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Long-chain polyunsaturated fatty acid (LCPUFA) requirement for brain development: A personal view

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Abstract – Dietary docosahexaenoic acid (DHA) is known to accumulate in the infant brain and clinical trials have established that dietary DHA is associated with improvements in visual and neural function in preterm infants. Thus, an elevated DHA status is considered to be important throughout infancy for brain development. While DHA can be added directly to infant foods, there have been important studies to show that infants can partially meet their own DHA requirements by consuming adequate levels of omega 3 alpha linolenic acid (ALA). A key requirement to allow for the conversion of ALA to DHA and to maximise its incorporation into tissues is a diet that is also low in omega 6 linoleic acid (LA). Such diets are hard to find commercially because dietary guidelines dictate that ~3% energy of infant diets should be in the form of LA. These estimates were based on early animal experiments in which basal diets were devoid of both LA and ALA. However, recent animal experiments have indicated that the level of LA required to avoid essential fatty acid deficiency is much lower when ALA is also present in the diet. When a wide range diets are evaluated in animal systems, it is possible to see that the level of DHA found in the blood of animals fed diets containing only LA and ALA can reach levels similar to that of animals fed diets rich in fish oil, but only when the ALA:LA ratio is high and the total amount of dietary polyunsaturated fatty acids (PUFA) is low. Diets that are rich in either monounsaturates or saturates meet these requirements. Importantly, there are human infant studies that have tested such diets and demonstrated that human infants accumulate greater amounts of DHA than when diets are high in LA. It might be time to reconsider the dietary requirement of the two essential fatty acids LA and ALA in human infants in terms of their ability to enhance endogenous synthesis of DHA rather than more adult biomarkers like cholesterol levels.

Keywords: DHA / LCPUFA / infant / nutrition / dietary fats

Résumé – Besoins en acides gras polyinsaturés à longue chaîne (AGPI-LC) pour le développement du cerveau : point de vue personnel. L'acide docosahexaénoïque (DHA) alimentaire est connu pour s'accumuler dans le cerveau des nourrissons. Des essais cliniques ont établi que le DHA alimentaire est associée à l'amélioration de la fonction visuelle et cérébrale chez les nourrissons prématurés. Ainsi, un statut élevé en DHA est considéré comme important durant la petite enfance pour le développement optimal du cerveau. Bien que le DHA puisse être ajouté directement aux aliments pour nourrissons, certaines études montrent que les nourrissons peuvent partiellement répondre à leurs propres besoins en DHA en consommant des niveaux adéquats d'un acide gras oméga 3, l'acide alpha-linolénique (ALA). Afin de permettre la conversion de l'ALA en DHA et de maximiser son incorporation dans les tissus, il est primordial que le régime contienne également une faible quantité d'un oméga 6, l'acide linoléique (LA). De tels régimes lactés s'avèrent difficiles à trouver dans le commerce puisque que les directives diététiques dictent que ~3 % de l'énergie apportée par les aliments infantiles soit apporté sous la forme de LA. Ces estimations sont fondées sur les premières expérimentations animales dans lesquelles les régimes de base étaient dépourvus à la fois de LA et de ALA. Cependant, des expérimentations animales récentes ont indiqué que le niveau de LA nécessaire pour éviter une carence en acides gras essentiels est beaucoup plus faible lorsque l'ALA est également présent dans l'alimentation. Quand une large palette de régimes alimentaires sont évaluées sur des animaux, il est possible de voir que le niveau de DHA trouvé dans le sang des animaux nourris avec des aliments contenant uniquement du LA et de l'ALA peut atteindre des niveaux

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similaires à ceux d'animaux nourris avec des aliments renfermant de l'huile de poisson riche en DHA, mais uniquement lorsque le ratio ALA/LA est élevé et que la quantité totale d'acides gras polyinsaturés (AGPI) alimentaires reste faible. Les régimes qui sont riches en acides gras monoinsaturés ou saturés conviennent également. Des études cliniques ont testé cette approche nutritionnelle et démontré que les nourrissons humains accumulent de plus grandes quantités de DHA lorsque les régimes sont peu riches en LA. Il serait temps de réexaminer les recommandations nutritionnelles destinées aux nourrissons humains pour ces deux acides gras essentiels LA et l'ALA au regard de leur capacité à améliorer la synthèse endogène de DHA plutôt que de prendre en compte certains biomarqueurs de l'adulte comme le niveau de cholestérol circulant

Mots clés : DHA / AGPI-LC / nourrisson / nutrition / acides gras alimentaires

Dobbing (1973) in a series of papers highlighted the growth of the brain, particularly in the last trimester and post-natal period. Much later, we (Gibson *et al.*, 1996) showed that the types of fats in the diet were critical to brain composition and in particular the accumulation of DHA in the human brain was dependent on an adequate supply in the diet. Although arachidonic acid (AA) increased dramatically during this same period, its accumulation was independent of diet (Makrides *et al.*, 1994). Thus, I have formed the view that the most important issue in infant nutrition at the moment is providing enough DHA and that diets that allow infants to accumulate DHA will be better than those that do not.

We are now quite certain that DHA plays a role in the neurodevelopment of preterm infants. In the DINO trial we supplemented the diet of infants born less than 33 weeks gestation with DHA at a level equivalent to ~1% of fat, largely by supplementing the diet of the mothers with tuna oil that enriched their breast milk that they expressed for their infants. Mothers in the control group received an equivalent amount of vegetable oil without DHA but the natural level of DHA in their milk was about 0.2% fat. Our data (Fig. 1) showed that the higher level of DHA reduced the number of infants with mild (Bayley Mental Development Index MDI < 85, $P < 0.01$) and significant (MDI < 70, NS) mental delay when measured at 18 months (Makrides *et al.*, 2009).

So where do infants get their DHA? In utero there is a supply of DHA from mother via placenta, but preterm infants miss out due to the fact that they leave the womb early. In the DINO trial (Makrides *et al.*, 2009) we demonstrated that including DHA in the diet at a level of 1% of the total fats improved a range of neurological and clinical outcomes, compared with infants receiving the standard dose of DHA (~0.3% fats). This trial highlighted the importance of providing not just DHA, but DHA at a level that meets the needs of a growing infant.

Post birth infants can get DHA from breast milk but the level of DHA in human milk varies with the diet of the mother. We have shown a linear relationship between the level of DHA in milk and the amount in the maternal diet (Gibson *et al.*, 1997). In contrast, the level of AA in breast milk from around the world seems to be independent of a large range of diets (Yuhas R *et al.*, 2006). What has changed in recent years is the level of LA in breast milk as omega 6 polyunsaturated fats increasingly become part of our everyday diets. In Australia we have recorded LA levels in breast milk of around 20% with a concomitant decrease in breast milk DHA of 30% (Makrides *et al.*, 1995)

The recommendations surrounding omega 3 fatty acids are complex since infants may accumulate DHA as a result

Fig. 1. Proportion of Infants born less than 1250 g with mild and significant mental delay as measured by the Bayley Mental Development Index (MDI).

of converting ALA to DHA in the liver, a process which is thought to be slow, or by including preformed DHA in the formula. Both the endogenous conversion of ALA to DHA and the accumulation of preformed DHA into infant tissues are influenced by the level of LA in the diet. So the amount of LA in infant formulas is of some concern.

The level of LA in infant formulas is under the control of regulatory authorities in each country. The guidelines laid down by Codex indicate that LA should be provided at the rate of 300 mg/100 kcal. With the fat content of a formula of 4.4 g this equates to an LA content of around 6.8% of the total fatty acids. The Australian recommendations set by Food Standards Australia New Zealand set the range of LA in the fat of infant formulas to be between 9 and 26% of the total fatty acids. Given that the fat content of most infant formulas is set at 50% of the total energy it means that most infant formulas provide LA at a level of at least 3% of the total energy (6% fats) and many provide much higher levels.

So how much LA do infants need? Following the early report of Burr and Burr (1929) that an aspect of fat was essential for the normal growth and development of rats there was an explosion of research activity in animals and human infants by Arild E Hansen and colleagues (1963) that concluded the following:

1. Evidence of essential fatty acid (EFA) deficiency developed in young children who received a diet in which the fat provided less than 0.1% energy as LA.
2. Manifestations of the deficiency state (poor growth, dry-scaly skin) disappeared when the fat contained 1% energy or more as LA.

These numbers were widely accepted following elegant experiments in animals by Holman and colleagues (1971). However, in 1957 Cuthbertson, a respected nutritional biochemist that worked for the Glaxo company published a paper in the American Journal of Clinical Nutrition entitled “Essential fatty acid requirements in infancy” in which he challenged the accepted wisdom of these figures (Cuthbertson, 1976). He pointed out that children in the UK at that time were being fed routinely with a dried milk powder that contained only around 0.7% energy as LA and yet there were no reports of children succumbing to essential fatty acid deficiency. He concluded ... “it is believed that the minimum requirements for EFA have been set far too high and are in fact less than 0.5% of cal, so that a daily allowance of 65 mg/100 kcal (about 0.6% cal) should provide an ample margin of safety” (Cuthbertson, 1976).

This had little or no effect on the levels of LA that found their way into infant formula and in 1981 we reported that some infant formulas had levels of LA as high as 58% (29% energy) although most ranged from 5 to 14% energy (Gibson and Kneebone, 1981). So the question that I address in this paper is, were the early experiments accurate at estimating the requirement for LA?

It is important to remember that most early experiments of EFA deficiency explored different levels of dietary LA in the context of either fat free diets or diets containing fats that were extremely low in unsaturated fats. Most importantly nearly all of the experiments were conducted in the absence of ALA in the fat. Recently Philippe Guesnet and colleagues addressed the question of whether the absence of ALA in these early experiments resulted in a systemic error in the requirement for LA in the diet (Guesnet *et al.*, 2011). These workers found that rats consuming the zero LA/zero ALA diet had the lowest final body weight, lower LA and arachidonate in plasma and liver, and elevated Mead acid (20:3 n-9), the marker of EFA deficiency. However when ALA was added to the diet at only 0.5 en %, it completely prevented the lower growth and partly prevented the rise in 20:3 (n-9). Guesnet and colleagues concluded that providing dietary ALA at 0.5 en % reduces the rat’s physiological requirement for LA by an estimated factor of at least four (0.5 en % instead of 2 en %). These results have been confirmed by more recently experiments (Choque *et al.*, 2015). Most importantly, when we replot the effect of the diets used in these experiments (Guesnet *et al.*, 2011) on the plasma DHA levels it is possible to see the strong competitive effect of dietary LA levels above 0.5% en on the accumulation of DHA (Fig. 2). Whether this is due to inhibition of conversion of ALA to DHA or inhibition of DHA incorporation into plasma lipids is not clear. However, since LA requirements in humans are also based on the same model of EFA deficiency, it is plausible that they too have been overestimated and should therefore be reinvestigated.

In 1984 Martha Neuringer reported a seminal study of infant rhesus monkeys whose mothers and they had been fed either an ALA deficient diet (0.3% total fat) or an ALA sufficient (7.7% total fat) diet (Neuringer *et al.*, 1986). The reduced visual acuity detected in the young monkeys fed the ALA deficient diets sent shock waves through the field as the ALA deficient diet was very similar to an human infant formula of the time. Shortly after Uauy and coworkers (Uauy *et al.*, 1990)

Fig. 2. Conversion of ALA to DHA is inhibited by LA. Data adapted from Guesnet *et al.* 2011. DHA level was measured in the rat plasma (% of total fatty acids). The dietary content of LA and ALA was expressed in % energy.

published the first human trial in healthy preterm infants born 27–33 week gestation. Treatment commenced at full enteral feeds with one of 3 infant formulas – a corn oil formula containing only 0.5% ALA, a soy based formula containing 2.7% ALA and a soy/marine formula containing 1.4% ALA +0.65% eicosapentaenoic acid (EPA) and 0.35% DHA (% of total fatty acids in the diet). The results were remarkably similar to those reported by Neuringer, that is, the infants fed the lowest level of ALA showed the poorest VEP acuity results. As all the formulas used in both the Neuringer study and the Uauy study were very high in LA we are left with the conclusion that a balance of LA and ALA is important to animals and human infants alike.

So how do high LA diets regulate DHA levels? There have been two ways proposed, inhibition of omega 3 long-chain polyunsaturated fatty acids (LCPUFA) incorporation into tissues and inhibition of omega 3 synthesis. Several years ago we reported on primates (marmosets) fed EPA (~3% of the fats) in the context of either a high LA diet (> 30% of the fats) or a low LA (atherogenic) diet (~7% of the fat) (McMurchie *et al.*, 1990). Levels of EPA were higher in the low LA diet group as were the metabolically converted products docosapentaenoic acid (DPA) and DHA indicating that both incorporation of pre-formed EPA and its conversion to DPA and DHA were inhibited.

We explored this further in a rat study in which we fed weaning rats a total of 54 separate diets prepared with commonly used oils to obtain 3 levels of total fat–5, 10, 20% (12, 22, 39% energy), 7 levels of total PUFA – 2, 5, 10, 15, 20, 30, 45%, and 5 LA:ALA ratios – 10:1, 5:1, 2:1, 1:1, 0.5:1. This resulted in a range of dietary LA from 0.1–18% energy and ALA from 0.1–12% energy (Gibson *et al.*, 2013). The results (Fig. 3) clearly show the interrelationship between LA and ALA in the diet, with the maximal conversion of ALA to DHA occurring when the total PUFA was less than 2 en % and the LA:ALA ratio was low. The peak of plasma phospholipid DHA (> 8% total fatty acids) was attained as a result of feeding a narrow dietary range of 1–3 en % ALA and 1–2 en % LA but was suppressed to basal levels (~2% total fatty acids)

Fig. 3. Plasma phospholipid DHA in rats fed a range of diets varying in the proportion of LA and ALA. DHA production is highest when the total level of PUFA is low and the ratio of LA to ALA is also low.

at dietary intakes of total (PUFA) above 3 en %. We conclude it is possible to enhance the DHA status of rats fed diets containing ALA as the only source of n-3 fatty acids but only when the level of dietary PUFA is low (< 3 en %).

So what fats should we be using in infant foods? In my opinion they need to be low in LA, have an LA to ALA ratio between 2:1 and 0.5:1, be high in monounsaturates and contain modest level of saturates. Are there any fats available with these properties? Although not now commonly used, dairy fat blends meet most of these criteria. Dairy fats were used in the past before regulators became concerned about the levels of saturates and cholesterol in our diets. It is interesting to note that human breast milk fats contain both these compounds. In fact, there is evidence to suggest that formulas based on dairy fats are superior to vegetable oil based formulas as far as omega 3 LCPUFA status is concerned. Sanders and Naismith demonstrated that the EPA and DHA levels of term infants fed dairy based formulas were elevated compared with vegetable oil based formula and were closer to breast fed levels (Sanders and Naismith, 1979). In addition, Courage and co-workers in 1998 demonstrated that the levels of DHA in the red cells of term infants fed evaporated whole cow milk was equivalent to the levels in breast fed infants when measured at 3 and 6 months (Courage *et al.*, 1998).

Formulas based on an appropriate blend of vegetable oils also have potential to be modified to increase the DHA status. In term infants we showed that DHA accumulation can be increased in plasma by lowering the LA:ALA ratio either by reducing dietary LA or increasing ALA (Clark *et al.*, 1992). In this small but unique study we compared infants fed a standard infant formula of the time with around 14% of the fats as LA and less than 1% as ALA (LA:ALA ratio = 20:1), with a formula in which the LA:ALA ratio was reduced by decreasing the LA content to about 4% of the fats (LA:ALA ratio = 3:1) and another in which we attained a similar LA:ALA ratio by increasing the ALA content to 3.5% of the fats but keeping

the LA level at about 13%. Both formulas with the reduced LA:ALA ratio improved the erythrocyte DHA status relative to the standard infant formula. This experiment highlights the fact that infant formulas could benefit from a re-examination of their fatty acid composition and in particular their LA and ALA content.

Given that there is a clear inverse relationship between LA intake and DHA status why is there so much LA in infant formulas? In many ways it is largely historical. The required level of LA was established in an era where EFA deficiency had been seen in human infants and adults and authorities were keen to avoid it. It was also an era when LA was seen as not only harmless but also useful in lowering plasma cholesterol levels. While it is clear that replacing saturated fats with oils rich in LA can lower the cholesterol of adults this has little relevance to infants given that breast milk is rich in both saturates and cholesterol. Does it matter whether our children are eating so much LA? This is unclear at the moment. Certainly high LA diets inhibit both the synthesis and the incorporation of DHA so adding high levels of LA to infant formulas seems counterproductive since it would have the negative effect of increasing the amount of DHA required in the formula. There is work to suggest that LA can give rise to oxidised metabolites (Ramsden *et al.*, 2012) that have physiological effects that deserves assessment in formula fed infants. What is clear is that there is a need to reassess the LA levels currently in infant formulas.

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