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EARNEST

EARly Nutrition programming- long term follow up of Efficacy and Safety Trials and integrated epidemiological, genetic, animal, consumer and economic research

Instrument: Integrated Project

Thematic Priority 5.4.3.1: Food Quality and Safety

Final public report on activity 1.3.2

Follow-up of infants randomised to LCPUFA supplemented or standard formulas during infancy: effects on neurodevelopmental outcome and blood pressure

Start date of project: 15.04.2005 Duration: 5,5 Years

Organisation Name of Lead Contractor for this report: UCLON

Activity 1.3.2 Follow-up of infants randomised to long-chain polyunsaturated supplemented or standard formulas during infancy: effects on neurodevelopmental outcomes and blood pressure.

Introduction:
Long-chain polyunsaturated fatty acids (LCPUFAs) are important constituents of cell membranes in particular neural and retinal cells but are also functional compounds acting as precursors for eicosanoids that control inflammation, immunity and adipogensesis[1]. Higher levels of LCPUFAs are found in the tissues and blood of breast fed infants compared to formula fed infants leading investigators to hypothesise that it is the LCPUFAs in breast milk (but not in formula) that confer the cognitive and visual developmental advantages seen in breast fed infants when compared to their formula fed peers.

LCPUFAs are derived from the two families of essential fatty acids (n-3 linolenic acid and n-6 linoleic acids), infants can form LCPUFAs endogenously, however this is considered energetically expensive and, particularly for preterm infants, this synthesis may not be efficient. Both families compete for the same enzymes (a series of desaturases and elongases) but the fatty acids are not able to interconvert in between families due to the position of the last double bond with respect to the methyl end of the fatty acid. The rate of LCPUFA deposition increases rapidly during the last trimester of pregnancy, with around 30% of DHA, (decosahexanoic acid 22:6(n-3)) being laid down during this time, leaving preterm infants in potential deficit at delivery. Nevertheless, recent Cochrane meta-analyses[2,3] of supplementation studies in infancy have concluded that up to 2 years of age there are no benefits or detrimental effects of using LCPUFA supplemented formulas on neurological, visual, and growth outcomes in term or preterm infants. However, as LCPUFAs are multi-functional it is plausible that longer term studies of supplementation in infancy may yield effects on cognition, growth or body composition. One long term report by Forsyth et al[4] has associated LCPUFAs in infant formula with reduced blood pressure at 6 years, other studies have yielded inconsistent results and frequently with discrepancies between the genders [5,6].

This Activity consisted of three tasks:
Task 1: Follow-up at 14 – 16 years of a cohort of infants born at term who were randomised a standard or LCPUFA supplemented formula for the first 6 months of life. The LCPUFAs were sourced from egg phospholipids and fish oil. This cohort was initially followed-up until 18 months[7] and then when 4-6 years[8], at the end of the first period, there were no differences between randomised groups, however at 4-6 years the LCPUFA supplemented children had significantly lower IQ (5.9 points), there were no differences between visual maturity between randomised groups.
Task 2: Follow-up at 14-16 years of a cohort of infants born preterm who were randomised to a standard or LCPUFA supplemented formula until discharge from the neonatal unit[9], LCPUFAs were sourced from evening primrose (borage oil) providing γ-linolenic acid (a precursor to DHA) and egg lipids (DHA and AA). This was a short intervention used whilst the infants were still on the neonatal units, with a mean intervention period 31 days for the LCPUFA group and 33 days for control group. At the end of the study when the subjects were 18 months corrected age, the

supplemented infants were shorter compared to the controls and no significant cognitive difference between the groups.

Tasks 1 + 2 were organised as one project as both cohorts were born in the same hospitals in the cities of Leicester and Nottingham. Research psychologists, one for each centre, were appointed to assess both cohorts using the same protocols, the researchers were aware of which cohort the subject belonged to but were blind to the randomised dietary group.

Task 3: Follow-up at age 10 years of a cohort so infants born preterm between 1995-97, who were randomised to standard or LCPUFA supplemented formula (fish oil and borage oil) until 9 months corrected age, the n-6 LCPUFA was provided as a γ-linolenic acid (18:3(n-6)) as a precursor of AA (20:4(n-6))[10]. At 18 months corrected age, the supplemented infants showed greater weight and length gain compared to the control infants and the supplemented boys showed higher cognitive development at 18 months compared to controls.

Follow-up protocol.

All three cohorts followed similar protocols including cognitive and physiological assessments, with small differences to reflect the different ages of the cohorts. The cognitive and physiological tests were carried, which were chosen to look at global IQ, attention and problem solving, neurological function, behaviour and school achievement. The tests used included:

1. WASI – the Weschler Abbreviated Scales of Intelligence to measure global IQ
2. NEPSY II using sub-tests to measure attention/executive function, memory and language.
3. Tea-Ch – the Test of Everyday Attention
4. WIAT II – the Weschler Individual Achievement Test to look at reading and numerical ability
5. BADSc – the Behavioural Assessment of Dysecutive syndrome
6. CBCL and TRF – the Child Behaviour Check-list and Teacher Report forms, completed by the subject (if over 12 years), their parent and their teacher to detect behavioural problems
7. Additional memory measures – the word-pairs sub-test from the Children's Memory Scale, which tests association-learning and is a test of hippocampus function

Physiological assessments included anthropometry, body composition (using skinfold thicknesses, deuterium and bio-electrical impedance), self-assessment of puberty, atopic disease and blood pressure. Data on atopy was collected by interview based on the SCORAD system for assessing atopic dermatitis. The assessments took 4 -5 hours on one occasion, usually in the local clinic but occasionally at the subject's home. The subjects were invited to take part by letter, prior to sending an invitation letter their GP had been contacted to check that it would be appropriate to write to the subject.

Task 3 was carried out between 2006-2008 in by one researcher at the Hospital for Sick Children, Yorkhill, Glasgow, the study was approved by the ethics committee of

Yorkhill Sick Children's Hospital. Tasks 1 and 2 were delayed in starting as initially there was a short-fall in funding for the costs of carrying these tasks. There were also some problems with gaining ethics and R+D approval for these two tasks, which had not been experienced with the Task 3 follow-up. The Nottingham 1 Research Ethics Committee (REC), would not approve letters being sent directly to the last known address we knew of these subjects, so we were obliged to write to the GPs of each subject and ask the GP to forward a letter on our behalf, adding a further step and delay in contacting the cohorts. The data collection for this study is currently being carried out by two research psychologists in each city centre and commenced in March 2010.

Results:

Task 1 and Task2: due to unexpected delays in gaining ethics and R+D approval for this combined project, the data collection is currently on-going. Approximately, 40 subjects have been followed-up to date; no interim analysis has been carried.

Task 3: One hundred and seven out of the original cohort of 237 (45%) took part in the follow-up. Those who took part were compared to those who did not take part in the follow-up, a greater proportion of those followed up received mothers breast milk (64% vs 47%, $p = 0.01$), be older at randomisation (16 days vs 12 days, $p = 0.002$) and have a higher birth weight SD score (-0.41 vs -0.70, $p = 0.03$). Those followed-up, 107 were randomised to control formula and 100 to LCPUFA formula, it was found that the LCPUFA group had mothers who were older and from higher social background, these factors were controlled for in later analysis.

There were no differences between the randomised groups for growth, blood pressure and cognitive development for the whole cohort. However, in pre-planned gender analyses, girls who had received LCPUFA formula had significantly higher scores in reading and word based tasks. Girls were also heavier, had greater sum of skinfold thicknesses and higher blood pressure. The analyses were adjusted for confounding factors known to effect growth and blood pressure, the differences seen between groups with respect to weight SD score, BP and sum of skinfolds remained after adjustment, however the differences for blood pressure were lost when analyses were adjusted for current weight.

Conclusion:
This is the longest follow-up of an LCPUFA supplementation that we know of and has shown, contrary to our hypothesis, that LCPUFA supplementation of preterm infant girls increases weight, adiposity, blood pressure at age 10 years. Whether these findings have consequences for later health is unclear. Girls who received the LCPUFA supplemented formula did, however, perform better in certain tests of literacy. No effects were seen in the boys from this cohort.

Although overall there were no differences in cognitive function between randomised groups, in the sub-group of infants who received no breast milk in the neonatal period (n=39), LCPUFA-supplementation was associated with beneficial effects on verbal IQ (98.2 (13.3) pts vs 86.3(7.8) pts, $p = 0.03$), which was reflected in full scale IQ cognitive function (97.0 (11.1) vs 87.6 (9.8) pts, $p = 0.04$). in addition, there was also a significant difference in the word-pair learning scaled score from the Children's

Memory Scale, which is believed to be related the hippocampus function (13.1 vs 10.3, p = 0.004).

Although our study has limitations – in particular cohort attrition – our findings are typical of the inconsistent results seen in trials of LCPUFA supplementation in both preterm and term infants, the majority of which have so far only reported outcome data in infancy. Our unexpected results emphasise the importance of longer-term follow-up of these cohorts, and the need to measure health outcomes such as body composition and blood pressure as well as cognitive function. Data from this follow-up will be combined with those from follow-up of our remaining two cohorts, and the cohort of Professor Mijna Hadders-Algra (EARNEST partner) – all conducted using the same protocol and outcome measures) to provide increased sample size and power.

Dissemination:

This Activity has generated two scientific papers:

1. Kennedy K, Ross S, Isaacs EB, Weaver LT, Singhal A, Lucas A, Fewtrell MS. The 10-year follow-up of randomised trial of long-chain polyunsaturated fatty acid supplementation in preterm infants: effects on growth and blood pressure. Arch Dis Child 2010; 95: 588-595.
2. Isaacs EB, Ross S, Kennedy K, Weaver LT, Lucas A, Fewtrell MS. Cognitive function at 10 years of age in a randomised, double blind trial of long-chain polyunsaturated fatty acid supplementation of formula fed to preterm infants. Submitted to Pediatrics, October 2010.

The original data from the cohorts in Tasks 1 and 2 have been combined in two publications in collaboration with EARNEST colleagues from LMU, Munich and the University of Groningen as below:

1. Beyerlein A, Hadders-Algra M, Kennedy K, Fewtrell MS, Singhal A, Rosenfeld E, Bouwstra H, Koletzko B, von Kries R. Infant formula supplementation with long-chain polyunsaturated fatty acids has no effect on Bayley Developmental scores at 18 months of age: Individual patient data meta-analysis of 4 large clinical trials. J Pediatr Gasterenterol Nutr 2010; 50: 79-84
2. Rosenfeld E, Beyerlein A, Hadders-Algra M, Kennedy K, Singhal A, Fewtrell M, Lucas A, Koletzko B, von Kires R. Individual patient data meta-analysis shows no effect of long-chain polyunsaturated fatty acid supplemntaiotn on infant growth at 18 months. Acta Pediatr 2099; 98: 91-97.

In addition a review article, ‘Gender specific differences in essential fatty acid metabolism’, has been submitted (June 2010) to American Journal of Clinical Nutrition as an EARNEST collaboration with Tamas Decsi (University of Pecs, Hungary) and Kathy Kennedy (UCL, UK).

References:

1. de Roos B et al. Identification of potential serum biomarkers of inflammation and lipid modulation that are altered by fish oil supplementation in healthy volunteers. Proteonomics 2008; 8: 1965-7
2. Simmer K et al. Long-chain polyunsaturated fatty acid supplementation in preterm infants. Cochrane database Syst Rev 2008; 1: CD000375.
3. Simmer K et al. Long-chain polyunsaturated fatty acid supplementation in term infants. Cochrane database Syst Rev 2008; 23: CD000376.
4. Forsyth JS et al. Long-chain polyunsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow-up of a randomised controlled trial. BMJ 2003; 326: 953.
5. Ryan AS et al. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. Am J Hum Biol 1999; 11: 457-67
6. Makrides M et al. Neurodevelopmental outcomes of preterm infants fed high dose DHA: a randomised controlled trial. JAMA 2009; 301: 175-82.
7. Lucas A et al. Efficacy and safety of long-chain polyunsaturated fatty acids of infant formula milk: a randomised trial. Lancet 1999; 354 (9194): 1948-54.
8. Singhal A et al. Infant nutrition and stereoacuity at age 4-6 years. AJCN 2007; 85 (1): 152-9.
9. Fewtrell MS et al. Double-blind randomised trial of Long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics 2002; 110: (73-82).
10. Fewtrell MS et al. Randomised, double blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. J Pediatr 2004; 144: 471-9.