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# Recruitment of mothers with infants in an intervention trial – initial findings from the PINGU-study[1]

## PINGU – Multimodal optimisation of the dietary supply of infants with polyunsaturated fatty acids in complementary food: background and project structure

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## Summary

The (partially) double-blinded, randomised, controlled intervention trial PINGU investigates for the first time the effects of an optimised omega-3 fatty acid complementary food on fatty acid status, as well as on the cognitive and visual development of infants in the second half of the first year of life.

Subject recruitment took place both directly and indirectly and lasted one and a half years. Only German speaking adult mothers were recruited who lived in the area of Dortmund and who had a healthy term new born infant.

Recruitment was more time consuming than in the preceding DINO study, as only 1 in 14 of the mothers asked was willing to participate in the PINGU-study. Apparently the most effective recruitment method is directly to address women in childbed at maternity clinics, followed by a phone call follow-up.

**Keywords:** recruitment, intervention studies, infants, mothers, complementary food, infant nutrition

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## Introduction

Successful recruitment of study subjects needs a comprehensive recruitment plan which is specifically adapted to the study population. For the nutrition intervention trial PINGU on the optimisation of omega-3 fatty acid supply in complementary food, mothers with young infants were recruited through both direct and indirect pathways. The present article describes the challenges of the recruitment period and presents the apparent influence factors.

### Background

There has been increasing scientific interest in recent years in long-chain polyunsaturated fatty acids (LC-PUFA). Apart from preventive effects in adulthood, e.g. the reduction of coronary heart disease mortality [1], there is also evidence for beneficial impacts of LC-PUFA supply – primarily docosahexaenoic acid (DHA) – in the prenatal and postnatal period on infant's cognitive and visual development [2–4]. As infants grow rapidly – particularly the brain –, the DHA requirement is specifically high.

In the first 4–6 months of life, milk (breast milk and/or enriched formula) is the exclusive exogenous source of LC-PUFA for infants. Complementary food that is introduced in the beginning of months 5 to 7 normally contains low levels of LC-PUFA. As a consequence, LC-PUFA supply decreases in the second six months of life.

LC-PUFA may not only be supplied in the preformed state, but can be synthesised endogenously from the essential PUFAs linoleic acid (LA) and α-linolenic acid. This conversion re-

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quires desaturation and elongation reactions, catalysed by specific enzymes. There is evidence that even infants are capable of performing this synthesis [5, 6].

If the ratio n-6 (LA):n-3 (ALA) PUFA is favourable, the synthesis of n-3-LC-PUFA and thus of DHA is supported [7]. Therefore, if cooking oil, e.g. rapeseed oil with a favourable n-6/n-3 PUFA ratio (2:1), and fatty fish, e. g. salmon with preformed DHA, are used, this might be an alternative or additional strategy for DHA supply in the complementary feeding period, in which the requirements for brain growth are still relatively high.

There have not yet been any conclusive results to what extent the nutritional supply of omega-3 fatty acids in complementary food influences the fatty acid status in the blood and thus the development of the infant [8–11]. In an earlier double-blinded, randomised, controlled intervention trial, DINO (Dortmund Intervention Trial for Optimization of Infant Nutrition), conducted by the Research Institute of Child Nutrition FKE a positive correlation was found for the first time between the addition of rapeseed oil to complementary food and the plasma DHA values of infants – in comparison with the addition of corn oil, a n-6 PUFA rich oil [11].

The aim of the PINGU-study (**P**oly-unsaturated fatty acids **in** child nutrition – A **G**erman m**u**ltimodal optimisation study) – a (partially) double-blinded controlled intervention trial – is to verify these results with additional biomarkers and to investigate the supply of preformed DHA via fish as an alternative strategy.

The study has been in the field period since April 2011. Most time has been spent on recruiting the participants, which has been made more difficult by limiting factors. The present article maps the course of recruitment on the basis of initial data from the recruitment period. Factors are identified that might influence the success of recruitment in a nutrition intervention trial in infants.

## Project structure and research questions

PINGU is a multimodal, interdisciplinary collaborative research project with research partners from nutrition science, paediatrics and psychology[2] and consists of main and secondary studies to examine primary and secondary questions (◆ Figure 1). A positive vote of the Ethics Committee of the Rheinische Friedrich-Wilhelm-Universität Bonn was obtained (Lfd. Nr. 282/10).

### Intervention design

The study includes three study groups with different nutrition strategies: The "rapeseed oil group" and the "fish group" are the intervention groups and the "corn oil group" is the control group. Study food are commercial meals containing meat or fish (HiPP GmbH & Co. Vertrieb KG). The "rapeseed oil group" and the "corn oil group" only differ with respect to the sort of added cooking oil (rapeseed oil, rich in ALA, or corn oil, rich in LA). The "fish group" receives the same menus as the "corn oil group", but two fish menus per week (salmon, rich in DHA) replace two meat menus.

Study participation starts with the mother's written consent, that must be given within eight weeks of the birth. The nutrition intervention starts with the introduction of the complementary food in the period between the beginning of the 5th and 7th months of the child's life and ends with the transition to family food at the end of the 10th month. Therefore, the intervention period lasts 4 to 6 months.

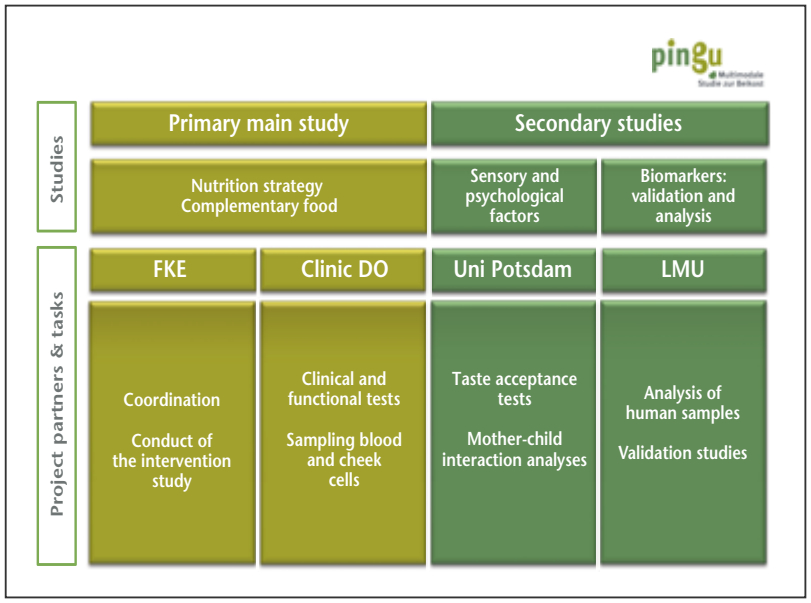


Fig. 1: **Collaborative structures in the PINGU Study 2010–2013**
FKE = Research Institute of Child Nutrition; Clinic DO = Clinic Dortmund; Uni Potsdam = University of Potsdam; LMU = Ludwig-Maximilians-Universität Munich

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## Study program

A special consultation has been set up for the PINGU-study in the Paediatric Clinic Dortmund. In the course of the medical examinations, samples of blood and cheek cells are taken from the infant and mother, in order to determine the current fatty acid status and any genetic polymorphisms.

Samples from the mother are taken at the first appointment in the clinic ($T_0$) at the end of the child's 8th week of life. This also includes a breast milk sample in order to estimate fatty acid supply to the infant. Besides maternal food consumption during pregnancy and postpartum as well as sociodemographic data are recorded. Immediately after this appointment, the infant's nutrition is recorded in daily weighted dietary records. (◆ Figure 2)

The paediatric examinations ($T_1$; $T_2$) serve to monitor physical development. Functional examinations are conducted at the same time, covering visual development with visual evoked potentials (VEP) and cognitive development with the age-specific Bayley II Test. (◆ Figure 3)

House visits ($H_1$; $H_2$) take place during the 4th and 7th months of the child's life. The study personnel deliver the study food (baby menus month 4, junior menus month 7) and advise the mother about current recommendations for infant nutrition.

Data in the secondary psychological sensory study are recorded in accordance with the nutrition intervention program. Taste acceptance tests and a mother-child interaction analysis of the feeding situation under video observation are conducted on a subsample immediately after the child is habituated to solid food. All the mothers are given a set of self-administered questionnaires to investigate their attitudes e.g. to fish.

## Research questions

### *Primary questions*

In relation to the efficacy of the three different nutrition strategies in the complementary food period (see intervention design and study program) – data base: main study on nutrition strategies (biomarkers and functions):

- If there is a favourable n-3-PUFA supply (rapeseed oil), can the DHA status be significantly improved by enhanced endogenous synthesis of n-3-LC-PUFA in infants, allowing for polymorphisms (SNPs) in the genes of enzymes for fatty acid metabolism (FADS1 and 2)?
- What are the effects on the fatty acid status of infants when they receive preformed n-3-LC-PUFA via salmon?
- What effects do these alternative nutrition strategies of fatty acid supply have on the cognitive development and the visual acuity of the infants?

### *Secondary questions*

In relation to the feasibility of the alternative nutrition strategies for consumers – data base: secondary psychological and sensory studies and a representative population survey:

- What effect does fish consumption have on taste development in infancy?
- What effect do maternal attitudes and control styles have on fatty acid-optimised food selection in complementary food?

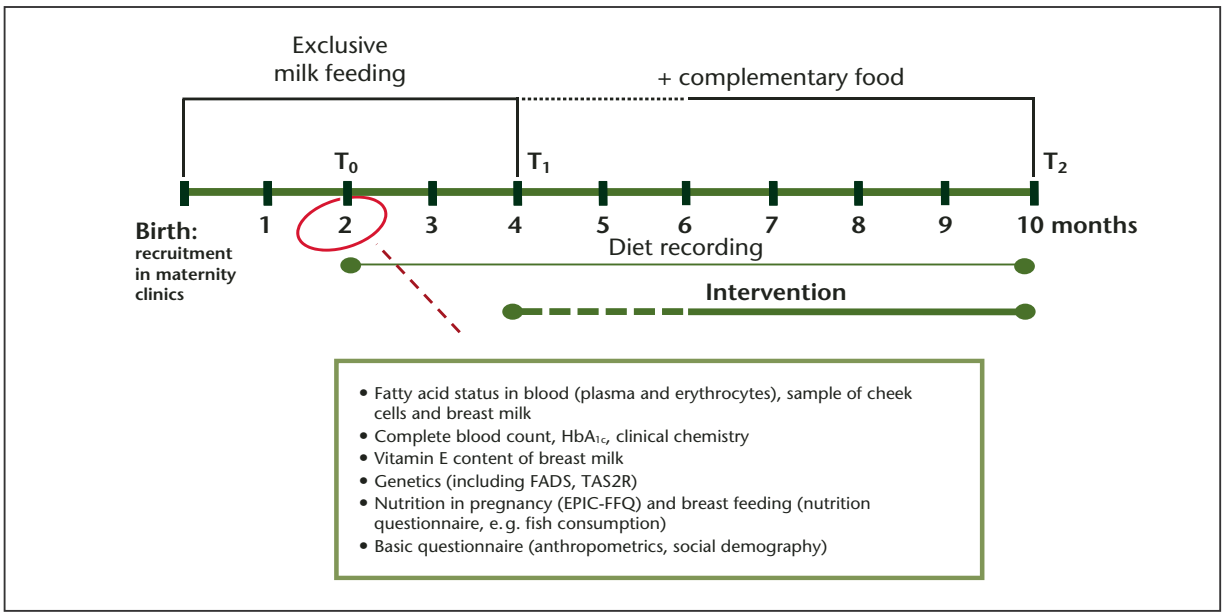


Fig. 2: **Study program mother ($T_0$): overview of the parameters to be recorded at the end of the child's second month**
EPIC-FFQ = European Prospective Investigation into Cancer and Nutrition-Food Frequency Questionnaire
FADS = fatty acid desaturase; TAS2R = taste 2 receptor (bitter taste receptor)
$T_0$ = mother's hospital appointment; $T_1$ and $T_2$ = child's hospital appointments

– What are the attitudes, behaviour patterns or knowledge of young mothers in relation to these nutrition strategies?

In relation to the validation of biomarkers – data base: secondary methodological and analytical study:
– Are cheek cells suitable as non-invasive biomarker for the fatty acid status and for the detection of potential intervention effects in comparison to the established biomarkers of erythrocytes and plasma?

***Recruitment of the study population***

The following recruitment criteria apply for PINGU:

Mother:
– adequate knowledge of German
– mother must be of age
– residence in the Dortmund area

Child:
– healthy, term newborn
– single birth

The women in childbed and their neonates were recruited by personal contact with the study personnel in 9 maternity clinics in Dortmund and region. If the mothers were interested, they were given additional printed information material. The objective of this first contact step was to obtain the contact data of mothers who were interested in principle. They were then contacted again by e-mail and telephone in the period up to the 8th week of the child's life.

An *a priori* power analysis indicated that the sample size should be 189 (including 10 % drop-out). The recruitment period was originally planned to last one year and started as planned in mid April 2011. As few mothers were willing to participate, recruitment was extended through the end of October 2012.

## Initial recruitment results

***Recruitment in maternity clinics***

In the recruitment period between April and November 2011, 1,100 women in childbed were adressed. 30 % of these mothers provided their contact data, whereof 24 % stated that they were willing to participate in the study. Thus, approximately 1 mother in 14 of those adressed in the maternity clinics agreed to participate in the study. The mothers who provided no contact data (70 %) either failed to fulfil the criteria of the study protocol (e. g. intention to prepare the complementary food themselves) or were not interested. Some mothers who did not provide their contact information were nevertheless interested in obtaining printed study information material (n = 181). However, none of these mothers contacted the study personnel again. (◆ Figure 4)

24 % of the mothers who provided their contact data also provided their written consent to participation by the 8th week of their child's life. 39 % of the other mothers (76 %) could not be reached through a phone call, announced in advance, about 6 weeks after the birth (pp). 61 % refused to participate when telephoned.

The main reason for this refusal was that the study was very demanding or the study program was too extensive (49 %). A smaller proportion of the mothers (25.5 %) either could

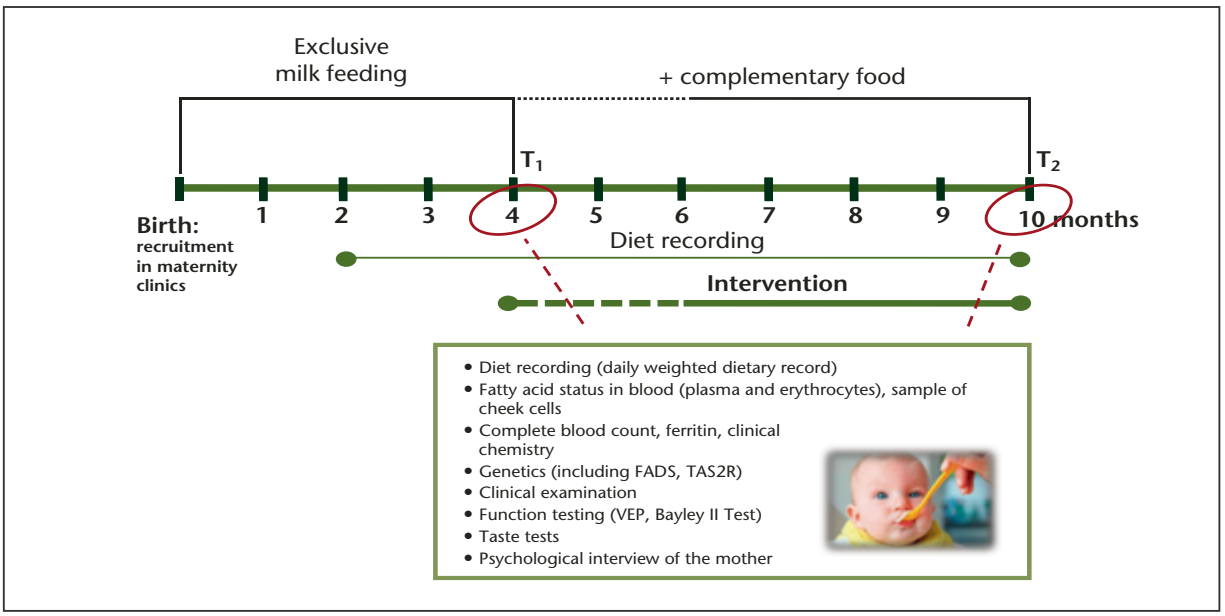


Fig. 3: **Study program for the child ($T_1$; $T_2$): overview of the parameters to be recorded at the end of the 4th and 10th months of the child's life**
FADS = fatty acid desaturase; TAS2R = taste 2 receptor (bitter taste receptor)
$T_0$ = mother's hospital appointment; $T_1$ and $T_2$ = child's hospital appointments

not or would not comply with the instructions of the study protocol (e. g. intention to prepare the complementary food themselves), were generally uninterested (14.5 %) or lived too far away (11 %). (◆ Figure 5)

***Indirect recruitment by health care professionals or the media***

Other methods of contacting the mothers were tried because direct recruitment in the hospital was difficult and the period for recruitment was limited in the study design.

- Birth preparation courses, mother and child groups and midwives were visited by the study personnel and e. g. PINGU flyers were distributed.
- Gynaecologists and paediatricians were personally informed by study management and were given PINGU flyers to pass on to potentially suitable mothers.
- The public was informed in a press report to the local media (newspapers, radio) and through a PINGU website (www.pingu-studie.de).

As these recruitment pathways were implemented in parallel, it is difficult to quantify them. However, the success was very poor. There were only enquiries from few interested mothers immediately after the announcement in the local media.

## Discussion

Hospital recruitment of women in childbed was found to be much more difficult with the PINGU-study than with the preceding DINO study. In contrast to the DINO study in which 1 in 7 adressed women in childbed agreed to take part, twice as many women had to be contacted for the PINGU-study. In the DINO study, five appointments were planned for the volunteers – three hospital appointments with blood sampling and two house visits for distributing the study food. In the PINGU-study, there was a total of seven appointments - three hospital visits, two appointments in the FKE and two house visits. In both studies, the infant's diet had to be recorded daily from the age of 8 weeks.

The main reason for the greater recruitment effort in the PINGU-study was that the study program was more extensive, and this was frequently given as a reason not to participate. In addition, mothers interested in nutrition and therefore potentially interested in participating in studies already have fixed ideas about handling complementary food these days. They stated that the reason they refused to participate was that they intended to prepare the complementary food themselves and/or to use rapeseed oil or fish.

Additional attempts in the surroundings of young parents were not successful in the recruitment period evaluated here. One reason for this might be that recruiting by health care professionals during prenatal and postnatal medical care requires additional time and cannot be accounted as part of medical care. Only a few interested mothers could be reached through courses for (future) mothers. This might be because it is difficult to discuss the trial and to record contact data during a course or that it is too early to discuss the study during pregnancy.

The last section of the recruitment period was more successful, as mothers who were already taking part were able to motivate their (pregnant) acquaintances with their favourable experiences. The announcement in the local media also excited interest, even though only a few mothers then expressed interest in participating in the study.

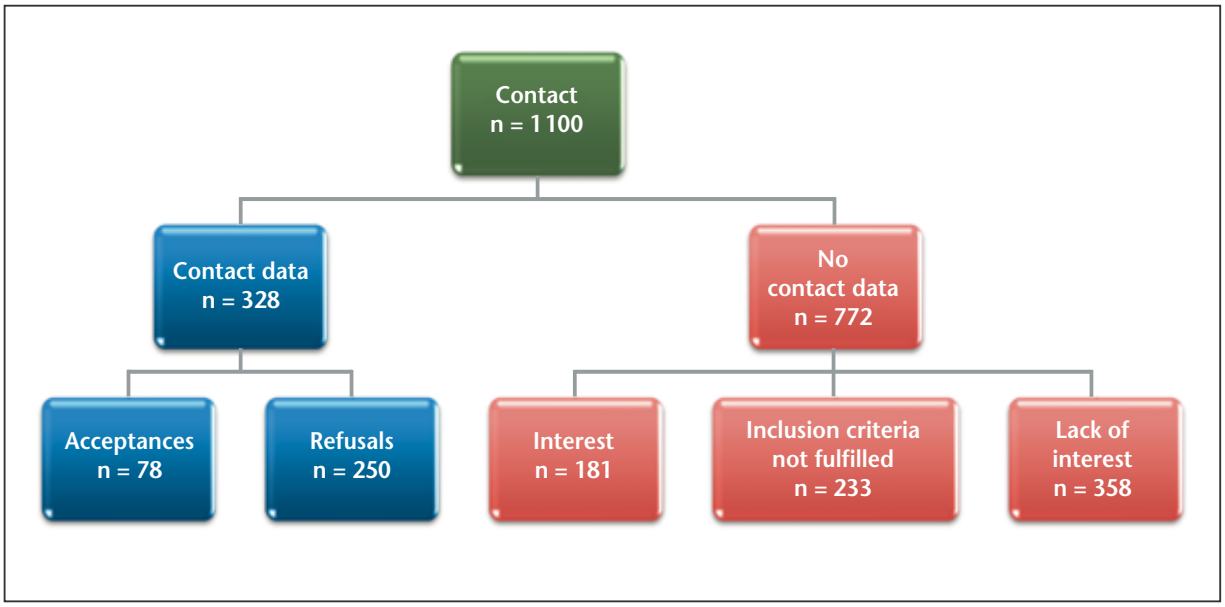


Fig. 4: **Recruitment results after contacting women in childbed in the hospital** (April–November 2011)

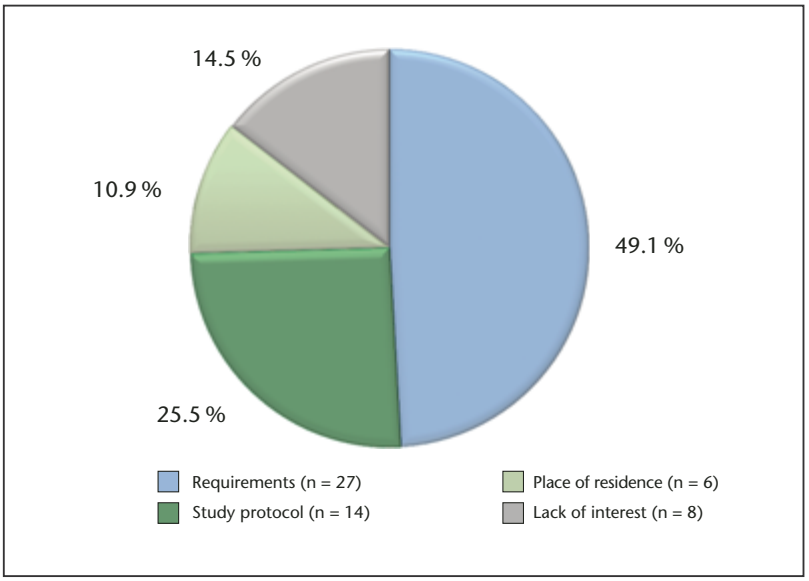


Fig. 5: **Reasons that mothers who were initially interested refuse to take part in the study when contacted by phone ca. 6 weeks after the birth** [n = 55]

## Conclusions

Taken together, the present results on the recruitment for the PINGU-study indicate that, in nutrition intervention trials with infants, it is still sensible to contact women in childbed directly, followed by a telephone call. Although this requires considerable effort for the study personnel, it provides early quantitative information for subsequent study planning and supports the decision whether additional recruitment measures are needed. Even though the additional involvement of the rest of the health care service in contact with young families failed to achieve short term increases in recruitment, it can encourage local support for the study in synergy with the local media. However, a great deal of patience is needed before word of mouth takes effect. Moreover, the results on the PINGU recruitment indicate that it is desirable to reduce the scope of the study program as far as it is scientifically defensible. Once cheek cells have been validated as a non-invasive biomarker for fatty acid supply in the PINGU-study, it will, for example, become clear whether it will be possible to dispense with taking a blood sample for specific questions.

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**Conflict of Interest**
The authors declare no conflict of interest according to the guidelines of the International Committee of Medical Journal Editors.

## References

1. *Deutsche Gesellschaft für Ernährung (Hg). Evidenzbasierte Leitlinie Fettkonsum und Prävention ausgewählter ernährungsmitbedingter Krankheiten. Bonn (2006)*
2. *Campoy C, Escolano-Margarit MV, Anjos T et al. (2012) Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. Br J Nutr 107 Suppl 2: 85–106*
3. *Simmer K, Patole SK, Rao SC (2011) Long-chain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database Syst Rev 7(12): CD000376*
4. *Eilander A, Hundscheid DC, Osendarp SJ et al. (2007) Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids 76(4): 189–203*
5. *Uauy R, Mena P, Wegher B et al. (2000) Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. Pediatr Res 47: 127–135*
6. *Salem N Jr, Wegher B, Mena P et al. (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl Acad Sci USA 93: 49–54*
7. *Makrides M, Neumann MA, Jeffrey B et al. (2000) A randomized trial of different ratios of linoleic to alpha linolenic acid in the diet of term infants: effects on visual function and growth. Am J Clin Nutr 71(1): 120–129*
8. *Luukkainen P, Salo MK, Visakorpi JK et al. (1996) Impact of solid food on plasma arachidonic and docosahexaenoic acid status of term infants at 8 months of age. J Pediatr Gastroenterol Nutr 23: 229–234*
9. *Hoffman DR, Theuer RC, Castaneda YS et al. (2004) Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. J Nutr 134: 2307–2313*
10. *Makrides M, Hawkes JS, Neumann MA et al. (2002) Nutritional effect of including egg yolk in the weaning diet of breast-fed and formula-fed infants: a randomized controlled trial. Am J Clin Nutr 75: 1084–1092*
11. *Schwartz J, Dube K, Sichert-Hellert W et al. (2009) Modification of dietary polyunsaturated fatty acids via complementary food enhances n-3 long-chain polyunsaturated fatty acid synthesis in healthy infants: a double blinded randomised controlled trial. Arch Dis Child 94(11): 876–882*

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