Early behavior and development are influenced by the n-6 and n-3 status in prematures*

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Abstract: *In a cohort of premature infants, consecutively included in the study at birth and followed to 18 months of age, the neonatal status of essential fatty acids and long-chain polyunsaturated fatty acids (LCPUFA) were investigated and correlated to the development at 40 and 44 weeks gestational age and at 3, 6, 10 and 18 months corrected age. The diet reported by the mothers contained low EFA, 98% had an intake < 1 energy% of n-3 fatty acids. Fatty acid analyses were performed in early breast milk and in mothers' and infants' plasma phospholipids early after birth and at gestational age 40 and 44 weeks. The development of the infants were assessed with Brazelton Neonatal Behavioral Assessment Scale (BNBAS) at 40 and 44 weeks and with Bayley's Scales of Infant Development (Second Edition (BSID-II) at 3, 6, 10 and 18 months corrected age. At 40 weeks and 3 months videotapes were made of the infants' spontaneous motor behavior to assess the quality of their general movements. Adjustments for confounding background factors were made in multiple logistic regression analyses and mothers' education had the highest impact of the background variables. At all ages tested the n-6 fatty acid concentrations, expressed as total concentrations, LA, AA or as ratios to n-3 fatty acids in breast milk and early plasma phospholipids were negatively associated with development. Positive associations with LCPUFA, especially DHA, were mainly found after 10 months of age. Both mental and motor developments had similar pattern of associations, fatty acid concentrations and background factors explaining 20-50% (R^2) of the developmental scores. This was only an observational study, and it cannot be excluded that the highly negative influence of n-6 fatty acids was an effect of the low intake of n-3 fatty acids, which in the context of the changes generally seen in Western diet imply urgent need for larger studies.*

Key words: *arachidonic acid, docosahexaenoic acid, linoleic acid, mental development, motor development, orientation*

Retrospective epidemiological studies have indicated the importance of adequate nutrition during fetal and early postnatal periods for later health and for the development of especially cardiovascular diseases, obesity and diabetes in the adults (Forsdahl, 1977; Barker *et al.*, 1986; Eriksson *et al.*, 2003). Animal studies have confirmed the influence of general and specific under-and overnutrition on later metabolism by programming during special sensitive periods of early life (Langley-Evans, 2004; Korotkova *et al.*, 2005; Tamashiro and Moran 2010; Sebert *et al.*, 2011). An increasing number of studies have now started to find mechanisms for this influence, by studying different epigenetic mechanisms both in humans, exposed to the Dutch famine during the Second World War (Tobi *et al.*, 2009), and in animal studies (Waterland and Michels, 2007). Prospective studies from early life in humans are hitherto relatively rare. Most studies during the latest decades have focused on supplementation with long-chain polyunsaturated fatty acids (LCPUFA) during pregnancy and/or lactation for the neurological development, recognizing the difference in the supply of these fatty acids between formula and breast feeding, but without consideration of the great variation of fatty acids in breast milk. Few observational studies have investigated the habitual intake of nutrients in relation to ordinary development.

We have been interested in the observation that late prematures with an uneventful history often have minor developmental differences later during childhood compared to those born at full term (Raju, 2006). The brain development is requiring a large surge for

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LCPUFA during the last trimester and early postnatal period, which is supplied by efficient intrauterine transport via the placenta and by the breast milk, which is especially providing docosahexaenoic acid (DHA 22:6n-3) (Sabel *et al.*, 2009). At premature delivery this supply is abruptly discontinued, especially if mother's milk is not available.

We hypothesized that premature delivery might by changes in fatty acid pattern influence the later development and therefore invited mothers delivering preterm infants to participate in an observational prospective study. The mothers, delivering at a community urban hospital, were included consecutively during 10 months, Fifty-one mothers and their infants were included, gestational age 25-36.9 weeks, mean (SD) 33 (2.6) weeks, with 75% of the infants being late prematures, born between 32 and 36 weeks, and 57% between 34-36.9 weeks. Mother's mean age was 29.8 (6.0) years and BMI 24.9 (3.9) kg/m^2. In 52% the birth weight of the infants were >2000 g and mean birth weight was 2010 (590) g. Infants with malformation or those needing intensive neonatal care were not included, like mothers with chronic diseases. Extensive characterization of the mothers and infants have been presented (Sabel *et al.*, 2009), and those data were used as confounding factors in the analyses. Mother's diet was registered for 3 days and mean fat intake was 31 (6) energy%, but the essential fatty acid intake was generally low, being <3 energy% in 33% of the mothers and 98% had n-3 fatty acid intake less than 1 energy% (Sabel *et al.*, 2009). Breast milk was analysed at one week and plasma phospholipid fatty acid pattern in cord blood and in mothers and infants at one week, and at 40 and 44 weeks gestational age. The infants were examined for General movements (GM) at 40 weeks and 3 months (Hadders-Algra, 2001), with Brazelton Neonatal Behavioral Assessment Scale (Brazelton and Nugents, 1995) at 40 and 44 weeks, with the Selfregulation Scale at 40 and 44 weeks (Lundqvist-Persson, 2000) and by Bayley's Scales of Infant Development (Second Edition BSID-II) at 3, 6, 10 and 18 months (Bayley, 1993). The results were compared to the early fatty acid analyses and adjusted for confounding factors in mothers and infants.

There was a strong correlation between mothers' fat intake and the intake of essential fatty acids [11], $r=0.69$ ($p<0.001$), reflecting both linoleic (18:2n-6, LA) and alpha-linolenic (18:3n-3, ALA) acids *(figure 1)*. Mead acid (20:3n-9; ETA) was negatively related to LA both in mothers' ($r=-0.64$, $p<0.001$) and infant's ($r=-0.37$, $p=0.016$) plasma.

In the premature infants LA increased by age and its corresponding LCPUFA, arachidonic acid (20:4n-6, AA) decreased *(figure 2)*, illustrating a strong inverse association between these fatty acids in infants' early plasma phospholipids ($r=0.90$, $p<0.001$), stronger than for ALA to DHA *(figure 3)*. This might be an unexpected finding since the LCPUFA, including arachidonic acid is considered favoured in breast milk secretion. Our finding is in agreement with the pattern found in cells and plasma of adults, i.e. that high linoleic acid is associated with low arachidonic acid (Spector *et al.*, 1981; Liou and Innis, 2009; Friesen and Innis, 2010). The finding, if confirmed, might be of concern in view of the high intake of LA generally in food and the importance of arachidonic acid for the early development (Schuchardt *et al.*, 2010).

The DHA concentration in the infants' plasma phospholipid was relatively constant in the breast fed infants to 44 weeks of gestational age but declined significantly in the mothers.

The opposite patterns was found in infants and mothers not fully breast fed or lactating at 40 and 44 weeks of gestation (Sabel *et al.*, 2009).

Similarly the ratio of n-6/n-3 fatty acids in infants' plasma phospholipids were constant in breastfed infants to 44 weeks of gestational age, but showed a remarkable increase from 8 to 12 in those exchanging to more and more formula during the corresponding time.

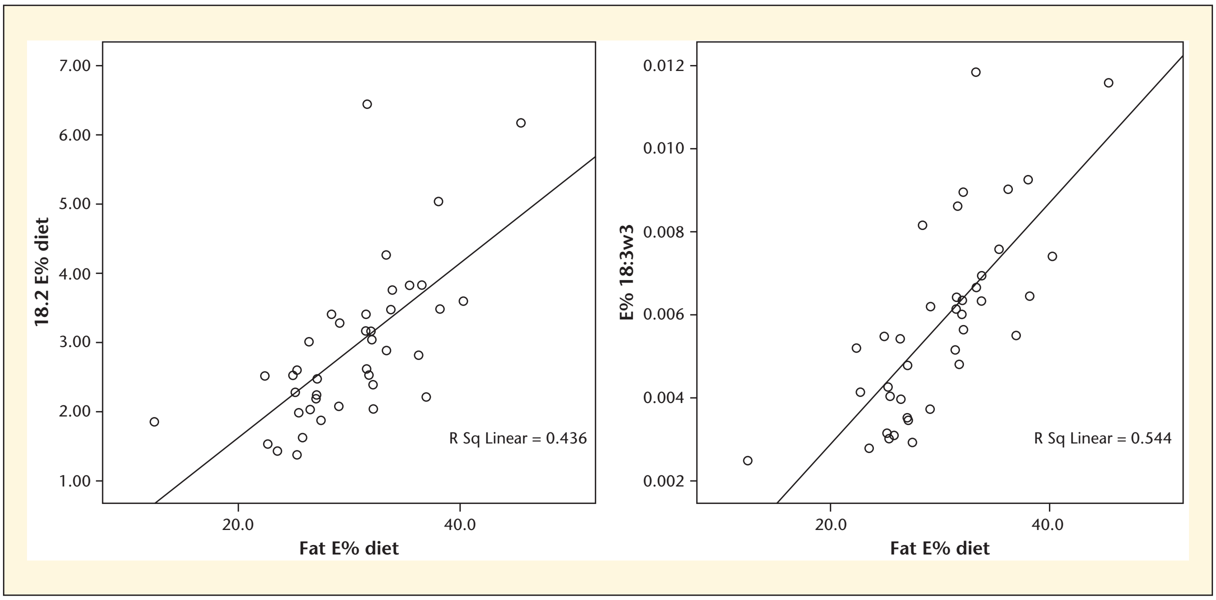

Figure 1. *The relation between maternal fat energy percentage intake and the energy percentage intake of linoleic acid (18:2n-6) (left) and alpha-linolenic acid (18:3n-3) (right). Data from Sabel* et al.*, 2009.*

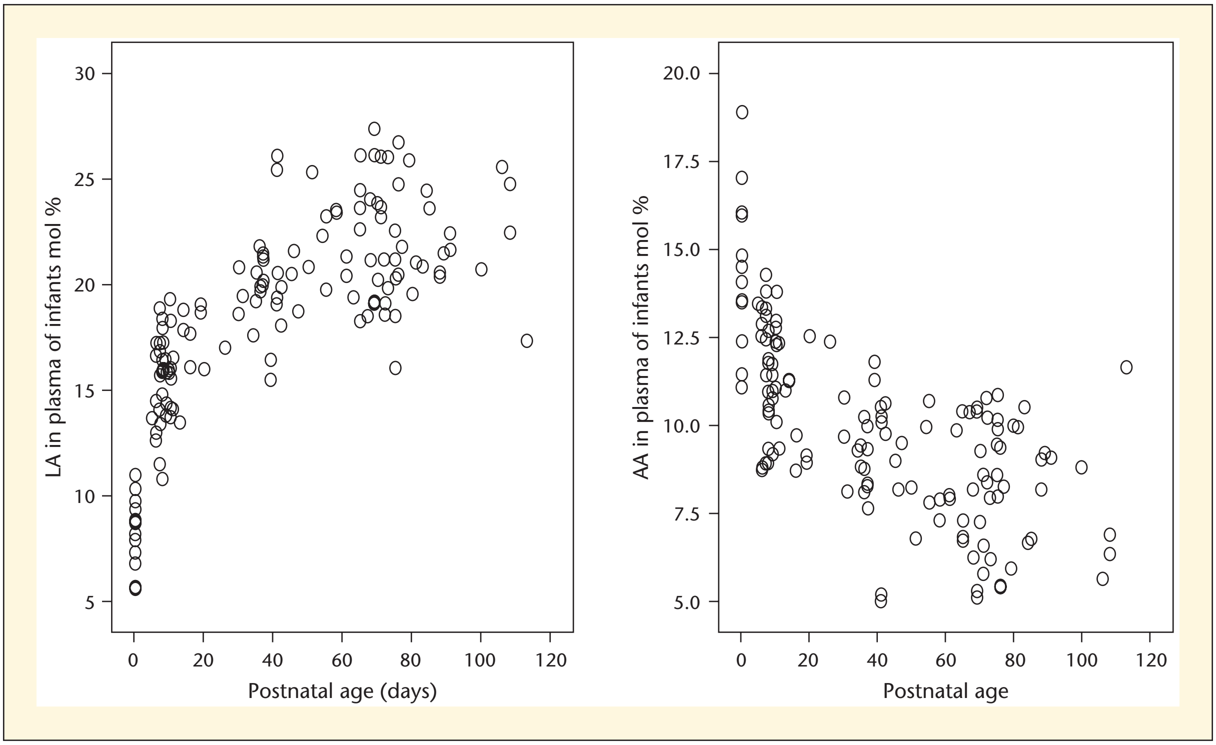

Figure 2. *The relation between linoleic acid (left) and arachidonic acid (right) and postnatal age in premature infants born between 25 and 36.9 weeks of gestation (Data from Sabel* et al.*, 2009).*

The GM quality at 40 weeks was inversely associated to the ratio n-6/n-3 in breast milk, and in multiple regression analysis to the LA/ALA ratio (β-0.64, $p < 0.001$). GM quality was also negatively correlated to the ratio of ETA/AA in breast milk and in multiple regression analysis to the Mead acid concentration (β-0.51, $p = 0.001$). Motor assessment according to BNBAS was in multiple regression analysis negatively correlated to the n-6/n-3 ratio in infants' early plasma phospholipids (β-0.39, $p = 0.016$) and to the AA/DHA ratio in infants plasma at 44 weeks (β-0.46, $p = 0.016$). The only

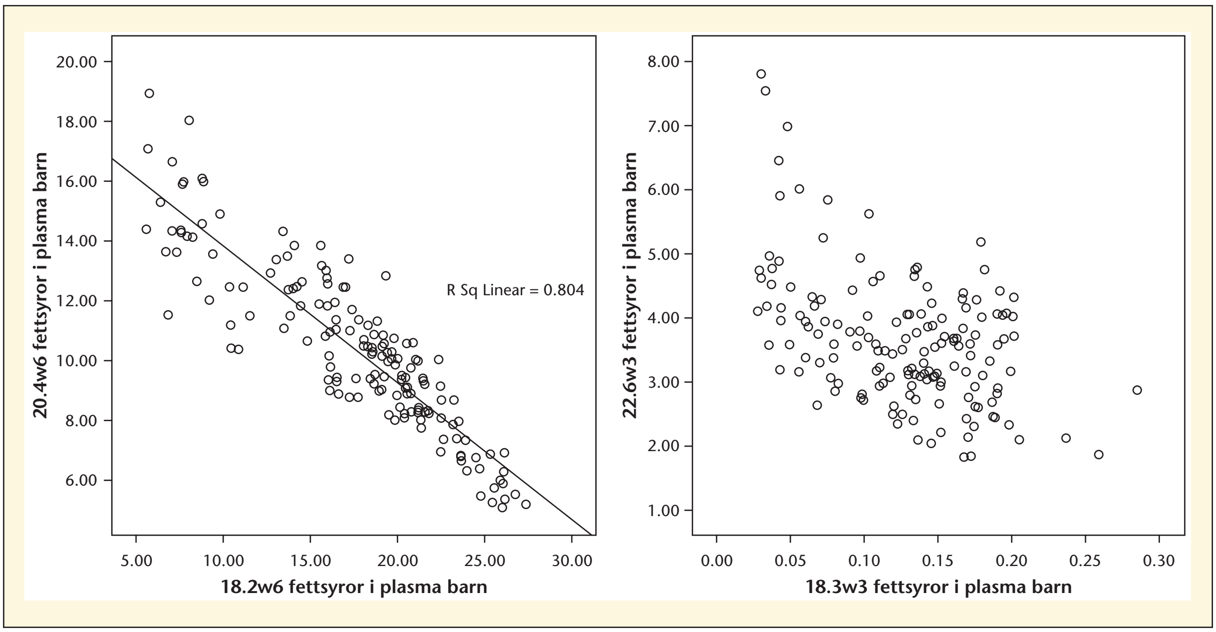

Figure 3. *The relation between linoleic acid and arachidonic acid (left) and between alpha-linolenic acid and docosahexaenoic acid (right) in infants' plasma phospholipids at one week of age (Data from Sabel* et al.*, 2009).*

positive correlation at that early age was found between autonomic stability (BNBAS) at 40 weeks and the P/S ratio (β-0.43, p = 0.009) and the w6 E% (β-0.84, p = 0.001) in mothers food intake. Positive association was also found between the change in autonomic stability between 40 and 44 weeks and the EPA concentration in infants plasma (Lundqvist-Persson *et al.*, 2010). Girls progressed more than boys when comparing BNBAS testing at 40 and 44 weeks.

Similar pattern, i.e. negative associations between GM quality and BNBAS items and different measures of n-6 fatty acids, was found in the Bayleys' Scales at 3 to 18 months of corrected age (Sabel *et al.* to be published). Positive association to DHA concentrations was only found in tests after 6 months of age and the strongest correlation was found to mental development at 18 months (β 0.417 (p = 0.004), after adjustment for confounders β 0.805, p = 0.038, R^2 0.50. Mothers' education was the strongest influencing confounding factor in all analyses. About 20-50% of the developmental measures could be explained by the early essential fatty acid and LCPUFA concentrations adjusted for the confounding factors.

In summary, there was a consistent trend that high n-6 fatty acids in early life were negatively correlated to motor and mental development up to 18 months. Since the general intake of n-3 fatty acids in the mothers was very low, it cannot be excluded that the negative influence of n-6 fatty acids more reflected the imbalance between the essential fatty acids than a pure negative effect of the n-6 fatty acids. On the other hand the inverse relation between linoleic and arachidonic acids might be of concern since arachidonic acid is important for early brain development (Schuchardt *et al.*, 2010). The expected positive influence of DHA was first seen after 6 months of age. Our results have to be confirmed in larger studies in both premature and term infants, also in view of the relatively high ratio of n-6/n-3 fatty acids in formula, which might be necessary to reconsider.

REFERENCES

Barker DJP, Cole O. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* 1986;1077-81.

Bayley N. *Manual for the Bayley scales of infant development.* 1993. 2nd Ed. The Psychological Corporation, San Antonio, US.

Brazelton TB, Nugents JK (eds). *Neonatal Behavioral Assessment Scale.* 1995. 3rd Ed Lippincott, Philadelphia, US.

Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJ. Early adiposity rebound in childhood and risk of Type 2 diabetes in adult life. *Diabetologia* 2003; 46: 190-4.

Forsdahl A Are poor living conditions in childhood and adolescence an important risk factor for arteriosclerotic heart disease? *Br J Prev Soc Med* 1977; 31: 91-5.

Friesen RW, Innis S. Linoleic acid is associated wtih lower long-chain n-6 and n-3 fatty acids in red blood cell lipids of Canadian prgnant women. *Am J Clin Nutr* 2010; 91: 23-31.

Hadders-Algra, M. Evaluation of motor function in young infants by means of the asssessment of general movements: a review. *Pediatr Phys Ther* 2001; 13: 27-36.

Korotkova M, Gabrielsson B, Holmäng A, Larsson BM, Hansson LÅ, Strandvik B. Gender related long term effects in adult rats by perinatal dietary ratio on n-6/n-3 fatty acids. *Am J Physiol Regul Integr Comp Physiol* 2005; 288: R575-9.

Langley-Evans S (Ed). Fetal Nutrition and Adult Disease. Programming of chronic disease through *fetal exposure to undernutrition.* Frontiers in nutritional science 2004. No 2.CABI Publishing, Cambridge, UK.

Liou YA, Innis S. Dietary linoleic acid has no effect on arachidonic acid, but increases n-6 eicosadiewnoic acid, and lowers dihomo-gamma-linolenic and eicosapentaenoic acid in plasma of adult men. *Prostaglandins Leukotrienes and Essential Fatty acids* 2009; 80: 201-6.

Lundqvist-Persson C. The newborn infant, capable and vulnerable: an interactional perspective. Thesis. 2000 Lund University, Lund, Sweden.

Lundqvist-Persson C, Lau G, Nordin P, Strandvik B, Sabel K-G. Early behaviour an development in breast-fed premature infants are influenced by omega-6 and omega-3 fatty acid status. *Early Hum Develop* 2010; 86: 407-12.

Raju T. The problem of late-preterm (near-term) birth: a workshop summary. *Pediatr Res* 2006; 60: 775-6.

Sabel K-G, Lundqvist-Persson C, Bona E, Petzold M, Strandvik B. Fatty acid patterns early afte premature birth, simultaneously analysed in mothers' food, breast milk and serum phospholipids of mothers and infants. *Lipids Health Res* 2009; 8: 20.

Sebert S, Sharkey D, Budge H, Symonds ME. The early programming of metabolic health: is epigenetic setting the missing link? *Am J Clin Nutr* 2011; 94: 1953S-8S.

Spector AA, Kaduce T, Hoak JC, Fry GL. Utilization of arachidonic and linoleic acids by cultured human endothelial cells. *J Clin Invest* 1981; 68: 1003-11.

Schuchardt JP, Huss M, Stauss-Grabo M, Hahn A. Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and abehaviour of children. *Eur J Pediatr* 2010; 169: 149-64.

Tamashiro KLK, Moran TH. Perinatal environment and its influences on metabolic programming of offspring. *Physiol Beh* 2010; 100: 560-6.

Tobi EW, Lumey LH, Talens RP *et al.* DNA methylation differences after exposure to prenatal famine are common and timing-and sex-specific. *Hum Mol Gen* 2009; 18: 4046-53.

Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr* 2007; 27: 363-88.