

Dietary Fatty Acids in Growth and Development.

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A newborn infant will double his/her weight in the first 3 months of life and double it again in the next year. Such a rapid growth rate demands adequate nutrition. Fat is a vital source of energy for the growing infant. In breast milk for example, 50% of the energy is in the form of fat. The fat of breast milk contains a full complement of polyunsaturated fatty acids (PUFA), including the two essential PUFA, linoleic acid (LA, 18:2 n-6) and α -linolenic acid, (ALA, 18:3 n-3) but also a range of long chain (LC) PUFA that have been shown to have benefits for both preterm and term infants. Until recently, infant formulas contained LA and ALA only, and this was thought to provide adequate fat nutrition since formula fed infants grew as well as breast-fed babies. LCPUFA are increasingly being added to infant formulas as the technical challenge relating to identifying sources that are stable and hence safe for infants have been overcome. The scope of this paper is to review some of the latest information available in the field of LCPUFA in infant nutrition, growth and development.

PUFA in the diet

While LA and ALA have biological activity in their own right, their activity is enhanced when they are converted to 20 and 22 carbon metabolites (LCPUFA) as they are readily incorporated into cell membranes and can be precursors of the potent hormones known collectively as eicosanoids. The main LCPUFA of interest during infancy are docosahexaenoic acid (DHA, 22:6 n-3) and arachidonic acid (AA, 20:4 n-6). DHA and AA are found in many non-vegetable foods (breast milk, fish, meat, eggs) so can be obtained directly from the diet as well as by conversion of LA and ALA in the body.

LCPUFA and growth in preterm infants

Growth is one of the most sensitive indicators of adequate nutritional status in infants and young children and for this reason is considered to be of key importance in all nutritional interventions. In the field of LCPUFA research the growth of infants randomly allocated to placebo or LCPUFA supplemented formulas has been used as a primary assessment of safety. One of the earliest and most cited trials involving preterm infants allocated to either a placebo, unsupplemented formula or one supplemented with a high EPA fish oil claimed that normalized weight and length of fish oil supplemented formula infants was less than that of infants fed unsupplemented formula at each assessment time between term and 12 months corrected age. (Carlson *et al.*, 1992; 1993a). It was postulated that low levels of AA, caused by the high n-3 LCPUFA content of the formula, were related to poor growth achievement in preterm infants. Phospholipid AA was correlated to normalized weight and length. (Carlson *et al.*, 1993a) However, it is interesting to note that more recent reports have not confirmed these findings. (Carlson *et al.*, 1992; 1993a) The failure of the later preterm studies to detect an effect of LCPUFA on growth has been suggested to be due to the balance of AA and DHA (together with

low EPA) in these diets (Faldella *et al.*, 1996; Vanderhoof *et al.*, 1997; Hansen *et al.*, 1997). It may also be that the growth of infants in these early studies was affected by factors other than the dietary fats. There are several studies underway to clarify this issue.

LCPUFA and growth in term infants

To test the effect of LCPUFA on growth, we undertook a study that was specifically designed to test the effects of supplementing the diets of term infants with either n-3 LCPUFA alone or given as a combination with AA. (Gibson *et al.*, 1997a). Our study induced large changes in infant levels of AA and DHA, and these changes were maintained for at least 12 months. Regressions examining the independent factors related to growth of formula-fed infants revealed that the most consistent factor to emerge at all time points for each of the growth parameters was gender. Maternal smoking had a consistently negative effect on infant length. Importantly, neither AA nor EPA, was associated with any of the outcome measures at any time points. When the models were constructed using the fatty acid data matching the assessment time, no LCPUFA emerged in any model. However when 16-week fatty acid data were used, DHA emerged as a factor predicting weight at 1 and 2 years of age. The magnitude of this effect was small, representing a mean weight increment of 205g (95% CI Alto -368) in ~13kg at 2 years.

Our trial was sufficiently powered to detect clinically relevant changes in weight and length and detected the well-known differential between breast- and formula-fed infants and between boys and girls. Although our regression analyses suggested an association between 16 week infant DHA status and weight at one and two years, these regression models accounted for less than 2% of the infant weight at these ages. Only one study has reported a relationship between growth and LCPUFA status in term infants (Jensen *et al.* (1997). Jensen *et al.* (1997) found an association between a range of n-6 LCPUFA (but not n-3 LCPUFA) at 17 weeks and weight at 17 weeks after correcting for birth weight. However only 13 infants were assessed from the group where lower weight gain was observed (Jensen *et al.*, 1997) raising questions about statistical power of growth effects.

In a trial where lactating women were randomly allocated to receive one of five doses of DHA and breast milk DHA concentration ranged from 0.1% to 1.7% total fatty acids, all infants had appropriate weight, length and head circumference for age (Gibson *et al.*, 1997b). These data, taken together with two other trials in term infants (Auestad *et al.*, 1997; Birch *et al.*, 1998) indicate that dietary LCPUFA supplementation does not adversely affect the growth of healthy, term infants.

LCPUFA and development: randomized clinical trials

One of the best ways to establish a causative link between dietary DHA and neural development is to conduct randomized controlled trials comparing infant formulas with and without DHA. Randomized trials of LCPUFA-supplemented formula have been undertaken with both preterm and term infants. However, their interpretation has proved difficult and perhaps some of the complicating factors have been differing level of supplementation, the choice of LCPUFA supplement and the fatty acid composition of the placebo formulas. For example, the level of DHA supplementation has varied between 0.1% to 0.35% total fatty acids or between 36 mg/L

and 126 mg/L (Makrides *et al.*, 1995; Agostoni *et al.*, Carlson *et al.*, 1995; Birch *et al.*, 1992a).

Breast milk levels of DHA vary with diet and range from 0.1-1.0% total fats and thus is a poor reference for choosing the level of DHA supplementation (Makrudes *et al.*, 1996). It is important to remember that the dose of DHA included in infant formulas has been largely determined by the level of OHA in breast milk rather than calculations of tissue requirements.

LCPUFA and development: the preterm infant

Most studies attempting to assess the effect of n-3 LCPUFA on neurodevelopment have chosen tests of visual function. The underlying hypothesis has been that any demonstrated effect on development of visual acuity may reflect an underlying, more widespread difference in neural maturation that might affect other aspects of brain function. This is based on the fact that the whole visual process is either an extension of the brain (embryologically) or is intimately involved in the interpretation of information from the retina. The rationale for the inclusion of DHA into infant formulas is based on the fact that primates raised on n-3 PUFA deficient diets have low tissue levels of DHA and poor visual function and that infants fed formulas that contain no DHA have lower brain DHA levels than infants fed breast milk containing DHA.

Despite the variation in the LCPUFA treatments, the results of randomized clinical trials with preterm infants are surprisingly consistent. Five studies conducted to date have used either electrophysiological or behavioral assessments of visual acuity as outcome measures (Faldella *et al.*, 1996; Birch *et al.*, 1992a; Birch *et al.*, 1992b; Uauy *et al.*, 1996a; Damli *et al.*, 1996). Three of the five studies demonstrate a positive effect of LCPUFA supplementation on visual acuity during the 4 months post-term (Birch *et al.*, 1992a; Carlson *et al.*, 1993b; Carlson *et al.*, 1996a; Damli *et al.*, 1996). Other researchers have chosen to assess the latency of the visual evoked potential (VEP) recorded at the occipital cortex in response to a flash stimulus (Feldella *et al.*, 1996). Although these workers report some differences in the VEP waveforms of LCPUFA supplemented and unsupplemented infants, the flash VEP cannot be used to evaluate acuity. Electroretinographic (ERG) studies have also been undertaken to investigate the effects of dietary LCPUFA on the functional integrity of the retina in two separate trials (Feldella *et al.*, 1996; Birch *et al.*, 1992b). Unfortunately only one of the randomized trials assessed ERG function according to the internationally accepted ISCEV standard (Marmor *et al.*, 1992b). The results of this trial demonstrate that infants fed the supplemented formula were able to respond to dimmer flashes of light and had larger responses to the bright flashes compared with unsupplemented infants (Birch *et al.*, 1992b). These observations were made only at 36 weeks post-conceptual age (PCA) and not at 57 weeks PCA, suggesting a transient dietary effect of n-3 LCPUFA.

Some trials have also measured behavioral outcomes that may be related to cognition as well as more global indices of development. Carlson and Werkman reported in both short (Carlson & Werkman, 1996) and long term (Werkman & Carlson, 1996) supplementation studies that there was altered visual attention (shorter and more discrete looks to familiar and novel stimuli) in healthy preterm infants who had been randomly allocated to receive n-3 LCPUFA compared with infants fed unsupplemented formula. The significance of these findings is not known although they parallel the observations reported in the monkey studies of Neuringer (Reisbick *et*

al., 1994). The infants in the Carison studies were also assessed at 12 months corrected age using the Bayley's Scales of Infant Development (Carson *et al.*, 1994). In only the short term feeding study was there a suggested benefit of n-3 PUFA supplementation on Bayley's mental development index (Carson *et al.*, 1994).

In summary, it would appear from the outcomes of the randomized clinical trials that preterm infants benefit from a supply of dietary LCPUFA. However, such a statement requires qualification. Almost all trials only included infants born less than 32 weeks gestation (Markrides & Gibson, 1998). Furthermore, the relatively healthy preterm infants were mostly studied. It is therefore difficult to extrapolate the results of trials with limited samples to the general preterm population, which can be variable with concomitant diseases and therapies that may affect or interact with the treatments or outcomes being measured.

LCPUFA and development: the term infant

The results of randomized trials of healthy, term infants fed either unsupplemented or LCPUFA-modified formulas have been more extensive compared with trials involving preterm infants but their results are less consistent. At least seven trials have evaluated visual acuity by either behavioral or electrophysiological measures (Gibson *et al.*, 1997a; Auestad *et al.*, 1997; Makrides *et al.*, 1995; Carlson *et al.*, 1996b; Jorgensen *et al.*, 1998; Birch *et al.*, 1996; Clausen *et al.*, 1996). Some research groups have reported improved acuity in infants fed breast milk and LCPUFA supplemented formula compared with infants fed standard, placebo formula in the first 6 months of life. Other investigators have shown differences in VEP acuity between a reference group of breast fed infants and infants randomly allocated to an unsupplemented formula (Jorgensen *et al.*, 1998). Infants fed the LCPUFA supplemented formula had VEP acuities that were similar to both breast and placebo formula-fed infants (Jorgensen *et al.*, 1998). Three trials have reported no differences in either electrophysiological (Gibson *et al.*, 1997a; Auestad *et al.*, 1997) or behavioral measures (Auestad *et al.*, 1997; Clausen *et al.*, 1996) of acuity between any randomly allocated formula feeding groups.

Developmental quotients (DO) of infants fed formulas with modified fat blends or breast milk have also been assessed. Agostoni and co-workers (Agostoni *et al.*, 1995) showed that infants randomized to receive a LCPUFA supplemented formula have better (higher) DQ as measured by the Brunet-Lezine compared with infants fed standard formula at 4 months of age. A follow-up assessment of these children at one and two years of age demonstrated no difference between the groups, although the authors reported a positive association between erythrocyte LCPUFA and DQ at two years of age (Agostoni *et al.*, 1997). Auestad and co-workers also reported no effect of diet on DQ as assessed by the Bayley's Scales at one year of age but claimed a negative association between erythrocyte phospholipid DHA and language comprehension in these infants at 14 months of age (Janowsky *et al.*, 1995). A recent study reporting a follow-up of infants who participated in a randomized trial of LCPUFA supplemented formula feeding between birth and 4 months demonstrated improved problem solving ability at 10 months in the infant fed LCPUFA supplemented formula compared with the unsupplemented formula group (Willatts *et al.*, 1998).

In trying to bring together the results of trials involving LCPUFA supplementation in healthy

term infants it would appear that a beneficial effect of dietary LCPUFA on neurodevelopment is equivocal - some trials demonstrate an improvement in neural indices while others show no effect. In theory trials involving term infants should be easier to interpret than trial with preterm infants as such trials are more extensive and are likely to involve less interactive or confounding variables likely to impact on the developmental outcome in question. However, this does not appear to be the case and a number of possible explanations deserve consideration. Firstly, the size of an effect of LCPUFA on a neural index may be smaller in term compared with preterm infants and therefore trials with larger numbers of infants in each dietary group would be required. But a major increase in the sample population being studied often requires trials with multiple centers or if conducted in a single center, the use of multiple testers. This of course has the potential to increase variability in the outcome being measured and a reduction in the likelihood of observing an effect, should it exist. It may be that the equivocal results partly derive from this fact (Gibson & Makrides, 1998).

It is important to remember that changes in the balance of LCPUFA have been associated with a variety of health improvements in adults including inflammatory responses and an inhibition of thrombotic potential. It would be surprising if LCPUFA in the diet of infants was not also associated with changes in physiological functions. We are currently investigating the potential role of LCPUFA in gastro-intestinal function and immunocompetence in term infants.

SUMMARY

There appears to be little evidence for concern relating to dietary LCPUFA and growth in either preterm or term infants. Our large study supplementing infant formulas with either tuna oil alone or a balance of LCPUFA in the form of egg phospholipids resulted in a large range of AA values which were similar to levels reported in preterm infants and yet we found no association between infant AA status and growth. The results of recent studies with preterm infants also show a similar lack of effect of LCPUFA supplemented formulas on growth. Breast milk also contains a range of LCPUFA that can be manipulated through changes in the maternal diet. Altering the balance of breast LCPUFA to strongly favour n-3 PUFA also had no effect on the growth of term infants. There seem to be few health concerns relating to the addition of LCPUFA to infant formulas.

There is evidence that LCPUFA can have subtle effects on aspects of infant development and this evidence is stronger and more consistent in preterm rather than term infants. It is important to remember the multifactorial nature of neurodevelopment. For this reason one would predict the effects of LCPUFA would require studies involving large numbers of children. It is somewhat surprising therefore that many of the trials have reported effects in infants with limited numbers, indicating that some physiological processes, such as visual perception, may be more sensitive than others. However, we have a poor understanding of the possible implications of altered physiological endpoints that have been used as outcome measures or the underlying mechanisms. For example, it is not known whether increased visual acuity is related to improved visual attention and the speed of processing that has been reported in studies with preterm infants (Carlson *et al.*, 1993b; Carlson & Werkman, 1996; Werkman & Carlson, 1996). While it is clear that a overt deficiency in n-3 LCPUFA can result in severe neurological abnormalities (Martinez,

1995) there is little consistent data to establish a dietary requirement for the development of optimal neural function. This has been made more difficult by issues relating to PUFA metabolism, the doses used in different studies, the choice of assessments by different investigators, and issues relating to trial design and conduct. This latter point is explored in detail in another review (Gibson & Makrides, 1998). The role of LCPUFA in neonatal development may be clarified by well-designed and conducted randomized clinical trials currently underway in several countries.

REFERENCES

Agostoni C, Trojan S, Belli R, Riva E, Bruzzese MG & Giovannini M (1997). Developmental quotient at 24 months and fatty acid composition of diet in early infancy: A follow up study. *Arch Dis Child* 6:421A.

Agostoni C, Trojan S, Belli R, Riva E & Giovannini M (1995). Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: The role of long-chain polyunsaturated fatty acids. *Pediatr Res* 38:2626.

Auestad N, Montalto MB, Hall RT, *et al.* (1997). Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. *Pediatr Res* 41:1-10.

Birch DG, Birch EE, Hoffman DR & Uauy RD (1992b). Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci* 33:2365-76.

Birch EE, Birch DG, Hoffman DR, *et al.* (1996). Visual maturation of term infants fed w3 long chain polyunsaturated fatty acid (LCPUFA) supplemented formula.. *Invest Ophthalmol Vis Sci* 37:SI 12 (abstr).

Birch EE, Birch DG, Hoffman DR & Uauy R (1992a). Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci* 32:3242-53.

Birch EE, Hoffman DR, Uauy R & Birch DG Prestidge C (1998). Visual acuity and the essentiality of docosahexanoic acid and arachidonic acid in the diet of term infants. *Pediatr Res* 44:201-9.

Carlson SE, Ford AJ, Werkman SH, Peeples JM & Koo WWK (1996b). Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatr Res* 9: 882-8

Carlson SE, Cooke RJ, Werkman SH & Tolley EA (1992). First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. *Lipids* 27:901-7.

Carlson SE, Ford AJ, Peeples JM & Werkman SH (1995). Visual acuity in term infants fed human milk or randomly assigned to a formula with or without long chain n-3 and n~fatty acids.

Pediatr Res 37:303A (abstr).

Carlson SE, Werkman SH & Tolley EA (1996a). Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr* 63:687-97.

Carlson SE, Werkman SH, Peeples JM, Cooke RJ & Tolley EA (1993a). Arachidonic acid status correlates with first year growth in preterm infants. *Proc Nat Acad Sci USA* 90:1073-7.

Carlson SE, Werkman SH, Peeples JM & Wilson WM (1994). Long-chain fatty acids and early visual and cognitive development of preterm infants. *Eur J Clin Nutr* 48 (Suppl 2):527-30.

Carlson SE, Werkman SH, Rhodes PG & Tolley EA (1993a). Visual acuity development in healthy preterm infants: effect of marine oil supplementation. *Am J Clin Nutr* 58:35A2.

Carlson SE & Werkman SH (1996). A randomized trial of visual attention of preterm infants fed docosahexaenoic fed until two months. *Lipids* 31:85-90

Clausen U, Damli A, Schenck UV & Koletzko B (1996). Influence of long-chain polyunsaturated fatty acids (LCPUFA) on early visual acuity and mental development of term infants. In *PUFA in Infant Nutrition: Consensus and Controversies*. November, 12(abst).

Damli A, Schenck UV, Clausen U & Koletzko B (1996). Effects of long-chain polyunsaturated fatty acids (LCPUFA) on early visual acuity and mental development of preterm infants. In *PUFA in Infant Nutrition: Concensus and Controversies*. November, 14(abst).

Faldella G, Govoni M, Alessandrini R, *et al.* (1996). Visual evoked potentials and dietary long chain polyunsaturated fatty acids in preterm infants. *Arch Dis Child Fetal Neonatal* 75:F108-12.

Gibson R, Neumann M & Makrides M (1997a). A randomised clinical trial of long chain fatty acids in term infants: effect on neural indices. *J Paediatr Child Health* 33:A8(abstr).

Gibson RA & Makrides M (1998). Polyunsaturated fatty adds and infant visual development: a critical appraisal of randomised clinical trials. *Lipids* (in press).

Gibson RA & Neumann MA, Makrides M (1997b). Effect of increasing breast milk docosahexanoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. *Eur J Clin Nutr* 51:57884.

Hansen J, Schade D, Harris C *et al.* (1997). Docosahexaenoic acid plus arachidonic acid enhance preterm infant growth. *Prostaglandins Leukot Essent Fatty Acids* 57:96(abstr).

Janowsky JS, Scott DT, Wheeler RE & Auestad N (1995). Fatty acids affect early language development. *Pediatr Res* 37:310A (abstr).

Jensen CL, Prager TC, Fraley JK & Chen HM, Anderson RE, Heird WC (1997). Effect of

dietary linoleidalpha-linolenic acid ratio on growth and visual function of term infants. *J Pediatr* 131:200-9.

Jorgensen MH, Holmer G, Lund P, Hernell O & Michaelsen, KF (1998). Effect of formula supplemented with docosahexaenoic acid and gamma-linolenic acid on fatty acid status and visual acuity in term infants. *Journal of Pediatric Gastroenterology & Nutrition* 26:412-21.

Makrides M & Gibson RA (1998). Are long chain polyunsaturated fatty acids essential nutrients in infancy? *Proceedings of the Fourth International Congress on Essential Fatty Acids and Eicosanoids* (in press).

Makrides M, Neumann MA, Simmer K, Pater J & Gibson R (1995). Are long-chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet* 345:1463-

Makrides M, Neumann MA & Gibson RA (1996). Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. *Eur J Clin Nutr* 50:352-7.

Marmor MF, Arden GB, Nilsson SEG & Zrenner E (1989). Standard for clinical electroretinography. *Arch Ophthalmol* 107:816-9.

Martinez M (1995). Polyunsaturated fatty acids in the developing human brain, erythrocytes and plasma in peroxisomal disease: Therapeutic implications. *J Inherit Metab Dis* 18:61-75.

Reisbick S, Neuringer M & Gohe E (1994). Increased look duration in paired comparisons by rhesus monkey infants with n-3 fatty acid deficiency (n-3 FAD). *Soc Neurosci Abstr* 20:1696.

Uauy R, Birch DG, Birch EE, Hoffman D & Tyson J (1993). Visual and brain development in infants as a function of essential fatty acid supply provided by the early diet. In: Dobbing J, (eds.) *Lipids, Learning, and the Brain: Fats in Infant Formulas*. Dobbing J, (eds.), pp. 21-38. Columbus, OH: Ross Laboratories.

Vanderhoof J, Gross S, Hegyi T, *et al.* (1997). A new arachidonic acid (ARA) and docosahexanoic acid (DHA) supplemented preterm formula: growth and safety assessment. *Pediatr Res* 41:242A (abstr).

Werkman SH & Carlson SE (1996). A randomized trial of visual attention of preterm infants fed docosahexanoic acid until nine months. *Lipids* 31:91-7

Willatts P, Forsyth JS, DiModugno MK, Varma S & Colvin M (1998). Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet* 352:688-91.