

1 *Koletzko B. Should Women Providing Milk to Their Preterm Infants Take Docosahexaenoic*
2 *Acid Supplements? Clin Perinatol. 2017 Mar;44(1):85-93. doi: 10.1016/j.clp.2016.11.002.*
3 *Review. PubMed PMID: 28159211.*

4

5 **Should women providing milk to their preterm infants take DHA supplements?**

6 Berthold Koletzko

7 Ludwig-Maximilians-Universität Munich

8 Div. Metabolism and Nutritional Medicine, Dr. von Hauner Childrens Hospital, University of
9 Munich Medical Center, Munich, Germany

10

11

12 Author's contact details:

13 Berthold Koletzko, Professor of Paediatrics

14 Ludwig-Maximilians-Universität Munich

15 Dr. von Hauner Children's Hospital, Univ. of Munich Medical Center, Campus Innenstadt

16 Lindwurmstr. 4, D-80337 Muenchen, Germany

17 Tel: +49 89 44005 2826, Fax: +49 89 44005 7742

18 E-mail: office.koletzko@med.lmu.de

19

20 **Key words:**

21 Arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, polyunsaturated fatty acids,
22 very low birthweight infants

23

24 **Key points:**

- 25 • Human milk globally has an average DHA content of about 0.3 % of fatty acids, with
26 large variation primarily due to different maternal DHA intakes from fish and seafood

- 27 • Breastfeeding usually meets DHA needs of term (100 mg/d) but not the much higher
28 requirements very low birthweight infants (VLBWI)
- 29 • To match intrauterine DHA accretion, VLBWI require a human milk DHA content of
30 about 1% that can be achieved by maternal supplementation with 3 g/d tuna oil
- 31 • A high milk DHA supply to VLBWI may enhance early visual and cognitive
32 development and reduce adverse events such as severe developmental delay,
33 bronchopulmonary dysplasia, necrotizing enterocolitis and allergies
- 34 • The supply, metabolism and biological effects of the omega-3 (n3) and the omega-6
35 (n-6) essential polyunsaturated fatty acids (PUFA) during pregnancy, infancy and
36 childhood has received considerable attention and has been addressed in numerous
37 research studies, as recently reviewed (1-4). The essential fatty acids omega-6 (n-6)
38 linoleic acid (18:2n-6, LA) and omega-3 (n-3) alpha-linolenic acid (18:3n-3, ALA)
39 found in plants and vegetable oils are the precursors of the biologically active long-
40 chain polyunsaturated fatty acids (LC-PUFA). The quantitatively pre-dominant LC-
41 PUFA are n-6 arachidonic acid (ARA, 20:4n- 6), n-3 eicosapentaenoic acid (20:5n-3,
42 EPA) and n-3 docosahexaenic acid (DHA, 22:6n-3). Both during pregnancy and
43 infancy, n-6 and n-3 LC-PUFA are accreted in relatively large amounts in fetal and
44 infant tissues. Particularly high concentrations are found in the brain grey matter and
45 in the rod outer segments of the retina and have been related to functional
46 development such as cognition and visual acuity (4). Some LC-PUFA, including n-6
47 dihomo-gamma-linolenic acid (20:3n-6), ARA, EPA and DHA also serve as
48 precursors of eicosanoids and docosanoids, such as prostaglandins, prostacyclins,
49 leukotrienes and resolvins. In low concentrations, eicosanoids and docosanoids are
50 powerful regulators of numerous physiological processes such as cardiovascular
51 function and the early postnatal closure of the ductus arteriosus Botalli, thrombocyte
52 aggregation and bleeding time, inflammation and immunity, and others. In fact, the
53 early availability of LC-PUFA has been associated with the immune functions and the
54 likelihood of the development of allergies and infections (1).

55 LC-PUFA can either be provided preformed via the placenta or the dietary sources such as
56 human milk, or they can be endogenously synthesized from the precursors LA and ALA by
57 consecutive desaturation and chain elongation. However, in humans the conversion rates
58 are rather low. It was estimated that only 0.1% to 10% of the precursor fatty acids are
59 converted to LC-PUFA, with a particularly low rate of synthesis for DHA (5-7). The rates of
60 conversion are also very variable depending on genotypes of the fatty acid desaturase
61 (*FADS*) gene cluster. Individuals with certain genetic haplotypes have extremely low rates of
62 ARA and DHA synthesis and thus depend even more on the supply of preformed LC-PUFA
63 to maintain plasma and tissue levels (1, 8-12). In infants, and particularly in preterm infants,
64 the rates of parent PUFA conversion to LC-PUFA are considered insufficient to achieve
65 biochemical and functional normality (13, 14).

66 *In utero*, ARA and DHA are supplied preformed to the fetus by way of an active and
67 preferential materno-fetal transport across the placenta that we have measured *in vivo* using
68 fatty acids labelled with stable isotopes (15, 16). The underlying mechanisms of this active
69 materno-fetal LC-PUFA transfer have been partly explored (17-19). Fatty acids from
70 maternal lipoproteins are released by two lipases expressed in placental tissue, lipoprotein
71 lipase (LPL) and endothelial lipase (EL). LPL hydrolyses triglycerides, whereas EL is a
72 phospholipase with little triacylglycerol lipase activity. EL continues to be expressed towards
73 the end of pregnancy, while LPL is virtually absent in the trophoblast. In addition, maternal
74 circulating NEFA can be directly taken up by placental tissue. The binding and transfer of
75 released fatty acids is mediated by membrane-bound proteins expressed in the trophoblast,
76 including FABPpm (fatty acid binding protein plasma membrane), p-FABPpm (placental
77 plasma membrane fatty acid-binding protein), FAT/CD36 (fatty acid translocase) and FATP
78 (fatty acid transport proteins) -1 to -6 (17, 19). In the cytosol, fatty acids are bound to fatty
79 acid binding proteins (FABPs) leading to interaction with subcellular organelles, including the
80 endoplasmic reticulum, mitochondria, lipid droplets and peroxisomes. FABPs are also likely
81 to function in the nucleus through the delivery of specific ligands to nuclear transcription
82 factors, such as the peroxisome proliferator-activated receptors (PPAR). This complex

83 system achieves an active placental materno-fetal transfer of ARA and particularly of DHA.
84 Given that intrauterine growth and body composition is generally considered the reference
85 that postnatal care of preterm infants should match as much as feasible, it appears prudent
86 to aim at approaching the degree of intrauterine provision of preformed LC-PUFA with
87 postnatal nutritional regimens.

88 After birth, breastfed infants always receive preformed ARA and DHA with human milk lipids.
89 The milk fatty acid composition is modified by maternal diet, lipolysis of body fat stores that
90 markedly contribute to milk fat synthesis, maternal genotype, and stage of lactation (11, 20,
91 21). Around the world, human milk provides a relatively stable ARA supply around 0.5 % of
92 milk fatty acids, whereas DHA is found at a mean level of 0.3 % but shows much more
93 variation primarily due to differences in maternal intake of dietary DHA sources such as fish
94 and seafood (21, 22). Full breastfeeding usually meets the recommended intakes for term
95 born infants of 140 mg ARA/day and 100 mg DHA/day (1, 23) but not the higher
96 recommended intakes for preterm infants. Although the milk of mothers of preterm women
97 contains slightly higher amounts of LC-PUFA (24), the recommended DHA supply of very low
98 birthweight infants can only be met through human milk if women obtain a markedly
99 increased DHA intake. The human milk DHA content is linearly related to the maternal DHA
100 intake, as we documented in a supplementation study of well-nourished mothers who fully
101 breastfed their infants born at term (25)

102

103 LC-PUFA supply to preterm infants

104 Increasing DHA provision to preterm infants through DHA supplements to the lactating
105 mother needs to be justified by indications for a benefit for clinical outcome. The recent
106 systematic review of the Early Nutrition Academy on the roles of pre- and postnatal long-
107 chain polyunsaturated fatty acids (LC-PUFA) included studies in preterm infants published
108 until 2013 (1). The LC-PUFA provision to preterm infants was also evaluated in a meta-
109 analysis of available studies (26) and in recent reviews (2, 27). Most of the available studies

110 in preterm infants evaluated DHA supplies with human milk or formula of about 0.2-0.3 % of
111 fat, as often provided to healthy infants born at term. However, this level of supply is not
112 sufficient to achieve the estimated daily intrauterine deposition of DHA of 43 mg/kg body
113 weight, which occurs along with an even high ARA deposition of about 212 mg/kg (28). It has
114 been estimated that the intrauterine DHA accretion rate may be matched postnatally in
115 preterm infants by DHA provision amount to 1% of human milk or formula fat (29). This
116 approach has been evaluated in randomized trials that studied the addition of LC-PUFA-rich
117 oils to human milk (30) or the supplementation of mothers providing human milk with LC-
118 PUFA-rich marine oil (31-34).

119

120 *Visual and cognitive development*

121 The Cochrane meta-analysis by Schulzke et al found no benefit of an added LC-PUFA
122 supply to preterm infants on cognitive outcomes at the age of 12 to 18 months in 4 out of 7
123 studies included in this meta-analysis (26). Of interest, the three studies that found such
124 benefits used the newer version II of the “*Bayley Scales of Infant Development*”, which raises
125 the question whether the older version of the Bayley test or other tests might not be as
126 sensitive to detect effects. Visual acuity was not found to be influenced by LC-PUFA supply
127 (26).

128 Of particular interest are the findings of two large studies providing much higher DHA
129 amounts with milk and hence approaching more closely the levels of intrauterine supply.
130 Henriksen et al (2008) randomized 141 very low birthweight infants to an intervention adding
131 an LC-PUFA-rich algal oil, mixed with soy oil and medium-chain triglyceride oil. The
132 intervention provided an added 32 mg of DHA and 31 mg of ARA per 100 mL of human milk
133 and started at one week after birth, with continuation until discharge from the hospital which
134 occurred on average after nine weeks. Cognitive development was evaluated at 6 months of
135 age by the “*Ages and Stages Questionnaire*” and event-related potentials, a measure of
136 brain correlates related to recognition memory. At the 6-month follow-up, the authors found a

137 better performance of the intervention group on the problem-solving subscore, compared
138 with the control group (53.4 vs 49.5 points). There was also a nonsignificant trend to a higher
139 total score (221 vs 215 points). The event-related potential data revealed that infants in the
140 intervention group had significantly lower responses after the standard image, compared with
141 the control group (8.6 vs 13.2). Further follow-up to the age of 8 years did not indicate any
142 significant differences at school age with regards to brain structure, cognition and behaviour
143 (35, 36).

144 Makrides and coworkers performed a very large randomized multicentre trial enrolling as
145 many as 657 preterm infants who were provided with a conventional (0.35 %) or high (1 %) DHA
146 supply from day 2-4 of life until term. The higher DHA provision was achieved by
147 supplementation of women providing human milk with a daily dose of 3 g of tuna oil, or by a
148 preterm formula with increased DHA content, along with about 0.5 % ARA (31-34). No
149 adverse effects of supplementation were observed in the infants. The higher DHA supply
150 improved visual acuity development at the corrected age of 4 months, with an acuity that was
151 1.4 cycles per degree higher than in the control group (Table 1) (37). The Bayley test of
152 infant development (version II) was applied at an age of 18 months, corrected for gestational
153 age. While no significant benefit of the intervention was detected in the total study
154 population, improved cognitive development was found in girls, and in the group of smaller
155 infants with a birthweight less than 1250 g (Table 1). Probably of even greater clinical
156 importance, the rate of children with severe developmental retardation (Mental Development
157 Index <70) was reduced by half (Table 1). Later follow-up to the age of 7 years did not
158 indicate any significant differences at school age with regards to cognition, behaviour and
159 visual function (38-40).

160

161 *Effects on other outcomes*

162 In the previously cited trial with 657 preterm infants provided with conventional (0.35 %) or
163 high (1 %) levels of DHA in the milk supplied, the occurrence of chronic lung disease

164 (bronchopulmonary dysplasia, BPD), defined by the need for oxygen treatment at a
165 postmenstrual age of 36 weeks, was reduced by high DHA supply in boys (relative risk [RR]:
166 0.67 [95% confidence interval (CI): 0.47-0.96]; P=.03) and in all infants with a birth weight of
167 <1250 g (RR: 0.75 [95% CI: 0.57-0.98]; P=.04) (32). Since this was a secondary endpoint, a
168 new trial to revisit this important effect has been initiated (41). A meta-analysis including 18
169 randomized controlled trials found that n-3 LC-PUFA supply was not associated with a
170 decreased risk of BPD in all studied preterm infants, but trials that included only infants born
171 at ≤ 32 weeks of found a trend toward reduced BPD (pooled RR 0.88, 95% CI 0.74-1.05, 7
172 studies, n = 1156 infants) along with a reduction in the risk of necrotizing enterocolitis (pooled
173 RR 0.50, 95% CI 0.23-1.10, 5 studies, n = 900 infants (42).

174 The preterm trial by Makrides et al. providing either 0.3 % or 1 % of milk fatty acids as DHA
175 also evaluated the incidence of atopic conditions up to the age of 18 months. (32). There was
176 no effect on duration of respiratory support, admission length, or home oxygen requirement.
177 There was a reduction in reported hay fever in all infants in the high-DHA group at either 12
178 or 18 months (RR: 0.41 [95% CI: 0.18-0.91]; P=.03) and at either 12 or 18 months in boys
179 (RR: 0.15 [0.03-0.64]; P=.01), while there was no effect on asthma, eczema, or food allergy.
180 A recent Cochrane review evaluated published data on effects of pre- or postnatal n-3 LC-
181 PUFA supplementation to women during pregnancy or the or breastfeeding period on allergy
182 outcomes in their children (43). Although the data were derived from a limited number of
183 informative randomized trials, an added n-3 LC-PUFA supply reduced the risk of any
184 medically diagnosed, IgE mediated allergy in children aged 12 to 36 months (risk ratio 0.66,
185 95% confidence interval 0.44 to 0.98) but not after the age of 36 months Food allergies were
186 no different after the age of 1 year, but a clear reduction was seen for infants. Also, medically
187 diagnosed IgE-mediated eczema was reduced by maternal n-3 LC-PUFA supply at the age
188 of 1-3 years but not at other ages (43). These data point to possible benefits of a higher n-3
189 LC-PUFA supply in early life on allergic and atopic disorders.

190

191 **Conclusion**

192 Based on the available data, a high milk DHA supply to very low birthweight infants and to
193 extremely low birthweight infants at levels that support tissue accretion rates similar to the
194 high rates of intrauterine deposition has the potential to enhance the early visual and
195 cognitive development, and to reduce the occurrence of adverse events such as severe
196 developmental delay, bronchopulmonary dysplasia, necrotizing enterocolitis and allergic
197 manifestations in infancy and early childhood. Lapillonne described the dose-response
198 relationship between the level of DHA in the milk provided to preterm infants and the
199 achieved mental development (Figure 2) and concluded that a DHA intake near 1 % of fatty
200 acid supply is desirable (44). Based on current knowledge, DHA should always be supplied
201 along with ARA, which human milk always provides (45).

202 Current recommendations stipulate for very low birthweight infants a daily DHA supply of at
203 least 18 mg but preferably 55-60 mg/kg bodyweight (≈ 1 % of total fatty acid supply) and a
204 daily ARA supply of at least 18 mg but preferably 35-45 mg/kg ($\approx 0.6-0.75$ %) (1, 27). It is
205 possible that subgroups of preterm infants achieve greater benefits, for example more
206 immature infants or those with lower birthweights, as well as infants with genotypes
207 predicting a low rate of endogenous LC-PUFA formation. Women providing milk to their very
208 low or extremely birthweight infants can achieve the desirable DHA content in their milk by
209 taking a supplement of 3 g tuna oil per day ($\approx 1\%$ of total fatty acid supply) (29).

210

211 **Acknowledgments:**

212 The work of the author is financially supported in part by the Commission of the European
213 Community, the 7th Framework Programme Early Nutrition (FP7-289346), the Horizon 2020
214 research and innovation programme DYNAHEALTH (No 633595), and the European
215 Research Council Advanced Grant META-GROWTH (ERC-2012-AdG – no.322605). This

216 manuscript does not necessarily reflect the views of the Commission and in no way
217 anticipates the future policy in this area.

218

219 **Table 1:** Providing preterm infants with milk with a higher DHA dose (about 1% of fatty acids,
 220 compared to 0.3%) improved early visual function and reduced markedly abnormal
 221 developmental outcomes at age 18 months (29). Later follow-up did not indicate any
 222 significant developmental differences at school age.

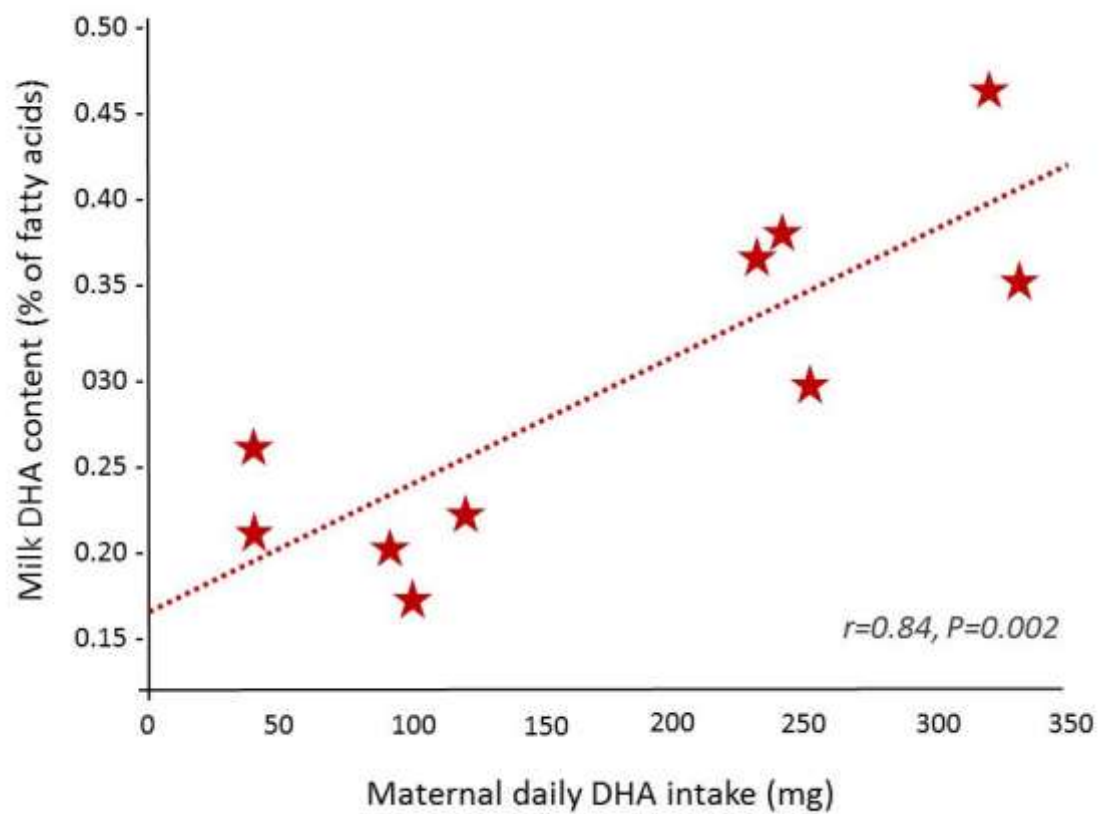
	High DHA (≈1%)	Standard DHA (≈0.3%)	Significance
Visual acuity (cycles per degree, cpd) , age 4 months (corrected for gestational age)			
	9.6 (3.7)	8.2 (1.8)	P=0.025
Mental development index (MDI), age 18 months (corrected for gestational age)			
Girls	99.1(13.9)	94.4 (17.5)	P=0.03
Boys	91.3 (14.0)	91.9 (17.2)	n.s.
Markedly abnormal development index (MDI) , age 18 months (corrected for gestational age)			
MDI <70	17 (5%)	35 (11%)	P=0.03
MDI <85	64 (20%)	90 (27%)	P=0.08

223

224

225 **Figure 2:** The DHA content in mature human milk is linearly related to the DHA intake of the
226 breastfeeding mother. Data from a randomized supplementation trial, redrawn from (25).

227

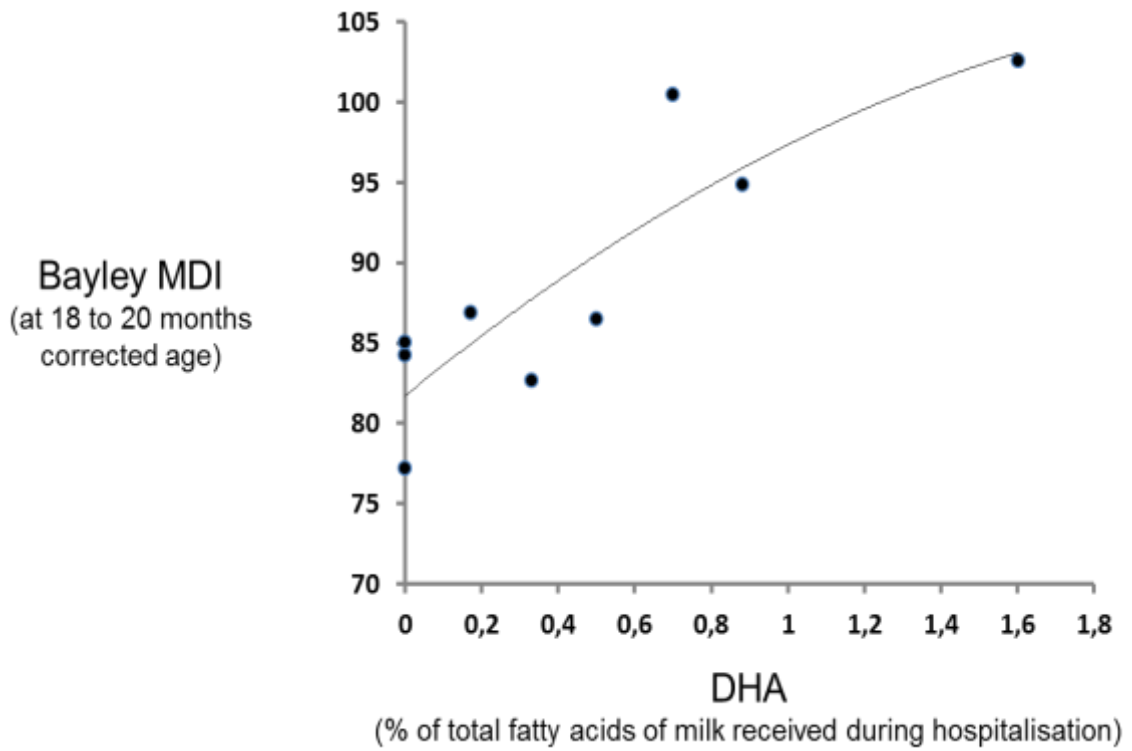


228

229

230 **Figure 1:** Dose-response relationship between milk DHA supply to preterm infants and the
231 Bayley Mental Development Index (Bayley MDI) at age 18-20 months, corrected for
232 gestational age. Reproduced from (44), with permission (currently being requested).

233



234

235

236

237 References

- 238 1. Koletzko B, Boey CCM, Campoy C, Carlson SE, Chang N, Guillermo-Tuazon MA, et al. Current
239 information and Asian perspectives on long-chain polyunsaturated fatty acids in pregnancy, lactation
240 and infancy. Systematic review and practice recommendations from an Early Nutrition Academy
241 workshop. *Ann Nutr Metab.* 2014;65(1):i49-80.
- 242 2. Lapillonne A, Moltu SJ. Long chain polyunsaturated fatty acids and clinical outcomes of
243 preterm infants. *Ann Nutr Metab.* 2016:in press.
- 244 3. Makrides M, Best B. DHA and preterm birth. *Ann Nutr Metab.* 2016:in press.
- 245 4. Calder PC. Docosahexaenoic acid. *Ann Nutr Metab.* 2016:in press.
- 246 5. Vermunt SH, Mensink RP, Simonis AM, Hornstra G. Effects of age and dietary n-3 fatty acids
247 on the metabolism of [13C]-alpha-linolenic acid. *Lipids.* 1999;34 Suppl:S127.
- 248 6. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N, Jr. Physiological compartmental analysis of
249 alpha-linolenic acid metabolism in adult humans. *Journal of lipid research.* 2001;42(8):1257-65.
- 250 7. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man.
251 *Current opinion in clinical nutrition and metabolic care.* 2002;5(2):127-32.
- 252 8. Lattka E, Klopp N, Demmelmair H, Klingler M, Heinrich J, Koletzko B. Genetic variations in
253 polyunsaturated fatty acid metabolism - implications for child health? *Ann Nutr Metab.*
254 2012;60(Suppl. 3):8-17.
- 255 9. Glaser C, Lattka E, Rzehak P, Steer C, Koletzko B. Genetic variation in polyunsaturated fatty
256 acid metabolism and its potential relevance for human development and health. *Matern Child Nutr.*
257 2011;7 Suppl 2:27-40.
- 258 10. Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C. Genetic variants of the fatty acid desaturase
259 gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty
260 acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. *Am J*
261 *Clin Nutr.* 2011;93(1):211-9.
- 262 11. Lattka E, Rzehak P, Szabo E, Jakobik V, Weck M, Weyermann M, et al. Genetic variants in the
263 FADS gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5
264 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and trans-9-
265 octadecenoic acid concentrations over the duration of lactation. *Am J Clin Nutr.* 2011;93(2):382-91.
- 266 12. Steer CD, Lattka E, Koletzko B, Golding J, Hibbeln JR. Maternal fatty acids in pregnancy, FADS
267 polymorphisms, and child intelligence quotient at 8 y of age. *Am J Clin Nutr.* 2013.
- 268 13. Uauy R, Castillo C. Lipid requirements of infants: implications for nutrient composition of
269 fortified complementary foods. *J Nutr.* 2003;133(9):2962S-72S.
- 270 14. Uauy R, Dangour AD. Fat and fatty acid requirements and recommendations for infants of 0-2
271 years and children of 2-18 years. *Ann Nutr Metab.* 2009;55(1-3):76-96.
- 272 15. Gil-Sanchez A, Larque E, Demmelmair H, Acien MI, Faber FL, Parrilla JJ, et al. Maternal-fetal in
273 vivo transfer of [13C]docosahexaenoic and other fatty acids across the human placenta 12 h after
274 maternal oral intake. *Am J Clin Nutr.* 2010;92(1):115-22.
- 275 16. Larque E, Demmelmair H, Berger B, Hasbargen U, Koletzko B. In vivo investigation of the
276 placental transfer of (13)C-labeled fatty acids in humans. *J Lipid Res.* 2003;44(1):49-55.
- 277 17. Larque E, Ruiz-Palacios M, Koletzko B. Placental regulation of fetal nutrient supply. *Curr Opin*
278 *Clin Nutr Metab Care.* 2013;16(3):292-7.
- 279 18. Prieto-Sanchez MT, Ruiz-Palacios M, Blanco-Carnero JE, Pagan A, Hellmuth C, Uhl O, et al.
280 Placental MFSD2a transporter is related to decreased DHA in cord blood of women with treated
281 gestational diabetes. *Clin Nutr.* 2016.
- 282 19. Larque E, Pagan A, Prieto MT, Blanco JE, Gil-Sanchez A, Zornoza-Moreno M, et al. Placental
283 fatty acid transfer: a key factor in fetal growth. *Ann Nutr Metab.* 2014;64(3-4):247-53.
- 284 20. Del Prado M, Villalpando S, Elizondo A, Rodriguez M, Demmelmair H, Koletzko B.
285 Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a
286 low-fat diet. *Am J Clin Nutr.* 2001;74(2):242-7.

- 287 21. Koletzko B, Agostoni C, Bergmann R, Ritzenthaler K, Shamir R. Physiological aspects of human
288 milk lipids and implications for infant feeding: a workshop report. *Acta Paediatr.* 2011;100(11):1405-
289 15.
- 290 22. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM.
291 Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin*
292 *Nutr.* 2007;85(6):1457-64.
- 293 23. EFSA-Panel-on-Dietetic-Products. Scientific Opinion on nutrient requirements and dietary
294 intakes of infants and young children in the European Union. *EFSA Journal* 2013;11(10):3408.
- 295 24. Genzel-Boroviczeny O, Wahle J, Koletzko B. Fatty acid composition of human milk during the
296 1st month after term and preterm delivery. *Eur J Pediatr.* 1997;156(2):142-7.
- 297 25. Fidler N, Sauerwald T, Pohl A, Demmelmair H, Koletzko B. Docosahexaenoic acid transfer into
298 human milk after dietary supplementation: a randomized clinical trial. *J Lipid Res.* 2000;41(9):1376-
299 83.
- 300 26. Schulzke SM, Patole SK, Simmer K. Long-chain polyunsaturated fatty acid supplementation in
301 preterm infants. *The Cochrane database of systematic reviews.* 2011(2):CD000375.
- 302 27. Koletzko B, Poindexter B, Uauy R, editors. *Nutritional Care of Preterm Infants.* Basel: Karger;
303 2014.
- 304 28. Kuipers RS, Luxwolda MF, Offringa PJ, Boersma ER, Dijck-Brouwer DA, Muskiet FA. Fetal
305 intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates.
306 *Prostaglandins Leukot Essent Fatty Acids.* 2012;86(1-2):13-20.
- 307 29. Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, et al.
308 Neurodevelopmental Outcomes of Preterm Infants Fed High-Dose Docosahexaenoic Acid A
309 Randomized Controlled Trial. *Jama-J Am Med Assoc.* 2009;301(2):175-82.
- 310 30. Henriksen C, Haugholt K, Lindgren M, Aurvag AK, Ronnestad A, Gronn M, et al. Improved
311 cognitive development among preterm infants attributable to early supplementation of human milk
312 with docosahexaenoic acid and arachidonic acid. *Pediatrics.* 2008;121(6):1137-45.
- 313 31. Atwell K, Collins CT, Sullivan TR, Ryan P, Gibson RA, Makrides M, et al. Respiratory
314 hospitalisation of infants supplemented with docosahexaenoic acid as preterm neonates. *Journal of*
315 *paediatrics and child health.* 2013;49(1):E17-22.
- 316 32. Manley BJ, Makrides M, Collins CT, McPhee AJ, Gibson RA, Ryan P, et al. High-dose
317 docosahexaenoic acid supplementation of preterm infants: respiratory and allergy outcomes.
318 *Pediatrics.* 2011;128(1):e71-7.
- 319 33. Collins CT, Makrides M, Gibson RA, McPhee AJ, Davis PG, Doyle LW, et al. Pre- and post-term
320 growth in pre-term infants supplemented with higher-dose DHA: a randomised controlled trial. *The*
321 *British journal of nutrition.* 2011;105(11):1635-43.
- 322 34. Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of two doses of docosahexaenoic acid
323 (DHA) in the diet of preterm infants on infant fatty acid status: results from the DINO trial.
324 *Prostaglandins, leukotrienes, and essential fatty acids.* 2008;79(3-5):141-6.
- 325 35. Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Grydeland H, Walhovd KB, et al. Diffusion
326 tensor imaging and behavior in premature infants at 8 years of age, a randomized controlled trial
327 with long-chain polyunsaturated fatty acids. *Early Hum Dev.* 2016;95:41-6.
- 328 36. Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Walhovd KB, Fjell AM, et al. Long-chain
329 polyunsaturated fatty acids and cognition in VLBW infants at 8 years: an RCT. *Pediatrics.*
330 2015;135(6):972-80.
- 331 37. Smithers LG, Gibson RA, McPhee A, Makrides M. Higher dose of docosahexaenoic acid in the
332 neonatal period improves visual acuity of preterm infants: results of a randomized controlled trial.
333 *Am J Clin Nutr.* 2008;88(4):1049-56.
- 334 38. Smithers LG, Collins CT, Simmonds LA, Gibson RA, McPhee A, Makrides M. Feeding preterm
335 infants milk with a higher dose of docosahexaenoic acid than that used in current practice does not
336 influence language or behavior in early childhood: a follow-up study of a randomized controlled trial.
337 *Am J Clin Nutr.* 2010;91(3):628-34.

- 338 39. Collins CT, Gibson RA, Anderson PJ, McPhee AJ, Sullivan TR, Gould JF, et al.
339 Neurodevelopmental outcomes at 7 years' corrected age in preterm infants who were fed high-dose
340 docosahexaenoic acid to term equivalent: a follow-up of a randomised controlled trial. *BMJ open*.
341 2015;5(3):e007314.
- 342 40. Molloy CS, Stokes S, Makrides M, Collins CT, Anderson PJ, Doyle LW. Long-term effect of
343 high-dose supplementation with DHA on visual function at school age in children born at <33 wk
344 gestational age: results from a follow-up of a randomized controlled trial. *Am J Clin Nutr*.
345 2016;103(1):268-75.
- 346 41. Collins CT, Gibson RA, Makrides M, McPhee AJ, Sullivan TR, Davis PG, et al. The N3RO trial: a
347 randomised controlled trial of docosahexaenoic acid to reduce bronchopulmonary dysplasia in
348 preterm infants < 29 weeks' gestation. *BMC Pediatr*. 2016;16:72.
- 349 42. Zhang P, Lavoie PM, Lacaze-Masmonteil T, Rhoads M, Marc I. Omega-3 long-chain
350 polyunsaturated fatty acids for extremely preterm infants: a systematic review. *Pediatrics*.
351 2014;134(1):120-34.
- 352 43. Gunaratne AW, Makrides M, Collins CT. Maternal prenatal and/or postnatal n-3 long chain
353 polyunsaturated fatty acids (LCPUFA) supplementation for preventing allergies in early childhood.
354 *Cochrane Database Syst Rev*. 2015(7):CD010085.
- 355 44. Lapillonne A. Enteral and parenteral lipid requirements of preterm infants In: Koletzko B,
356 Poindexter B, Uauy R, editors. *Nutritional care of preterm infants Scientific basis and practical
357 guidelines*. Basel: Karger; 2014. p. 82-98.
- 358 45. Koletzko B, Carlson SE, van Goudoever JB. Should infant formula provide both omega-3 DHA
359 and omega-6 arachidonic acid? *Ann Nutr Metab*. 2015;66:137-8.

360