EXTREMELY LIMITED SYNTHESIS OF LONG CHAIN POLYUNSATURATES IN ADULTS:

IMPLICATIONS FOR THEIR DIETARY ESSENTIALITY AND USE AS SUPPLEMENTS

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Running head: Extremely limited synthesis of long chain polyunsaturates

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1

Abstract

There is considerable interest in the potential impact of several polyunsaturated fatty acids (PUFA) in mitigating the significant morbidity and mortality caused by degenerative diseases of the cardiovascular system and brain. Despite this interest, confusion surrounds the extent of conversion in humans of the parent PUFA – linoleic acid or α-linolenic acid (ALA) - to their respective long chain PUFA products. As a result, there is uncertainty about the potential benefits of ALA versus eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Some of the confusion arises because although mammals have the necessary enzymes to make the long chain PUFA from the parent PUFA, *in vivo* studies in humans show that ~5% of ALA is converted to EPA and <0.5% of ALA is converted to DHA. Because the _capacity_ of this pathway is very low in healthy, non-vegetarian humans, even large amounts of dietary ALA have a negligible effect on plasma DHA, an effect paralleled in the $\omega 6$ PUFA by a negligible effect of dietary linoleic acid on plasma arachidonic acid. Despite this inefficient conversion, there are potential roles in human health for ALA and EPA that could be independent of their metabolism to DHA through the desaturation-chain elongation pathway.

Key words:

Abbreviations:

AA - arachidonic acid ($20:4\omega6$)

ALA - α-linolenic acid ($18:3\omega3$)

DGLA - dihomo-γ-linolenic acid ($20:3\omega6$)

DHA - docosahexaenoic acid ($22:6\omega3$)

DPA - docosapentaenoic acid ($22:5\omega3$ or $22:5\omega6$)

EFA - essential fatty acid

EPA - eicosapentaenoic acid ($20:5\omega3$)

GLA - γ-linolenic acid ($18:3\omega6$)

LA - linoleic acid ($18:2\omega6$)

PUFA - polyunsaturated fatty acid(s)

SDA - stearidonic acid ($18:4\omega3$)

Introduction

Government regulatory agencies in North America and Europe recently began to require the addition of AA and DHA to infant milk formulas. This was an important milestone in recognizing the significance of limited conversion of LA to AA and ALA to DHA in infants. With an acceptance that infants are not able to make *enough* DHA from ALA to meet their needs, the concept of extremely limited efficiency of the desaturation – chain elongation pathway in humans has finally gained some acceptance, at least as applied to infants. Resistance to including DHA or AA in infant formulas was founded principally on a misunderstanding about the supposed efficiency of conversion of the parent to long chain PUFA in humans, a problem that arose primarily from inappropriate extrapolation from *in vitro* and animal studies to humans. Interest in this pathway is now focussed on whether one or more long chain $\omega 3$ PUFA are needed for adult health. As the use of fish oils to maintain cardiovascular and neurological health grows, a clearer understanding of the role of individual dietary PUFA in nutrition and metabolism is needed precisely because there is limited conversion of the parent to the long chain PUFA in humans.

Given the current interest in the health implications of PUFA, it seems surprising now how difficult it was to establish that the two parent PUFA, LA and ALA, are in effect vitamins or at least pro-vitamins. Using mostly rat models, the pioneers like Ralph Holman, James Mead, Erik Aaes-Jorgensen, and Rodolfo Brenner made heroic efforts to unravel the main features of the biochemistry, metabolism and nutritional importance of PUFA. As in several fields of nutrition, extrapolation from PUFA studies done in animals to the human condition wasn't easy. The present-day discussion surrounding the efficacy of the desaturation-chain elongation pathway show that several issues remain. For practical and ethical reasons, our understanding of the biological roles of and requirements for $\omega 6$ and $\omega 3$ PUFA in humans still

4

leans heavily on animal studies, but they have contributed to at least two important sources of confusion: the model known as 'EFA deficiency', and the extent of conversion of parent to long chain PUFA.

This review focuses on two related questions concerned with the nutritional importance of ω6 and ω3 polyunsaturates (PUFA) in adult humans – (i) estimating the % conversion through the desaturation – chain elongation pathway of the shorter chain 'parent' PUFA to their principal respective long chain PUFA, i.e. metabolism of LA to AA or ALA to DHA, and (ii) implications of inefficient conversion of parent to long chain PUFA in humans for the nutritional or potentially health protective roles of individual PUFA. The context is that the extremely limited conversion of parent to long chain PUFA in humans presents an opportunity to investigate the clinical effects individual PUFA, especially ALA, EPA and DHA, with minimal confounding due to conversion of ALA or EPA to DHA.

Historical Context: Two Important Sources of Confusion

Almost 70 years ago it was discovered that normal growth and development in rats depends on the presence of LA and ALA in the diet. These two fatty acids are both eighteen carbon PUFA with LA having two and ALA having three double bonds (18:2ω6 and 18:3ω3, respectively). Plants but not mammals can insert double bonds into the eighteen-carbon homologue – oleic acid (18:1ω9) – to sequentially make LA and then ALA. The absence of either LA or ALA from the diet of rats induces clinical symptoms that cannot be corrected by any known saturated or monounsaturated fatty acids. The discovery that dietary fat deficiency caused specific symptoms due to insufficient ω6 and ω3 PUFA ushered in the brief era of 'vitamin F'.

By the 1950s, AA was available in pure form and although it too could relieve some symptoms of fat deficiency, it was generally less efficient than LA. Based probably on the 'essential amino acid' model, and the fact that several different fatty acids seemed to be involved, vitamin F was replaced by the term 'essential fatty acid' (EFA). Initially, three PUFA were EFA – LA, AA and ALA, but it was soon learned that rats could synthesize AA from LA so AA was subsequently de-listed. Despite the broadly similar efficacy of both LA and ALA in preventing or correcting the gross symptoms of total dietary fat deficiency in rats (Greenberg and Deuel 1950; Mohrhauer and Holman 1963), the syndrome of 'EFA deficiency' became associated primarily with deficiency of $\omega 6$ PUFA, specifically LA.

During the 1950-1990s, the few human studies examining the amount of PUFA needed to correct total dietary fat deficiency almost all involved 'artificial' feeding, either in infant formulas substituting for breast milk or in total parenteral nutrition for surgical patients. Both these human models showed that LA prevented the skin and gastrointestinal symptoms of total dietary fat deficiency. As in the concurrent rat studies of the day, these studies did not look carefully enough at the role of $\omega 3$ PUFA in the symptoms that were observed. Hence, there was little resistance to decreasing the number of EFA from two to just one - LA – which was declared in the early 1970s to be the one and only EFA (Holman 1971; Wene et al. 1975). The situation favouring attention on the $\omega 6$ PUFA was encouraged by parallel discoveries in the early 1970s linking two of the long chain $\omega 6$ PUFA – dihomo-γ-linolenic acid (DGLA) and AA - to the newly discovered prostaglandins, which are now grouped under the superfamily of eicosanoids.

The Problem with EFA Deficiency

Whether in rodents or in humans, modelling dietary requirements for ω6 PUFA is most commonly based on inducing and then correcting EFA deficiency. In the early studies, EFA deficiency was achieved by providing a diet as totally depleted of fat as possible. It was subsequently accepted that since fat is normally present in the diet, EFA deficient diets should also contain some fat. In order to assure total PUFA deficiency, the dietary sources of fat clearly had to rigidly exclude unsaturated fatty acids. At a result, EFA deficient diets contain neither monounsaturates, ω6 PUFA, nor ω3 PUFA.

The lack of both ω6 and ω3 PUFA in EFA deficient diets creates a problem of both principle and practice (Cunnane 2003a). The problem related to principle is that ω6 and ω3 PUFA are chemically and nutritionally two distinct families of nutrients, so one should be present when deficiency of the other is being evaluated experimentally and *vice versa*. Otherwise, any deficiency symptoms observed cannot be attributed directly to either one or other PUFA family. The practical problem is that ALA actually <u>protects</u> against ω6 PUFA depletion (Bourre et al. 1989; Greenberg and Deuel 1950; Hansen and Jensen 1983). This means that the double depletion, i.e. EFA deficiency, is a more extreme situation than the specific deficiency of either ω3 or ω6 PUFA alone (Cunnane 2003a; Guesnet et al. 2006). In hindsight, it is clear that the early research on EFA deficiency propelled the PUFA towards a legitimate and much needed seat at the essential nutrient table. The cost of that visibility was the inappropriate skewing of the nutritional importance of PUFA heavily towards to ω6 family, specifically LA, an imbalance the repercussions of which are still felt today.

The EFA deficiency model also contributes to difficulties of extrapolating conversion of the parent to the long PUFA. This is not only because rats normally have more efficient conversion of the parent to long chain PUFA than humans but also because EFA deficiency

7

further increases this conversion. Hence, it was common to do studies on the desaturation – chain elongation pathway using EFA deficient rats (Holman 1971; Sprecher 1968). The stimulation of the desaturases in EFA deficient animals presumably occurs because declining tissue levels of the long chain PUFA feed back on the desaturation-chain elongation pathway to stimulate their own synthesis. These two confounders – the rat model and the stimulatory effect of EFA deficiency – were important contributors to the ongoing misunderstanding about the inefficient synthesis of long chain PUFA in humans.

The Need for Long Chain PUFA in Infants

By the 1970s, studies on early development raised concerns towards the prevailing attitude that ω3 fatty acids were not really important (Benolken et al. 1973; Lamptey and Walker 1976; Sinclair and Crawford 1972). In fairness, symptoms of ω3 PUFA deficiency are mild even under well controlled laboratory conditions in the most susceptible model known – the rapidly growing rat (Bourre et al. 1989; Moriguchi et al. 2004), so it took meticulous, painstaking, multi-generation studies to push this field forward. In the 1980-1990s, establishing PUFA requirements for healthy development in infants became the key battleground that helped refocus nutritional attention on the role of PUFA in free living humans instead of correcting EFA deficiency in patients or modelling PUFA requirements in lab animals.

The hard wrought recognition that pre-formed DHA was necessary in formula milk for human infants was driven home by two key observations: First, the brain of the breast-fed infant acquires about 50% more DHA compared to the formula-fed infant receiving no DHA (Farquharson et al. 1992; Makrides et al. 1994). Hence, infants not given DHA are not able to make, or at least accumulate, as much DHA as those who were receiving DHA from breast

8

milk. Second, Zellweger syndrome is a rare but devastating inherited peroxisomal disease in which neurological and physical retardation are profound and death is likely within the first few years of life. DHA synthesis and brain DHA accumulation are severely impaired in Zellweger syndrome but the symptoms are not immediately apparent in breast-fed cases because they obtain DHA from breast milk and from limited body DHA stores (Martinez 1989). Zellweger cases that are bottle-fed a formula containing no DHA rapidly become symptomatic but also respond positively though very modestly to DHA supplementation (Martinez 1989). Combined with much associated research, these two observations were pivotal in dissipating the long debate about the nutritional importance of some *pre-formed* (dietary) DHA in infants.

The challenge in this process was that studies on PUFA metabolism needed to be done in healthy infants. This was necessary both ethically and to reduce confounders associated with prematurity or low birth weight. The problem was that healthy term infants are not only able to make a small amount of DHA, but they also have considerable DHA stores at birth (Cunnane et al 2000) so they have the most 'resistance' to the absence of incoming pre-formed DHA. Hence, paradoxically, the most suitable infant model was the one in which it was hardest to show the need for pre-formed DHA. Incidentally, although AA is present in all breast milk and a need for pre-formed AA has been legislated into infant formulas along with DHA, the role of dietary AA in healthy early development is much less clear than for DHA.

The Desaturation-Chain Elongation Pathway

The pathway by which LA and ALA undergo conversion to their respective longer chain PUFA involves a sequence of desaturation and chain elongation steps that figuratively occur in two dimensions: In the first dimension, desaturation adds a double bond across two carbons of a fatty acid. In the second dimension, chain elongation adds two carbons at the

carboxyl end of a fatty acid. Not only PUFA but also saturated and monounsaturated fatty acids undergo desaturation and chain elongation; this is how plants convert palmitic acid (16:0) to stearic acid (18:0), oleic acid (18:1ω9) and on to LA and ALA. The desaturation - chain elongation pathway has several broadly consistent, well recognised features across species and in different tissues (Table 1).

At each step, the pathway tends to favour either desaturation or chain elongation but, in fact, all the known PUFA can be both desaturated and chain elongated to a greater or lesser extent. For example, the most commonly known first downstream product of LA is the Δ^6 desaturase product - γ-linolenic acid (GLA; 18:3ω6) – which can be elongated and further desaturated to AA. Nevertheless, LA's lesser known product, eicosadienoic acid (20:2ω6), is also present in most tissues but is formed not by desaturation but by direct chain elongation of LA.

The bidimensional nature of PUFA conversion and the flexibility of substrates for desaturation-chain elongation means that membership in each of the ω6 and ω3 PUFA families has burgeoned from 3-4 known fatty acids in the 1940s and 1950s, to a latticework of many possible fatty acids *within* each of these two families. At least eight double bonds can be present and chain lengthening out to more than 34 carbons has been described (Suh and Clandinin 2005), but these 'ultra-long' PUFA occur in very low amounts and their biological role is completely unknown at this time. Chemically, almost any member fatty acid can be converted in either dimension but, biologically, alternating desaturation and chain elongation tends to be favoured. In practical terms, this means that the downstream conversion of each 'parent' PUFA produces a total of about 12 PUFA that are commonly measurable in human or animal tissues (Table 2).

Quantitatively, the main $\omega 6$ PUFA product of desaturation-chain elongation is AA, which is synthesized from LA in three steps – (i) Δ^6 desaturation to GLA, (ii) chain elongation to DGLA, and (iii) Δ^5 desaturation to AA. In the $\omega 3$ PUFA series, the equivalent fatty acid to AA is eicosapentaenoic acid (EPA), which is derived from ALA after the same three step sequence of two desaturations separating a single chain elongation step. However, EPA is a quantitatively (if not functionally) minor fatty acid. The broadly equivalent $\omega 3$ PUFA to AA as a membrane constituent is not EPA but DHA, the synthesis of which requires further desaturation and chain elongation.

Originally, synthesis of DHA from EPA was thought to occur via two chain elongations separated by a single Δ^4 desaturation but direct evidence for the Δ^4 desaturase has so far been extremely elusive. Rather, the more convoluted 'Sprecher pathway' seems to be the only way to produce DHA (Voss et al. 1991). This pathway involves elongation of EPA to $\omega 3$ docosapentaenoic acid (DPA; 22:5$\omega 3$) which is then Δ^6 desaturated to 22:6$\omega 3$ and then chain elongated to 24:6$\omega 3$. The final step in making DHA is a two carbon chain shortening which is believed to occur in peroxisomes. The equivalent steps convert AA to $\omega 6$ DPA (22:5$\omega 6$), an isomer of $\omega 3$ DPA.

In Vitro Evidence for Desaturation-Chain Elongation in Humans

For many years, lipid enzymologists especially those studying plants had been assaying desaturase activity in order to understand the controls on synthesis of oleic acid from stearic acid, a step employing the Δ^9 desaturase. They therefore were armed and ready to take the first crack at these measurements in animals, first with the same Δ^9 desaturase and then to measure the conversion of LA and ALA to their respective long chain PUFA. Enzymologists prefer to use purified enzyme preparations to determine rates, optimal pH,

cofactors, and other parameters controlling enzyme activity. Desaturation and chain elongation occur in the endoplasmic reticulum so isolation of tissue microsomes and purification of the enzymes magnifies many fold the conversion efficiency of each step.

With the rare exception of genetic anomalies such as Zellweger syndrome, humans have a fully functional desaturation-chain elongation pathway in the liver (and in other organs) that can convert <u>some</u> LA and ALA to their respective long chain PUFA. This has been established by several groups using *in vitro* assays on cells in culture or assays of the semi-purified desaturase enzymes themselves (Aeberhard et al. 1978; Biagi et al. 1990; de Gomez Dumm and Brenner 1975). However, results of the desaturase assays vary a lot between studies and have not yielded consistent results (Blond et al. 1981). Nevertheless, the question – do humans have the *enzyme machinery* to convert LA or ALA to their respective long chain PUFA? - can unequivocally be answered in the affirmative.

ALA Conversion to DHA: *in Vivo* Studies

Although humans technically possess the ability to desaturate and chain elongate PUFA, it is critical to be able to estimate net *in vivo* conversion through the pathway, especially in the ω3 PUFA family. This is because although ALA is the dominant ω3 PUFA in the diet of most countries, it is DHA that is the dominant ω3 PUFA in membranes. DHA is not only found in relatively large amounts in membranes but, functionally, it is by far the most important ω3 PUFA, especially during early development (Salem et al. 1996). DHA is also associated with lower risk of degenerative diseases of the brain and cardiovascular system (Freund-Levi et al. 2006; Gebauer et al. 2006). Hence, it is important to clearly establish the extent to which ALA is useful as a source of membrane DHA and whether pre-formed dietary DHA is needed by adults as well as infants.

Biochemically, the same issue applies to the conversion of LA to AA as applies to ALA conversion to DHA. However, nutritionally, the context is the opposite for the ω6 compared to the ω3 PUFA because there is no known risk linking AA deficiency to cardiovascular or brain health in adults. Indeed, excess intake of ω6 PUFA, especially LA, may well be contributing to the risk of cardiovascular disease (see ω3 and ω6 PUFA and Human Diseases – A Brief Overview).

The two commonest and most ethical ways to estimate desaturation-chain elongation of PUFA in *intact* humans are either using isotopically-labelled tracer fatty acids, or by analysis of plasma fatty acid profiles after dietary supplementation with the parent PUFA of interest. Both methods are indirect and both generally draw their information from a limited pool of body fatty acids, usually only blood plasma. Though they are very different approaches to the question, in omnivorous healthy adults, they nevertheless produce remarkably similar results.

Using stable isotopes or radioisotopes of LA or ALA to measure PUFA metabolism, *in vivo* studies show that humans can synthesize the respective long chain PUFA. From a strictly qualitative perspective, the *in vivo* data therefore agree with the *in vitro* data. However, analysis of the disappearance of the label from the precursor PUFA and its appearance in long chain PUFA suggests that ALA conversion through to DHA is almost universally <0.5% (Table 3). Hence, using tracers, the best attempts to quantify this conversion *in vivo* lead to essentially the opposite conclusion to that derived from the *in vitro* data, i.e. that, in humans, conversion of ALA to DHA is extremely limited, indeed, negligible (<0.1%) in many studies.

The response of plasma DHA to raised intake of ALA is the other common way to study conversion in the ω3 PUFA pathway. Flaxseed (linseed) oil contains 50-60% ALA so it has been used widely for this purpose. Several studies of similar dietary design show that

plasma DHA does not change significantly after supplementation with flaxseed oil or flaxseed itself (Table 4A). Like tracer studies, dietary supplementation studies of PUFA conversion do not fully agree on the apparent degree of conversion. Supplementation studies are also constrained by sampling being limited primarily to plasma with frequently no more than one blood sample before and one after the supplementation period.

The ALA supplements used often exceed the normal ALA intake of ~1.5 g/d by 5-10 fold (Table 4A) so it is legitimate to ask whether data from these supplementation studies are really suitable for understanding desaturation-chain elongation in humans consuming an 'average' diet. By definition, the supplementation model requires a raised dose of the precursor PUFA or else the change in plasma fatty acid profile cannot be detected. Hence, this is a flaw that cannot easily be eliminated from the model but may nevertheless skew the data towards an underestimate of true desaturation-chain elongation.

Equally importantly for the supplementation model – is plasma DHA too tightly regulated to allow for an increase after ALA supplementation? Here, the answer is clearer than with the substrate loading question: Plasma DHA does rise after supplementation with pure DHA (reviewed by Arterburn et al. 2006), which clearly shows that the combined effects of some β-oxidation, clearance to tissues, or further metabolism to other products do not prevent an increase in dietary DHA from raising plasma DHA. Hence, if DHA synthesis from ALA is occurring, it should be detectable by a measurable rise in plasma DHA after ALA supplementation. With two exceptions, this is rarely the case.

The first exception is vegetarians/vegans. Vegans strictly exclude all animal foods including fish. Hence they consume no known source of DHA. If they couldn't make DHA, they should have very low plasma DHA and, more importantly, should have symptoms of DHA deficiency such as impaired vision and cognition. However, although they have lower

plasma DHA, healthy vegans do not have clinical evidence of DHA deficiency (Geppert et al. 2005; Melchert et al. 1987; Sanders et al. 1978). Intuitively, therefore, they must be making some DHA or reducing its turnover. One would therefore expect plasma DHA in vegetarians to rise in relation to ALA supplementation but this is not necessarily the case (Li et al. 1999, Fokkema et al. 2000). Vegans/vegetarians are an important window on the ALA conversion question but to better understand these apparent inconsistencies, more detailed studies are needed.

The other exception in which plasma DHA generally rises after ALA supplementation is severe $\omega3$ PUFA deficiency (Table 4B). However, in the few reported cases, the rise in plasma DHA is much less obvious in adults (Bjerve et al. 1989) than in the two reported cases involving children (Bjerve et al. 1988; Holman et al. 1982) and is confounded by the clinical histories and multiple nutritional insufficiencies of the patients. As shown in *in vitro* studies (Table 1), tissue content of the product (DHA) probably has an important impact on its own synthesis, i.e. when tissue content of DHA is very low ($\omega3$ PUFA deficiency) ability to synthesize it from ALA would not surprisingly increase.

Low tissue content of DHA may not stimulate enough DHA synthesis to prevent clinical symptoms, at least in infants. A rare glimpse into the *tissue* DHA response to different $\omega3$ PUFA intakes is available from two similar studies done in infants (Farquharson et al. 1992; Makrides et al. 1994). In both studies, the comparison is imperfect in the sense that breast-fed babies were compared to those receiving a milk formula containing only ALA as the source of $\omega3$ PUFA. Both studies showed that 2-6 month old infants receiving ALA as the only $\omega3$ PUFA accumulate much less brain DHA than breast-fed infants of the same age. Breast-fed infants obtain at least 50-60 mg/d of pre-formed DHA from breast milk and accumulate brain DHA at about 5 mg/d over the first 6 mo of life but the brains of formula-fed infants

accumulated only 2.5 mg/d of DHA (Cunnane et al. 2000). To prevent the depressed

trajectory of brain DHA accumulation in infants receiving the ALA-based formulas, it was

calculated that they would theoretically need to be able to convert at least 5% of dietary ALA

to brain DHA. In other words, if ALA-fed infants not receiving dietary DHA achieve only 50%

of the brain DHA accumulation of breast-fed infants, their ability to convert dietary ALA to

brain DHA must be well under 5% (Cunnane et al. 2000).

Both the tracer and dietary supplementation methods offer only indirect estimates of

conversion of ALA to DHA synthesis but, overall, they concur in showing extremely low

(<0.5%) net response of plasma DHA to ALA (tracer or supplement) in 'average, healthy'

adult humans. They also show that this is the norm and is not a side effect either of

incorporation of newly synthesized DHA elsewhere or high loss of DHA via β-oxidation (see

'Other Metabolic Processes'). Hence, it seems clear that the main reason plasma DHA is

essentially unaffected by dietary supplementation with ALA is because of very low synthesis.

Though imperfect, this estimation process using three very different models (isotopically

labelled ALA, ALA supplements, brain DHA accumulation in ALA–fed infants) seems robust

enough to provide fairly secure boundaries of probability within which conversion of ALA to

DHA is unlikely to ever normally exceed 1% in humans. Other reviews on this topic have

come to the same conclusion (Brenna 2002; Burdge 2006; Cunnane 2003b; Gerster 1998).

Conversion of Other PUFA

ALA to EPA. Whether using dietary supplementation studies with ALA or by tracing

[13]C-ALA metabolism, it is clear that conversion of ALA to EPA is higher than for ALA

conversion to DHA and is on the order of ~5% (Tables 3, 4). Hence, depending on the dose

of ALA used, plasma EPA usually rises by 20-100% after supplementing humans with ALA.

ω3 DPA may or may not rise modestly after ALA supplementation or after administration of a tracer dose of ^{13}C-ALA (Goyens et al. 2006; McCloy et al. 2004). The change in ω3 DPA after ALA supplementation is noticeably less than for EPA but more than for DHA (Cunnane 2003b). Collectively, these data show that conversion of ALA to ω3 long chain PUFA really bogs down after EPA.

Stearidonic acid to long chain ω3 PUFA. Stearidonic acid (SDA; 18:4ω3) is the immediate Δ^6 desaturase product of ALA. It naturally occurs at moderately elevated levels in only a couple of foods, notably blackcurrant and borage oils. Few studies have evaluated metabolism of SDA in humans but one clearly shows that plasma EPA doubles but ω3 DPA and DHA do not change after SDA supplementation (James et al. 2003). By providing a pre-formed dietary source of SDA, any 'rate-limiting' effect (Table 1) of the Δ^6 desaturase that would supposedly restrain ALA's conversion would be bypassed but this did not have any effect on plasma levels of longer chain ω3 PUFA in this study. Since β-oxidation of 18 carbon fatty acids increases with each additional double bond from 0 (stearic acid) to 3 double bonds (ALA; Cunnane et al. 2003a), the fourth double bond in SDA might further enhance the β-oxidation of SDA, thereby reducing its availability for further desaturation and chain elongation.

EPA to DHA. Supplementation of pure EPA has not yet been studied exhaustively in humans but available data suggest that supplements of pure EPA do not change plasma DHA levels in healthy adults (Boston et al. 2004; Horrobin et al. 2002; James et al. 2003). This is consistent with the lack of effect of SDA or ALA supplements on plasma DHA and confirms that , in humans, desaturation-chain elongation-retroconversion of ω3 PUFA beyond EPA is minimal. The implication is that the steps beyond ω3 DPA that involve peroxisomal processing of the fatty acid are the bottleneck in this slow to minimal conversion through to DHA.

LA to AA. LA tracer (Table 3C) and supplementation (Table 5A) studies in healthy adults uniformly suggest that <0.1% dietary of LA is converted to AA a value that may rise depending on overall nutritional status or ω6 PUFA deficiency (Table 5B). There are four main reasons why conversion of LA to AA has attracted much less interest than the conversion of ALA to DHA: (i) people in industrialized countries have abundant LA intake, (ii) non-vegetarians have abundant AA intake, (iii) there is concern about side effects of excess ω6 PUFA giving rise to increased risk of degenerative diseases (Hamazaki and Okuyama 2003; Yam et al. 1996), possibly through a chronic overload of '2 series' eicosanoids derived from AA (Lands 2005), and (iv) unlike with DHA, the literature provides no clear reason to worry about adults achieving adequate intakes or tissue levels of AA.

It is unclear whether infants may have a requirement for preformed dietary AA so it is not yet appropriate to be complacent about role of dietary AA in infant development. Although AA synthesis seems to be very low in infants (Demmelmair et al. 2001), it may be under tight regulation because, unlike with DHA, brain AA levels in infants seem not to depend on the presence of incoming AA (Farquharson et al. 1992). Nevertheless, AA may promote fat cell development in infants, an effect that warrants close scrutiny (Massiera et al. 2003).

GLA and DGLA to AA. Plasma AA appears to be essentially as unaffected by GLA as by LA supplementation (Horrobin 1990; Manku et al. 1988). Pure DGLA given as a supplement (El Boustani et al. 1986; Stone et al. 1979) or as a tracer (El Boustani et al. 1986; Stone et al. 1979) is converted to AA.

Why the Disconnect Between *in Vitro* and *in Vivo* Data?

Desaturation – chain elongation is a complex pathway because it is responsive to – (i) hormones, nutritional and metabolic changes, (ii) differences in substrate availability and

substrate competition, and (iii) high or low levels of the end-products. Hence, without reference to data describing the efficiency of the conversion of parent to long chain PUFA in an intact organism, the *in vitro* information about a certain desaturase step being 'rate-limiting' (Table 1) is meaningless for understanding the implications for dietary requirements or health attributes of the long chain PUFA in humans.

An important consideration with isotopically-labelled molecules is that they are assumed to behave like the unlabelled molecules they are intended to mimic, i.e. they are 'tracers' imitating the 'tracee'. In order to be a true tracer, the smallest dose that can measure the process of interest should be used but this has not always been the case, either *in vitro* or *in vivo*. Some human stable isotope studies looking at conversion have used relatively high doses of tracer equivalent to 50-100+% of the daily intake of the PUFA in question (Burdge et al. 2002; Burdge and Wootton 2002; Emken et al. 1994; Hussein et al. 2005; Pawlosky et al. 2001). High amounts of tracer or substrate may reduce *in vivo* conversion of ALA to DHA in healthy adults by as much as 50% (compare Table 3A to 3B). However, this skewing effect can also occur *in vitro,* where the amount of 'free' substrate seen by the desaturase enzymes probably greatly exceeds what they see *in vivo*. Parallel issues involving the amount of 'free' substrate also seem to occur in trying to resolve why both fatty acids and cholesterol seem to so easily traverse the blood-brain barrier when injected into the blood but not when given orally (Cunnane et al. 1999; Edmond et al. 1991; Jurevics and Morell 1995).

Another challenge with stable isotope studies of PUFA conversion is that interpretation of both the supplementation and tracer studies is largely based on the plasma pool, which actually retains a very small proportion of the tracer or supplement administered. Plasma itself also has no capacity to effect conversion which takes place principally in the liver. Hence, small errors in analysis or small differences in study design can potentially significantly affect

the calculation of % conversion. Examining other pools like adipose tissue (McCloy et al. 2004) or excretion of the tracer in breath is occasionally done (Burdge 2006; DeLany et al. 2000), which is an additional window on PUFA metabolism (though not on the PUFA conversion pathway itself) that can increase confidence in this methodology.

Some investigators have used deuterated (^{2}H) PUFA while others use ^{13}C-enriched PUFA as the tracer. Some have used fatty acids labelled at just one carbon (DeLany et al. 2000), while others commonly use uniformly labelled tracers (Table 3). Other differences in study design that also affect the tracer-tracee ratio such as the PUFA content or ω3/ω6 PUFA ratio of the background diet (Goyens et al. 2006) could contribute to inter-study differences in apparent PUFA conversion rate or efficiency. Vegetarianism does appear to have a significant impact on ALA conversion to DHA (see next section) but this has not been studied in the tracer model. Pregnancy and lactation may also affect synthesis of long chain PUFA in humans but are not the subject of this review.

Other Metabolic Processes

If desaturation and chain elongation were the principle determinants of the degree of conversion of parent to long chain PUFA, AA and EPA rather than AA and DHA would be found in equivalent amounts in tissues. However, even the fatty acid composition of the microsomal preparations doing the desaturation does not change in ways predictable from experimentally induced changes in desaturase activity (Poisson and Cunnane 1991). Hence, although the ω6 and ω3 PUFA undergo the same sequence of desaturation and chain elongation, other processes affecting their metabolism must be relevant to determining which of the parent or long chain PUFA actually accumulate in membrane phospholipids.

These other processes that affect PUFA metabolism *in vivo* include rapid clearance of the derived long chain PUFA out of the plasma and into tissue membranes, differential esterification into membrane lipids, extensive conversion to further downstream products, or loss to lipid peroxidation, β-oxidation or carbon recycling. If these processes were ongoing at a sufficient rate, it would be wrong to assume that a low rate of conversion from ALA was occurring if, in fact, it was high DHA turnover (or degradation) that prevented a rise in plasma DHA.

Stable isotope studies are the best way to assess fatty acid turnover via β-oxidation. Very preliminary estimates suggest that <5% of DHA is β-oxidized in healthy, omnivorous adult humans over an 8 day period, whereas >60% of ALA is β-oxidized (McCloy et al. 2004, Freemantle et al. 2006). Hence, stable isotope studies can clearly distinguish high from low rates of β-oxidation of PUFA and 5% oxidation of newly synthesized DHA is still insufficient to account for the lack of change in plasma DHA during ALA supplementation. If accompanied by somewhat higher ALA conversion to DHA, lower β-oxidation of DHA in vegans might help account for their relative resistance to DHA deficiency. This question is pivotal in understanding DHA homeostasis but an answer to it awaits further study.

Synthesis of downstream products of DHA, whether neuroprotectins/docosanoids (Lukiw and Bazan 2006), ultra-long chain PUFA or peroxidation products, might also make it difficult to accurately distinguish actual from net DHA synthesis. However, the absolute amounts of these products of DHA are several orders of magnitude lower than DHA itself, so their synthesis cannot reasonably be expected to drain the plasma DHA pool in the time frame of tracer or ALA supplementation experiments (mostly <1 wk).

PUFA carbon recycling refers to the recovery of carbon in non-PUFA from PUFA undergoing β-oxidation (Cunnane et al. 2003). Carbon recycling excludes the down-stream

metabolites like hydroxy-fatty acids, eicosanoids, docosanoids/neuroprotectins or products of peroxidation, but includes newly synthesized lipids (and possibly amino acids or other molecules) into which carbon can be incorporated after fatty acid β-oxidation. Up to 100 times more carbon from ALA is used by neonatal rats and primates to make other lipids like cholesterol and saturated or monounsaturated fatty acids than is used to make DHA (Cunnane et al. 2003; Demar et al. 2005; Menard et al. 1998; Sheaff Greiner et al. 1996; Sinclair 1975). In rats, especially neonatal rats, extensive carbon recycling of ALA occurs under extremes of very high total fat intake or very deficient ω3 PUFA intake (Cunnane et al. 2006; Taha et al. 2006) so this pathway could easily affect conversion of ALA to DHA by reducing the availability of ALA for desaturation. In a primate model, about 15% of ^{13}C-DHA was also recycled into newly synthesized fatty acids (Sheaff Greiner et al. 1996), so carbon recycling is not limited to the 'parent' PUFA ALA or LA. However, preliminary evidence suggests there is minimal recycling from AA (Cunnane et al. 2003). Detectable amounts of ALA recycling occur in adult humans (Burdge 2006) but it is much lower than in neonatal animal models. The point is that in contrast to the situation in rats or possibly primates, in adult humans, it seems unlikely that recycling of the parent PUFA contributes in any meaningful way to the very low apparent conversion to DHA or AA.

PUFA retroconversion is the process by which a longer chain PUFA raises the level of a *precursor* PUFA, i.e. DHA intake raising EPA levels. EPA can rise when DHA alone is given (Arterburn et al. 2006; Conquer and Holub 1997; Gronn et al. 1990) and stable isotope studies suggest this occurs by chain shortening of DHA (Lemaitre-Delaunay et al. 1999), hence, retroconversion. The available data do not indicate that retroconversion of DHA to EPA consumes all the DHA nor that it occurs fast enough to prevent detecting a change in plasma DHA that might occur after EPA (or ALA or SDA) supplementation.

The bottom line is that desaturation measured in isolated microsomal enzyme preparations is a model system that takes conversion out of context, i.e. the *in vitro* model doesn't represent the actual fate of these fatty acids in an intact liver or, more challenging still, in an integrated living organism. Hence, optimized *in vitro* conditions permitting the conversion of several pmoles of ^{14}C-LA to ^{14}C-GLA/min/mg of microsomal protein do not easily translate into an *in vivo* value permitting one to say that a certain percentage of dietary LA or ALA is converted to the first, second or third in the series of their respective long chain PUFA products. In a way, PUFA researchers themselves have propagated the misleading concept of effective conversion of ALA to DHA because they always show the pathway with arrows connecting the parent to the long chain PUFA when, in reality, the arrows are much fainter (almost non-existent) in some species like humans than others, especially rats and most other lab rodents. This is the reason for showing the fatty acids in Table 2 as a list not a pathway connected together by arrows.

The Issue is – do we Need Long Chain PUFA in the Diet?

Accepting that humans have a very limited capacity to convert the parent to long chain PUFA, the issue becomes one of determining whether there is an equivalent need for the long chain PUFA in adults as there is in infants. Assuming for the sake of argument that plasma stable isotope enrichment data, in fact, truly reflect the human body's ability to convert LA to AA, <0.5% conversion of the average North American LA intake (10 g/d) would yield about 50 mg/d of AA, the impact on long term eicosanoid production of '2 series' eicosanoids of which is still unknown.

Similarly, at a conversion rate of 0.5%, a daily intake of 1000 mg ALA would produce DHA at a rate of about 5 mg/d; a 2000 mg/d intake of ALA and somewhat lower conversion

(0.2%) would produce 2 mg/d of DHA. A DHA intake of about 200-300 mg/d is widely viewed as having a beneficial impact on cardiovascular outcomes (www.issfal.org.uk, Gebauer et al. 2006; Lands 2005). To obtain 200 mg/d of DHA from ALA would require converting an intake of 2000 mg/d ALA to DHA at a rate of 10%. No published data suggest 10% of ALA intake can be converted to DHA under any sustainable conditions. Furthermore, ALA intakes of 2000 mg/d are uncommon (Hu et al. 1999). Even if the 0.5% figure derived from several stable isotope studies underestimates actual conversion of ALA to DHA in healthy adults by several fold, the desaturation-chain elongation pathway does not appear to be designed to allow humans to make even 20 mg/d of DHA let alone the recommended 200+ mg/d. Recent data suggest the human brain turns over DHA at about 4 mg/d (Rapoport 2006), an amount that non-vegetarians would appear to have great difficulty making from ALA.

ω3 and ω6 PUFA and Human Diseases – A Brief Overview

Over thirty years of human clinical trials indicates that EPA and DHA hold considerable promise in the primary and secondary reduction of the risk of morbidity and mortality from cardiovascular disease (Lands 2005). EPA and DHA may well turn out to be important in mitigating other diseases, especially psychiatric, cognitive and neurodegenerative disorders, but reports on these applications are really only beginning to emerge (Freund-Levi et al. 2006). Fish, shellfish and encapsulated fish oils are by far the commonest dietary sources of EPA and DHA. Capsules containing EPA but no DHA or DHA but no EPA are becoming available and their clinical effects are being investigated as well (Arterburn et al. 2006; Boston et al. 2004) but they have still not been widely studied. The two main intermediate ω3 PUFA, SDA and ω3 DPA, are also starting to receive attention but as yet little is known of their health implications.

The potential health impact of ALA supplementation has been studied in relation to cardiovascular disease but its effects are enigmatic. On the one hand there is evidence of a modest, beneficial effect of ALA on cardiovascular health (Baylin et al. 2003; Cunnane et al. 1995; Djoussé et al. 2005; Dolecek 1992; Hu et al. 1999). This risk reduction may be achieved through reduction of specific markers associated with cardiovascular disease (Carlson and Walldius 1975; Ferrucci et al. 2006; Freese and Mutanen 1997; Goyens and Mensink 2006; Szapary et al. 2007; Zhao et al. 2004). On the other hand, EPA and DHA generally have a stronger protective on cardiovascular risk than ALA so with low conversion to EPA and negligible conversion to DHA, it is unclear whether ALA acts directly or through EPA. If part of the cardiovascular benefit of ALA is due to desaturation-chain elongation to EPA, at even ~5% conversion, a generous intake of 2000 mg ALA would only produce about 100 mg/d EPA, an amount roughly equivalent to current EPA intake in North America. About 100 mg/d of EPA could have a mild, beneficial cardiovascular effect involving inhibition of platelet aggregation, reduction of triglycerides, inhibition of 2 series eicosanoid synthesis and action, inhibition of LA conversion to AA, and stabilization of heart rhythm.

The potential health effects of AA in adult humans have attracted sporadic attention. Except in very low amounts suitable for infant milk formulas, AA has rarely been studied as a human dietary supplement. After 50 d supplementation with 1.5 g/d of AA, urinary excretion of 2 series eicosanoids increased but no other adverse symptoms were reported (Ferretti et al. 1997; Kelley et al. 1998). Clearly further work on this topic is needed. The health implications of encapsulated GLA (as evening primrose or borage oil) received considerable attention in the 1980s and 1990s (Horrobin 1990). The main interest in these intermediate ω6 PUFA was to try to exploit the low conversion especially of LA and GLA to AA and to use them to raise levels of the '1 series' eicosanoids derived from DGLA, thereby hopefully counteracting the

25

pro-inflammatory effects of excessive amounts of the 2 series eicosanoids derived from AA. The idea of using GLA or DGLA and 1 series eicosanoids to prevent the damaging effects of AA and the 2 series eicosanoids has shifted towards the ω3 PUFA and their own '3 series' eicosanoids derived from EPA or the docosanoids derived from DHA (Lukiw and Bazan 2006). At present, there is at best weak evidence to suggest that any ω6 PUFA can be exploited to reduce the risk of cardiovascular, psychiatric or neurological diseases.

With its emphasis on the primacy of LA, early EFA research in humans led to interest in the potential health effects of LA. It needs to be clearly stated that the risk of LA deficiency (indeed deficiency of all ω6 PUFA) is rare in non-surgical adult patients who consume adequate dietary energy. Furthermore, the intake of LA needed to prevent a risk of ω6 PUFA deficiency in adults is low (no more than 2 g/d) but current LA intakes exceed this 'adequate intake' by 3-10 fold in all countries for which data are available. Hence, it is fair to say that in most developed countries LA intakes are excessive and there is no need or even vaguely legitimate rationale to explore therapeutic uses of LA, certainly for the main causes of morbidity and mortality. Historically, interest in the health effects of LA focussed on cardiovascular protection by cholesterol lowering. However, there is much more to cardiovascular health than cholesterol lowering and many more effective nutritional interventions than LA supplements.

Hence, there is general agreement that diets of developed countries are heavily skewed in favour of the ω6 PUFA, especially LA. There is also general agreement that chronic low grade inflammation aggravates or promotes the degenerative processes that are the main causes of mortality and morbidity in developed countries. What is still more conjecture than fact is the hypothesized direct link between elevated ω6 PUFA intake (mainly LA), elevated 2 series eicosanoids, and increasing prevalence of degenerative disease

processes (Hamazaki and Okuyama 2003; Lands 2005). This broadly-cited concept makes intuitive sense but has as yet received little direct experimental support (Nelson et al. 1997) because good eicosanoid measurements are difficult to do, so insufficient data are available to support or refute it. Until this link is better substantiated, it will be difficult to target specific nutrient-based strategies towards the task of reducing chronic disease risk more effectively than is being done at present with fish, shellfish or encapsulated fish oils. While it is still unknown whether ω3 PUFA act more by inhibiting 2 series eicosanoids, stimulating 3 series eicosanoids or by their effects on membrane composition affecting cell signalling systems, their broadly beneficial clinical effects on cardiovascular and neurological health remain clear and well-supported.

Health Attributes of Individual PUFA

PUFA have several well accepted attributes in relation to preventing or mitigating the effects of degenerative cardiovascular or neurological disease processes in adults (Table 6). Concentrated dietary sources or supplements of ALA, EPA and DHA are now widely available so it is important to get beyond the general agreement that ω3 PUFA are generally beneficial and start examining whether some of their effects can be more effectively or specifically targeted to one or more of these fatty acids. DHA is a much more important membrane constituent than EPA (in all organs but especially in the brain) so it is reasonable to assess different neurological targets for DHA than for EPA. Furthermore, the limited evidence to date shows that there is very low to negligible conversion of EPA to DHA in humans (James et al. 2003, Boston et al. 2004) so it makes sense to explore different mechanisms of action for EPA compared to DHA. DHA's effects seem membrane-based whereas EPA seems to act more like a modulator, possibly of eicosanoids, but it may also affect energy substrate

availability or gene expression differently from DHA (Freemantle et al. 2006; Gottlicher et al. 1993).

ALA has cardiovascular health effects similar to but much milder than those of EPA and DHA. There is no obvious mechanism by which ALA would affect cardiovascular or neurological health except possibly through a mild inhibitory effect on desaturation-chain elongation of ω6 PUFA or mild inhibition of 2 series eicosanoids. ALA is a better substrate for fatty acid β-oxidation and ketogenesis than any other common dietary fatty acid (whether saturated, monounsaturated or PUFA; Cunnane 2003a). The therapeutic effects of chronic, mild to moderate stimulation of ketones are now under increasing scrutiny, especially in relation to 'brain health' (Veech 2004, Maalouf et al. 2007). Given the emerging link between brain hypometabolism and cognitive decline in the elderly, strategies to improve energy substrate availability to the adult or aging brain need further investigation (Hoyer 2004; Reger et al. 2004; Reiman et al. 2004; Veech 2004, Freemantle et al. 2006).

Though still highly speculative, it is conceivable that ALA could potentially become a tool in this strategy, not to increase EPA or DHA, but to improve brain fuel supply by mildly increasing ketogenesis (Freemantle et al. 2006). Insulin resistance is the main non-genetic risk factor for cognitive decline in the elderly and appears to contribute to impaired glucose availability to the brain (Steen et al. 2005). Hence, imaginative strategies to bypass the risk of deteriorating brain glucose uptake are needed and could involve exploiting ALA as a ketogenic substrate, EPA as a modulator of fatty acid β-oxidation and DHA as key structural component of synaptosomal function, learning and memory.

Implications of Extremely Limited Conversion: What is an EFA?

The term EFA is still in widespread use but with broader acceptance of the inefficient conversion of parent to long chain PUFA in humans, the usefulness of this term needs to be questioned. A detailed rationale for this concern has been published (Cunnane 2003a). Historically, the PUFA defined as EFA have already varied a lot because in the 1970s, the original three EFA - LA, ALA, and AA – were reduced to just one (LA). It then became much clearer in the 1980s that both ω3 and ω6 PUFA had unique and important nutritional roles. The research in this period really defined the subtlety of the roles associated with ω3 PUFA and in so doing provided crucial new insights into their physiological functions. With only LA and ALA as dietary PUFA, rats could normally produce sufficient amounts of their respective long chain PUFA to grow and reproduce normally, so ALA regained its stature as an EFA. Therefore in rats, by definition, only LA and ALA are EFA; the longer chain PUFA are strictly not EFA in rats but they are still commonly grouped as EFA for convenience.

We now see that conversion of parent to long chain PUFA is much less efficient in humans than in rats and that human infants definitely need pre-formed dietary DHA. If infants actually need pre-formed dietary DHA and ALA is so inefficiently converted to DHA, is ALA truly an EFA in humans? If so, by what criteria? Indeed, is ALA doing anything *specific* in humans? If EPA conversion to DHA is minimal, but it can be made from ALA, is EPA actually doing anything unique that qualifies it as an EFA? Infants need dietary DHA but do healthy adults? Adult humans can't normally make much DHA but, it still isn't clear that what conditions lead to clear and specific symptoms of DHA deficiency in adults.

Conditionality is the heart of the issue. Infancy is a 'condition' leading to a dietary need for DHA. Are there 'conditions' leading to a dietary need for DHA (or EPA, or AA) in adults and if so, what are they? For instance, is one or other PUFA needed in infants but not needed

29

by healthy adults, or needed in infants but also in adults suffering from disease X? Are different amounts needed to treat condition X versus condition Y? Do vegans actually need less DHA than omnivores (because they eat better diets or have fewer risk factors for cardiovascular disease)? If the conditionality of PUFA requirements can be acknowledged, such conditions will then be sought out and the field will move forward. Conditions for requiring a nutrient form the framework for a better understanding not only of that nutrient's function but also how to provide foods or supplements that will respond most effectively to meeting that need and preventing, reducing or eliminating the symptoms of deficiency. It may eventually be possible to estimate 'minimally sufficient' compared to 'optimal' DHA intakes but this depends on accepting the more basic concept of conditionality.

At least we can unreservedly say the DHA is 'conditionally indispensable' or 'conditionally essential' in infants. We can recommend DHA intake in adults but we can't yet call it 'conditionally indispensable' in adults because we can't really say what constitutes DHA deficiency in adults or how much DHA would be needed to prevent it. To be an essential nutrient, the molecule needs to be more than unique – it has to do something no other nutrient does; in adults, at least one of ALA, EPA, or DHA is required, but which one?

Now that a fair amount is known about the subtlety of PUFA metabolism, it can be seen that metabolism through the pathway is very slow but also somewhat flexible and vulnerable to inhibition. Definitions should therefore be flexible enough to correspond to the physiology of these fatty acids *in humans*. We need to be able to define and agree on the conditions of dietary essentiality at least of LA, AA, ALA, EPA and DHA – for the moment they look challenging enough! Acknowledging the conditionally indispensable nature of some PUFA simply means that the EFA join the ranks of other similar organic nutrients like the

'conditionally in/dispensable amino acids', which is the more correct and official term for the 'essential amino acids'.

Conclusions

We make the case that most adult humans are virtually unable to convert ALA to DHA. The consequence is that people not eating foods or supplements containing DHA have lower plasma and tissue DHA and have a higher risk of declining mental and cardiovascular function. Hence, in combination with EPA, dietary DHA may be useful in protecting those at higher risk (smoking, obesity, sedentary lifestyle, etc.). The clinical impact of supplemental EPA and DHA would, however, be expected to be lower in those who, for other reasons, are already at lower risk of declining mental or cardiovascular function. ALA has some mildly protective effects but probably more so in those at lower risk. Hence the potential role of these ω3 PUFA to protect brain and cardiovascular function is a moving target that is conditional on lifestyle, environmental risk factors and possibly genetic susceptibility. Defining the conditions that make ALA, EPA or DHA necessary nutrients in adults is therefore an important priority. Adults also make virtually no AA from LA but, at the moment, this appears to have no adverse health implications.

It has been argued that the low capacity of the human desaturation-chain elongation system is a result of humans evolving on diets that contained fish and shellfish (Broadhurst et al. 1998; Cunnane 2005). The idea is that the capacity of this pathway in our forebears was largely irrelevant because of the continuous consumption of pre-formed AA, EPA and DHA from fish and shellfish. It is therefore only with widespread abandonment of 'shore-based' foods, especially during the last fifty years, that issues of desaturation-chain elongation have become evident. Returning to the key nutritional attributes of a 'shore-based' diet, especially

higher ω3 and lower ω6 PUFA intake, is essential from a health perspective, or the

consequences could be disastrous as much for mental as for cardiovascular health (Andlin-

Sobocki et al. 2005).

Acknowledgements

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Table 1

Main features of the desaturation – chain elongation pathway observed *in vitro*.

1. Desaturation is usually a slower process that chain elongation.

2. Desaturation usually (but not exclusively) alternates with chain elongation.

3. Mole for mole, the desaturases favour $\omega 3$ fatty acids over $\omega 6$ (or $\omega 9$) fatty acids.

4. Double bond position in a PUFA determines its efficacy as a desaturase substrate.

5. Desaturation is linked to the electron transport chain.

6. Several similar desaturases act in succession, permitting conversion of LA and ALA to all the known long chain PUFA.

7. Desaturation is increased when the diet is low in either LA and/or ALA or when the membrane content of long chain PUFA is substantially reduced.

8. Overloading the desaturase assay with the substrate fatty acid, eg. ALA, or with the end-product, eg. DHA, causes substrate or end-product inhibition, respectively.

9. Certain cofactor nutrients (notably vitamin B_6, iron and zinc) as well as certain hormones (notably insulin) are involved in both desaturation and chain elongation to the extent that dietary deficiency of key cofactors, eg. zinc, or deficiency of insulin (type 1 diabetes) can effectively shut the pathway down.

10. Through truncated β-oxidation, the reverse of chain elongation (chain shortening by two carbons) is important in the synthesis of DHA.

11. DHA synthesis (or $\omega 6$ DPA synthesis) seems to involve a repeated Δ^6 desaturation followed by chain shortening in peroxisomes, a pathway that is widely but not yet universally accepted.

12. When multiple desaturations and chain elongations are needed, i.e. to convert ALA to DHA, the first desaturation is commonly viewed as the 'rate limiting' step

ALA = Alpha-linolenic acid, DHA = Docosahexaenoic acid, DPA = Docosapentaenoic acid, LA =

Linoleic acid, PUFA = Polyunsaturated fatty acids

Table 2

The common ω6 and ω3 polyunsaturated fatty acids.

linoleic acid (18:2ω6)	α-linolenic acid (18:3ω3)
γ-linolenic acid (18:3ω6)	stearidonic acid (18:4ω3)
dihomo-γ-linolenic acid (20:3ω6)	ω3 eicosatetraenoic acid (20:4ω3)
arachidonic acid (20:4ω6)	eicosapentaenoic acid (20:5ω3)
adrenic acid (22:4ω6)	ω3 docosapentaenoic acid (22:5ω3)
ω6 docosapentaenoic acid (22:5ω6)	docosahexaenoic acid (22:6ω3)

Table 3

Desaturation-chain elongation data obtained using stable isotopes in adult humans.

Reference	Subjects	Isotope	Dose (mg)	Blood fraction	Conversion to	
					EPA	DHA
A. ALA tracer – Low dose (< 100 mg)						
1. Vermunt et al. 2000	15 M/F	[U-^{13}C]	45	TL	5.1%	TR
2. McCloy et al. 2004	6 F	[U-^{13}C]	47	TL	1.5%	0.3%
3. Goyens et al. 2005	29 M/F	[U-^{13}C]	30 + 20 daily	TL	7%	0.07%
B. ALA tracer – High dose (> 100 mg)						
1. Emken et al. 1994	7 M	[^{2}H]	3500	TL	6%	3.8%
2. Pawlosky et al. 2001	8 M/F	[^{2}H] ethyl ester	1000	TL	0.2%	TR
3. Burdge et al. 2002	6 M	[U-^{13}C]	700	TL	7.9 %	TR
4. Burdge and Wootton 2002	6 F	[U-^{13}C]	700	TG	TR	TR
5. Burdge et al. 2003	14 M/F	[U-^{13}C]	700	TG + PC	TR	TR
6. Hussein et al. 2005	38 M	[U-^{13}C]	400	TL	0.03%	TR
C. LA tracer					AA	
1. Nichaman et al. 1967a	4 M	[1-^{14}C]	100 μl	PL + CE	2%	
2. McCloy et al. 2004	6 F	[U-^{13}C]	47	TL	0.9%	
3. Hussein et al. 2005	38 M	[U-^{13}C]	400	TL	0.2%	

Legend: U- = uniformly labeled, TL = Total lipids, PC = Phosphatidylcholine, PL = Phospholipids, CE = Cholesteryl esters, ALA = Alpha-linolenic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid, LA = Linoleic acid, DGLA = Dihomo-gamma-linolenic acid, AA = Arachidonic acid, TR = Trace (< 0.1%).

Table 4

Change in plasma EPA and DHA in humans after ALA supplementation

	Reference	Subjects	Duration (weeks)	ALA (g·d⁻¹)	ALA form	Blood fraction	Change in EPA	Change in DHA
A. Healthy Adults								
1.	Kelley et al. 1993	10 M	8	20.5	FO	TL	TR	+ 38%
2.	Nordstrom et al. 1995	22 M/F	12	9.6	FO	TL	+ 0.02%	+ 0.5%
3.	Harper et al. 2006	31 M/F	26	3	FOC	TL	+ 53%	+ 4%
4.	Szapary et al. 2007	30 M/F	10	40	F	TL	+ 12%	TR
5.	Mantzioris et al. 1995	15 M	4	13.7	M	PL	+ 138%	+ 14%
6.	Cunnane et al. 1995	10 M/F	4	9	F	PL	+ 33%	TR
7.	Li et al. 1999	17 M	6	3.7	M	PL	+ 13%	TR
		17 M	6	15.4	M	PL	+ 250%	TR
8.	James et al. 2003	15 M/F	3	1.5	FOC	PL	+ 23%	TR
9.	Finnegan et al. 2003	29 M/F	26	4.5	M	PL	+ 90%	TR
10.	Wallace et al. 2003	8 M	12	3.5	FOC	PL	+ 60%	+ 2%
11.	de Groot et al. 2004	29 F	26	2.8	M	PL	TR	TR
12.	Goyens et al. 2006	10 M/F	6	[1.1% of energy]	M	PL	+ 9.7%	+ 0.03%
B. ALA Deficiency								
1.	Bjerve et al. 1989	3 M/F (Adults)	8	0.12 mL/d 0.5 mL/d	EALA	TL	TR + 41%	TR + 18%
2.	Holman et al. 1982	1 F (Child)	32	1.625 g/d	ALA	PL	+ 0.68%	+ 0.45%
3.	Bjerve et al. 1988	1 F (Child)	20	0.51 g/d	ALA	TL	+ 278%	+ 180%

Legend: F = Flaxseed, FO = Liquid flax oil, FOC = Flax oil capsule, M = Margarine, TL = Total lipids, PL = Phospholipids, ALA = Alpha-linolenic acid, EALA = Ethyl alpha-linolenic acid ester, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid, TR = Trace (< 0.1%)

Table 5

Change in plasma AA in humans after LA supplementation

	Reference	Subjects	Duration (weeks)	LA $(g \cdot d^{-1})$	LA form	Blood fraction	% change in AA
A. Healthy Adults							
1.	Nichaman et al. 1967b	5 M	4	11 - 52	SO + SFO	PL	TR
2	Lasserre et al. 1985	24 F	20	15	SFO	PL	TR
3.	Manku et al. 1988	18 M/F	12	2.88	EPO	PL	TR
4.	Mantzioris et al. 1994	15 M	4	20.3	M	PL	TR
5.	Nordstrom et al. 1995	22 M/F	12	9.9	SO	TL	+ 0.08%
6.	de Groot et al. 2004	29 F	26	10.9	M	PL	TR
B. Undernourished children or EFA deficiency							
1.	Miller et al. 1987	5 M/F	4-6	2.3 mg/kg	Cutaneous SO	TL	TR
2.	Harper et al. 1990	5 M/F	3.5	16.9% energy	Isocal [TM]	PC PE	+ 13 % + 5 %

Legend: SO = Safflower oil, SFO = Sunflower oil, EPO = Efamol evening primrose oil, M = Margarine, TL = Total lipids, PL = Phospholipids, PC = Phosphatidylcholine, PE = Phosphatidylethanolamine, LA = Linoleic acid, AA = Arachidonic acid, TR = Trace (< 0.1%)

Table 6

Commonly accepted health attributes of individual PUFA.

1. ALA makes a mildly positive but inconsistent contribution to cardiovascular health. As the main dietary $\omega 3$ PUFA, especially for vegans and many vegetarians, the mechanism of this beneficial effect needs clarification.

2. ALA has no known benefit for 'brain health' or to reduce risk for any neurological, psychological or psychiatric disease.

3. EPA alone has received insufficient attention but in combination with DHA is beneficial for cardiovascular health.

4. DHA alone has received insufficient attention but in combination with EPA is beneficial for cardiovascular health.

5. Unlike with EPA, the focus of attention with DHA effects on neurological function in adults is in relation to cognition and reduction of the risk of Alzheimer's disease. Preliminary indications of a beneficial effect of DHA supplementation on cognition in the elderly are encouraging (Freund-Levi et al. 2006) but clear-cut results are not yet available (Maclean et al. 2005).

6. As a replacement for other dietary fats or oils, relatively large amounts of LA may contribute to cholesterol lowering but this effect justifies neither the current high intake of LA nor raising it.

7. There is not yet any good evidence that GLA, DGLA, AA or SDA have beneficial effects on the cardiovascular or neurological systems.

8. In contrast to the $\omega 3$ PUFA, there is currently little need or rationale to explore the effects of supplementary $\omega 6$ PUFA in cardiovascular or neurological disease.

AA = Arachidonic acid, ALA = Alpha-linolenic acid, EPA = Eicosapentaenoic acid, DGLA =

Dihomo-gamma-linolenic acid, DHA = Docosahexaenoic acid, GLA = Gamma-linolenic acid,

LA = Linoleic acid, SDA = Stearidonic acid