that originated from plant sources (Crawford & Sinclair, 1972). Hence there would have been a strong evolutionary advantage for the expansion of the human brain from a coastal habitat with access to the abundant food resources of the marine food chain. The marine and fresh water food chains are by far the richest in preformed DHA. Cod muscle phospholipids for example contain 47% DHA (Broadhurst et al., 2002). Bovid meat on the East African savannahs may contain less than 1% (Crawford et al., 1969).

We recognised, in the 1970s, that DHA was important to the brain. Thus it must be of singular importance to human nutrition as 70% of the human brain cells divide before birth and the remainder, mostly, in the first two years of life. This led to a series of publications on maternal nutrition (Wynn et al., 1994, Rees et al., 2005), breast milk composition (Crawford et al., 1974, Drury & Crawford, 1990) and on school children (Doyle et al., 1994). This also led to the publication of evidence of the potential role of arachidonic and docosahexaenoic acid deficits in preterm delivery and the failure to feed preterm infants adequately with regard to the lipids essential to that phase of brain development; as an important contributor to the neurodevelopmental damage so common at very low birth weights and preterm deliveries (Crawford et al., 2003).

A special case for ALA

One might ask the question- is there any real significance or use for ALA? The amount in the diet is relatively small except in herbivores. However, herbivores synthesize little DHA (Crawford et al., 1969, 1976). There has been very little published regarding the negative aspects of ALA in the diet, but one study did appear in 2006. Christiansen et al., provide support for a deleterious role of ALA in the development of prostate cancer. This data has been disputed by others and the same authors reported an advantage in a possible antiarrhythmic effect of ALA in women (Christiansen et al., 2005). Djousse et al. (2006) have shown that a higher consumption of dietary ALA is associated with higher plasma insulin, but not glucose levels, in non-diabetic subjects. Similarly Egert et al., (2006) have described how ALA benefited lipoprotein profiles but EPA and DHA led to oxidized LDL. In contrast, ALA enrichment did not enhance LDL oxidizability. Consistent with these findings Mustad et al., (2006) have described differential effects of omega-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse with some benefits for ALA. Importantly, Goyens et al., (2006a) present evidence that ALA might affect concentrations of LDL-cholesterol and apoB more favorably than EPA/DHA and that the level of ALA is of critical importance (Goyens et al., 2006b). Mandasescu et al., (2005) claim that dietary flaxseed significantly improves lipid profiles in hyperlipidemic patients and may favorably modify cardiovascular risk factors. Galliôs group in Milan have been demonstrating the use of good dietary sources of ALA as described by Marangoni et al., (2006).

The most powerful evidence comes from the Lyon study (de Lorgeril et al., 1999, 2004) in which a reduction of over 40% of sudden death was seen in the first year of the trial. One of the main differences between the diets of the test and controls was the ALA content. This trial has

led to increased interest in ALA and cardiovascular function (Albert et al., 2005, Breslow et al., 2006, Nannicini et al., 2006, Stark et al., 2008, Rodriguez-Leyva et al., 2010, Begg et al., 2010, Bassett et al., 2011, Leyva et al., 2011). The general consensus, based on available literature, is that ALA is a beneficial component of the diet and promotes heart health.

ALA in vascular and skin-hair involvement.

Deficiency studies in rodents (Sinclair and Crawford, 1972) and primates (Fiennes et al., 1973, Neuringer et al., 1988) have demonstrated a requirement for omega-3 fatty acids. These studies were done under conditions of an ALA deficiency. Moreover, in collaboration with the Hebrew University of Jerusalem it was demonstrated that competition between omega-6 and omega-3 fatty acids exists and it was shown that the balance is critical for brain development and structural integrity (Budowski & Crawford, 1985, Budowski et al., 1987). Evidence was found by Budowski et al. (1980, 1987) who carried out research on nutritional encephalomalacia (NE) in the chicken. NE is a field disease that killed whole flocks of chickens in the 1950s and 60s, with occasional outbreaks even today. It was attributed to peroxidative damage through the use of aged food. The model used to study this disease employed a linoleic acid rich oil such as corn or sunflower seed oil, stripped of vitamin E. Budowski recognized that this diet, which was fed to the hatchling chickens, was also deficient in alpha-linolenic acid. Notably the chickens would die with severe cerebellar encephalomalacia . The encephalomalacia started with micro haemorrhages which lead to microthrombi and severe inflammation. So the disease, like many other disorders in the brain, is fundamentally not a neural problem but vascular problem. This fact is notable, as the pathology in the primate deficiency of ALA included loss

of vascular elastic tissue (Fiennes et al., 1973) a condition that would readily lead to haemorrhage in rapidly growing blood vessels.

Deaths first occurred at about 12 days and the whole flock would be dead by 33 days. However, following the day ALA was added to the diet, no more chickens died. We took this experiment to the extreme of distilling the ALA from linseed oil that had been treated with nitric acid to remove any traces of anti-oxidants. There were three notable features of the study. (1) The condition required a deficiency of both anti-oxidant and ALA. (2) Purified ALA, stripped of any anti-oxidant, on its own would protect the chicks. (3) Deficiency was induced in the presence of large amounts of linoleic acid. The conclusion is that in the chicken at least, ALA is required for vascular development in the fast growing cerebellum.

In the ALA deficit capuchins a striking infiltration of fat in the liver, loss of hair and poor skin condition and repair leading to damage was observed. There was also a loss of elastic tissue in blood vessels. This loss would be consistent with the poor skin condition and it is likely that there is a specific requirement for ALA in vascular development and hair growth.

Anecdotally, sheep farmers in Wales tell us that if the winter is extended and spring grass delayed, then the sheep will lose their winter coats without replacement with their new summer coats. The impact is a high mortality. Moreover the new coat does not grow in until the green grass begins to sprout again. We saw the same with the Welsh ponies one of which we owned. At the end of the autumn Charlie had a massively thick coat. This protected him against all-weather outdoors in the winter. Come spring and the new green herbage, the winter coat fell out in handfuls and was replaced by a beautiful black shining new coat. During the winter he, as do the sheep, relied on eating dead food as in twigs, bramble vines and dead grass etc. In the

spring, the green grass brings to life the photosynthetic source of ALA, vitamins E and C and carotenoids together with other micronutrients.

Hence there seems to be an ecological link between the health of the skin and hair. This idea is supported by studies in guinea pigs. Studies on the metabolism of 1-14C-alpha-linolenic acid in the guinea pig showed the fur to have the highest specific activity of all tissues examined (Fu et al., 2000). They also found that linoleic acid was specifically incorporated into the skin and fur (Fu et al., 2001). The authors comment that there is a need to examine the role of these fatty acids not just as precursors for neural lipids but in other organs as well. This topic requires further research.

Marmoset wasting disease due to excess peroxidation and long chain PUFA

In support of the independent significance of ALA as opposed to DHA and EPA we can quote the problem of marmoset wasting disease that occurred in the Imperial Chemical Industryôs (ICI) colony which was the largest of its kind in the UK in the 1980-90s. These small primates would lose weight, failed to breed and have poor skin condition. At the Nuffield Institute of Comparative Medicine, at the Zoological Society of London, we were asked to investigate. We studied the animals in our laboratory fed on the ICI diet. We found that they were universally suffering from haemolytic anaemia. On examination of the diet we found it included fish oil. It seemed to us this could be the cause of the rupturing red cells as it was unlikely the marmoset would have been catching and eating fish in the canopy of South American Forests. The hand of peroxidation was probably also at work here as the little effort was made to control it in the food. When we removed the fish oil and reconstituted the diet

with a 5 to 1 ratio of linoleic acid to ALA, the hair and skin condition was restored and anemia vanished. The animals also started to breed again (Ghebremeskel at al., 1991). Outcomes from this study should serve as a warning in regards to excess consumption of fish oils.

A possible role for ALA in myelin synthesis?

In a study of the conversion of ALA to DHA in the brain Cunnane et al., (1999) found that only one in 50 molecules of ALA that reached the brain was converted to DHA. This result typifies the data on conversion in every animal studied- it is very poor, hence the argument for eating DHA preformed. However, the bulk of the ALA in the brain was used for the synthesis of palmitic acid and cholesterol. Now the brain does not import cholesterol but relies entirely on its own synthesis without which it cannot function. If cholesterol synthesis is poisoned during early development the brain simply fails to develop. Hence endogenous cholesterol synthesis by the brain is of crucial importance. Here may be a special role for ALA as it is one of the few substrates readily oxidised that pass into the brain. Both palmitate and cholesterol are especially important as precursors for myelin synthesis.

Conclusion

Most animal studies have been done in species which can readily convert ALA to EPA and DHA and benefits of ALA per se are difficult to isolate, however there is evidence of principle for ALA in its own right for brain health, vascular function, skin and hair condition. Recently, there has been an interest in this independent function and several new studies are discussed above. All are consistent with a function for dietary ALA independently in vascular protection.

Moreover, the fact that ALA is the precursor of all omega-3 fatty acids and genomes were set millions of years ago, implies that it has an intrinsic nutritional role to play. This role could come from the evidence currently available which could be for: skin; hair; elastic tissue; vascular function (from loss of elastic tissue); myelin synthesis (cholesterol & palmitic acid). Or to down regulate linoleic acid desaturation and chain elongation that may be especially relevant today, with a high LA to ALA ratio in the modern diet.

There is clearly a need for research into ALA as an independent nutrient. There is a case for an independent role for ALA just as there is for an independent function for linoleic acid, arachidonic acid, eicosapentaenoic acid and DHA. That is, the idea of lumping all omega-3 or all omega-6 fatty acids in one word (omega-3 or omega-6) is misleading.

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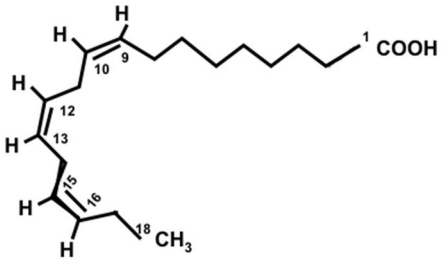

FIGURE 1 ALPHA-LINOLENIC ACID

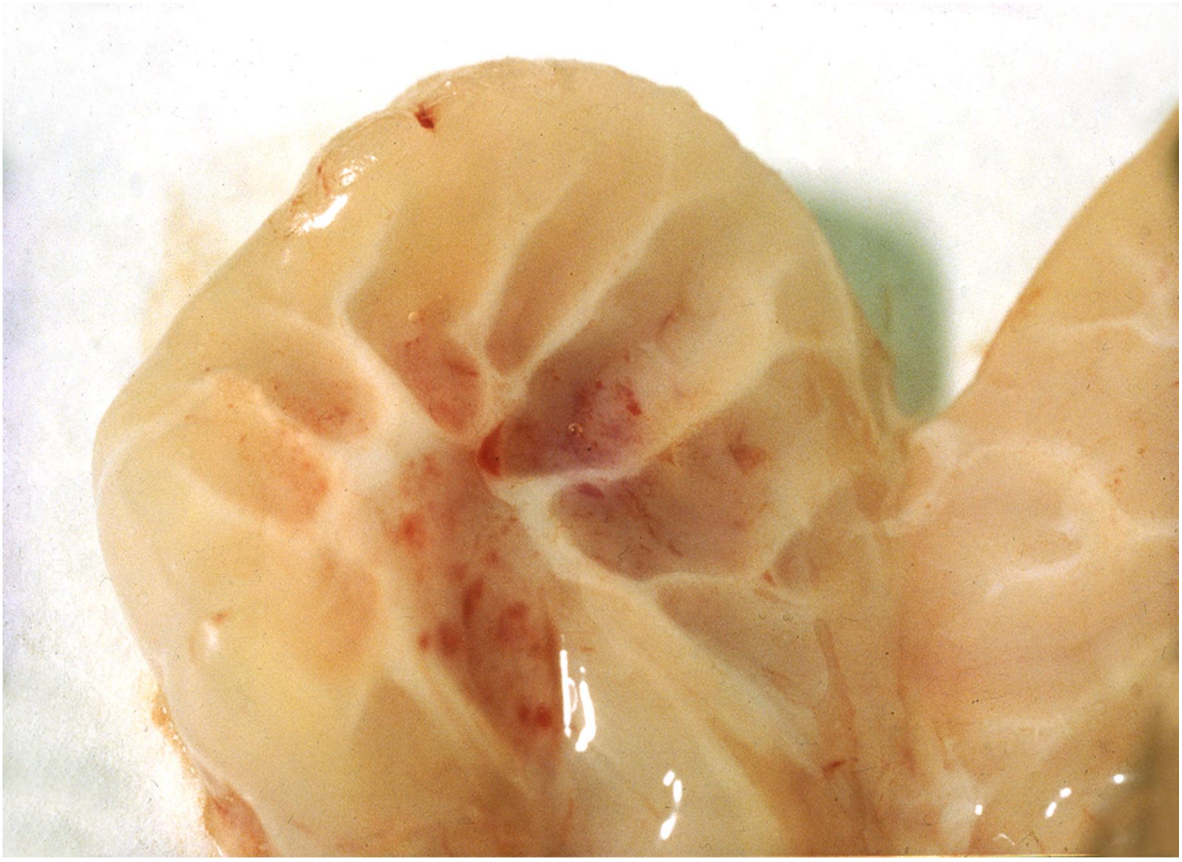

Figure 2: Hemmorhage in the cerebellum of a 14 day old chick fed a diet from hatching deficient in ALA and vitamin E.

