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Suplementacja kwasu dokozaheksaenowego w mleku modyfikowanym dla niemowląt: od modeli zwierzęcych do badań klinicznych. Przegląd literatury

A quick glance at docosahexaenoic acid fortification in formulated milk for infants, from animal models to clinical studies: a review

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Streszczenie

Kwas dokozaheksaenowy jest kwasem tłuszczowym występującym naturalnie w olejach roślinnych, owocach morza, siemieniu lnianym, algach i żółtkach jaj. Należy do długołańcuchowych nienasyconych kwasów tłuszczowych, które są istotne dla procesów biochemicznych organizmu ludzkiego. Jako istotny składnik substancji szarej kwas dokozaheksaenowy stanowi przedmiot intensywnych badań nad rozwojem układu nerwowego. Jest on niezbędny do rozwoju mózgu płodu przede wszystkim w trzecim trymestrze ciąży. Wiadomo też, że suplementacja tego kwasu u matki ma istotny wpływ na zaspokojenie zapotrzebowania płodu. Wciąż jednak trwają spory o to, czy kwas dokozaheksaenowy jest niezbędny w przypadku niemowląt, szczególnie w populacji dzieci urodzonych przedwcześnie. W przeszłości niektóre badania translacyjne wykazywały korzyści suplementacji kwasu dokozaheksaenowego u rozwijających się płodów i niemowląt. Z tego względu suplementy kwasu dokozaheksaenowego są od dawna dostępne na rynku w postaci mleka modyfikowanego z dodatkiem tego kwasu. Jednakże z dalszych badań u ludzi i badań klinicznych wynika, że korzyści z suplementacji są nadal dyskusyjne. Mimo to odkrycie genu desaturazy kwasów tłuszczowych i jego znaczenia w regulacji poziomu kwasu dokozaheksaenowego i wielonienasyconych kwasów tłuszczowych u ludzi wydaje się dostarczać dalszych podstaw do suplementacji kwasu dokozaheksaenowego u niemowląt. Niniejszy przegląd literatury jest próbą przedstawienia obecnego stanu wiedzy na temat korzyści klinicznych ze stosowania mleka modyfikowanego z dodatkiem kwasu dokozaheksaenowego u niemowląt – od badań podstawowych po badania kliniczne.

Słowa kluczowe: kwas dokozaheksaenowy, niemowlę, suplementacja, gen FADS, wielonienasycone kwasy tłuszczowe

Abstract

Docosahexaenoic acid is a fatty acid found naturally in plants oil, fish oil, fish meat, seafood flaxseed, algae, and egg yolk. It is one of the long-chain unsaturated fatty acids that are important for human biochemistry. As an important component of grey matter, docosahexaenoic acid is subject to intense research in the field of neurodevelopmental study. It is needed mainly in the third trimester of pregnancy for optimal foetal brain growth and mother's docosahexaenoic acid intake is known to be important in supplying the foetal needs. However, arguments still exist on whether docosahexaenoic acid status is essential or non-essential for infants, especially in the preterm infant population. In the past, strong arguments coming from translational studies showed the benefits of supplementation of docosahexaenoic acid in developing foetuses and infants. Hence, docosahexaenoic acid supplementation has long existed as commercially available docosahexaenoic acid-fortified formula milk. However, the benefit of this supplementation remains controversial after follow-up in human-based studies and clinical trials. The discovery of the fatty acid desaturase gene and its significance in regulating human docosahexaenoic acid and polyunsaturated fatty acids levels also seemed to give new evidence basis for docosahexaenoic acid supplementation in infants. This literature review attempts to explain the current understanding of clinical benefit of docosahexaenoic acid-fortified milk for infants, starting from the translational study level to clinical trials.

Keywords: docosahexaenoic acid, infant, supplementation, FADS gene, polyunsaturated fatty acids

INTRODUCTION

Docosahexaenoic acid (DHA) is one of long-chain polyunsaturated fatty acids (LC-PUFA) that are important for human biochemistry. The importance of DHA is especially highlighted due to its abundance in human brain, especially in grey matter[1,2]. Under normal condition, adult humans are able to sufficiently synthesise DHA from α-linoleic acid[3]. However, this synthesis is diminished in developing foetuses, creating a nearly-essential need for DHA for foetus. Due to its nearly essential nature for developing foetuses especially in the third trimester, DHA needs for foetuses rely heavily on their mother's DHA intake[1,4,5]. This review attempts to explain the current understanding of the clinical benefits of DHA-fortified milk for infants, from the translational study towards its established review on clinical trials.

DHA SOURCES AND SUPPLEMENTATION FOR INFANTS

Food known to be rich in DHA tends to come from various fat sources such as fish and plant oil. Fish oil, fish meat, seafood flaxseed, algae, and egg yolk, remain the common sources of DHA[6]. For young infants who were obviously unable to digest the mentioned DHA sources, natural DHA sources are available as breastmilk. Breastmilk naturally contains short-chain polyunsaturated fatty acids (SC-PUFA) as well as LC-PUFA, including DHA. Study showed that mothers' diet is reflected in the level of DHA in breastmilk. This was further investigated to the point where it was concluded that preterm infants erythrocyte DHA/AA level is correlated to their mother's DHA breastmilk level[7]. Interestingly, at community level, breastmilk DHA level is known to be manipulatable through education information dissemination[8]. This results in higher average DHA level from 2008 onwards compared to worldwide DHA level prior to 2007.

The question whether DHA should be supplemented in infants was raised based on argument on how infants fulfil their needs of fatty acids. It is argued that an infant is capable to synthetize LC-PUFAs from their SC-PUFA precursors[9]. This is further complicated with the confirmed existence of fatty acid desaturase (FADS) gene cluster variation in human population[10]. Recent COGNIS study (A Neurocognitive and Immunological Study of a New Formula for Healthy Infants) confirmed that even with DHA supplementation given at a supposedly sufficient and safe level, infants with FADS minor allele still suffer from lower DHA level[11]. This inspired numerous studies to claim that long-term supplementation of DHA is needed to achieve optimum infant neurobehavioral growth and development.

It is worth to mention that while DHA-rich food and supplements are widely available on the commercial market, DHA pure form is still hard to acquire through conventional purification methods. To add to this problem, the readily available common DHA food sources are difficult to digest for infants, thus to achieve higher breastmilk DHA level, mother must consume a higher amount of DHA-rich food. To bypass this problem, attempts to acquire a higher ratio of DHA-per-biomass have been made by harvesting and engineering DHA-rich microscopic food source such as algae *Crypthecodinium cohnii* and *Schizochytrium* sp.[12–14]. Because of their high DHA-per-biomass ratio, these sources are now used in the production of DHA-fortified formula milk[15,16].

DHA STUDIES IN ANIMAL MODELS

Since the discovery of DHA importance for neuronal biochemistry, animal model studies on DHA branched mainly towards three observational goals: 1) the effects of DHA deficiency, 2) the effects of DHA toxicity, and 3) DHA biochemistry. The outcomes of the mentioned studies may often be categorised into three major interests: 1) the effect of DHA on visual system, 2) the effect of DHA on neuroanatomy, neurophysiology, and neurobehaviors, 3) deeper understanding of DHA biochemistry. Common animals used for DHA pre-clinical studies are rat (*Rattus* sp.), piglet (*Sus scrofa*), rhesus macaque (*Macaca mullata*), guinea pig (*Cavia porcellus*) and mouse (*Mus musculus*)[17,18].

A study showed that pregnant and lactating rat brain DHA content is depleted when the diet supplies inadequate amounts of (n-3) PUFA and (n-6) docosapentaenoic acid (DPA)[19]. Pregnant rats fed with DHA-deficient diet have shown lowered hippocampal and hypothalamic brain-derived neurotrophic factor, which led to higher depression-like symptoms in their offspring[20]. Another study of DHA supplementation showed that maternal feeding of DHA prevented offspring's valproic acid-induced learning and memory impairment, as shown by the reduced rate of hippocampal neuron apoptosis and altered level of apoptosis related protein such as Bcl-2, Bax, and caspase-3. The study also related this result as a prevention method for childhood autism spectrum disorders[21].

Piglet has emerged as an important translational model for studying neurodevelopment due to the striking similarity of brain development pattern compared to humans[22]. Studies on pig and piglet have shown that breastmilk DHA content was increased by maternal supplementation of DHA-rich food. Furthermore, DHA-rich breastmilk has shown to increase piglets DHA level in plasma, liver, erythrocyte phospholipids, brain, and synaptic plasma membrane[23]. Study also showed that the addition of animal-based PUFA, such as fish oil, has led to improved DHA deposition in piglet tissue compared to piglet supplemented by plant-based PUFA. The same study also stated that maternal supplementation of PUFA increases piglet brain DHA that correlates with birthweight[24]. A study also showed that the addition of DHA in piglets' diet has shown to increase *N*-acylethanolamine, a type of cannabinoid receptor ligand, in specific brain regions such as brainstem,

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auditory cortex, cerebellum, and striatum, up to ten fold compared to DHA-deficient diet[25].

Rhesus macaque is another model animal that is often used for neuroanatomical and neurobehavioral studies due to similarities of postnatal neurodevelopment, and similar yield of magnetic-resonance image in regards of anatomical and functional map compared to humans[26]. Studies showed that DHA supplementation played crucial role in the modulation of large-scale system in rhesus macaque brain, mainly by increasing the density of neuronal connections which deal with visual acuity and cognitive pathway[27]. In DHA-deficient state, rhesus macaque has been observed to exhibit a lower level of DHA in brain and retina, as well as impaired visual function. Twelve-week supplementation of DHA was observed to resolve this deficiency state in rhesus macaque[28].

Studies have shown that dietary DHA supplementation produced moderate relative increases in DHA levels in retina and brain[29]. It was also shown that DHA supplementation in weaning guinea pig also reduce the retinal DHA depletion rate to more than half[30]. In DHA deficient guinea pig, electroretinography showed 42% reduction in response amplitude towards 30 Hz flicker. The same study also stated that 16 weeks of subsequent DHA supplementation provides complete retinal functional recovery towards DHA deficiency[31].

It is worth noticing that almost all of these animal studies did not use DHA-fortified milk as a method of administration. Because of this, a note must be taken when claiming that DHA fortification in man-made food is based on animal studies. Initial attempts to add PUFA, especially DHA and EPA, to common human food sources resulted in unwelcomed changes in food flavour and the appearance of fishy odour. This led to the invention of food processing methods for removing various odour sources from DHA-rich food[32]. In contrast, the mentioned studies used natural, unprocessed DHA food source such as safflower oil, fish oils, canola oil, or peanut oil[3,17,19–21,27,29,31]. Whether DHA processing for human consumption could reduce its bioavailability is still open to debate and requires further studies. However, the fact that DHA fortification has already reached infant formula milk in practice suggests that most of DHA studies in animal models have shown convincing benefit for their human counterpart.

CLINICAL STUDIES ON DHA-FORTIFIED FORMULA MILK

How DHA affects infant health is widely explored in many clinical studies. Moreover, systematic reviews and meta-analyses covering various results of DHA clinical trials on infant health exist in abundance. Supplementation of DHA is known to be beneficial in pregnancy, as it helps prevent low birth weight, mature the visual system, improve the child's attention and problem-solving ability[33–36]. However, DHA fortification in formula milk for infants remains a big controversy in itself. It is important to note that, although DHA is well known to be beneficial as maternal supplementation, many trials of DHA-in-formulated milk for infants actually yielded controversial results when compared to their animal and maternal model counterpart. Preterm infants have become a subject population for DHA-fortified formula milk study. This is based on the fact that premature infants are known to born with DHA deficiency[37]. Most DHA demand for foetal brain growth increases in the third trimester of pregnancy[1,4,5], in which any deficiency in that period may be fixed in the post-natal period. However, the review of eleven randomized trials on DHA supplementation in formula milk for preterm infants at first raised doubts concerning the importance of DHA supplementation despite of its nearly-essential nature[38]. Most trials yielded no significant results on visual acuity and limited significance for the neurodevelopment outcome, and anthropometric growth compared to infants who did not received DHA fortification. The review limits the neurodevelopmental outcome assessment using Bayley Scales of Infant Development (BSID) for children aged 12 to 24 months old. Visual development was assessed using Teller cards. Based on the review, the studies reported significant difference on neurodevelopmental outcome compared to the control group[39–41]. In the same review, five other studies were used for anthropometric growth evaluation, which has shown a significant increase in infant body weight and height up to two months old[42–46]. However, as optimistic as it may sound, the authors claiming the significant effect of DHA supplementation were warning about study limitations such as small sample size, strict inclusion criteria, radio diagnostic procedures, and limited diagnostic options on neurodevelopmental outcome assessment[40,41].

In 2011, Schulzke et al. conducted a similar review again while expanding its reference publication date[47]. By analysing publications from the start of 1966 through end of 2009, supplementation of DHA in preterm infant was reviewed again. The review recorded that neurodevelopmental outcomes were mostly assessed using BSID, Kindergarten Parent Survey (KPS) Screening Inventory, Fagan Infant Test, and MacArthur Communicative Inventories. Visual development was usually assessed using Teller cards and a few with rod electroretinogram. Seventeen studies, including thirteen that were categorised in the review as high-quality studies, still reported non-significant differences in visual acuity. In this review, three out of seven studies reported significant neurodevelopmental outcomes. However, after a meta-analysis of four studies conducted on 12 month old participants (n = 364) and three studies, which included 18 month old ones (n = 494), no significant neurodevelopmental outcomes were observed compared to the control group. The review also documented that only four from fifteen studies noted significant effects of DHA on anthropometric growth, especially weight and body length. The significant results were only documented for infants up to 2 months of age. Meanwhile, meta-analysis for 12 month

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old infant population (n = 271) and 18 month old infant population (n = 396) did not yield any significant results on infant body weight, body length, or head circumference. Not different from its preterm infant target population counterpart, another study showed that the addition of DHA in formula milk for full-term infants also yielded low to no benefit nor harm. Meta-analysis by Jasani et al. in 2017 concluded that there was no consistent beneficial outcome of DHA supplementation on visual acuity, and no beneficial effect on child neurodevelopmental and anthropometric growth[48]. Thirty one randomized controlled trials were identified for the study, but only fifteen were included for the review (n = 1,889). In the review, visual acuity was assessed by nine studies. Visual evoked potentials (VEP), two Teller cards, and a combination of both were used as the methods of assessment. The results were inconsistent, where beneficial effects were reported by four studies while the remaining ones were against the favour. Neurodevelopmental outcomes were measured by eleven studies. It appeared that Bayley Scales of Infant Development, version II (BSID II), is the most used method to assess infant development, in as many as nine trials. The remaining trials each used the Fagan Infant Test and Brunet and Lezine test. Overall, only two trials using BSID-II scores and one trial using Fagan Infant Test reported beneficial outcome of DHA-fortified formula milk in infants. Anthropometric measurements were performed by measuring body length, body weight, and head circumference, and this was done in thirteen studies. All studies reported neither harmful nor beneficial effect of DHA-fortified formula milk consumption.

Although it is out of the scope of this review, it is worth mentioning that, similar to its infant-targeted counterpart, supplementation of DHA for breastfeeding mothers also yielded weak significance. Delgado-Noguera et al. (2010) reviewed data from 1966 through November 2009 about the supplementation of DHA in breastfeeding mothers towards the cognitive and physical development of children[49]. The study found mostly non-significant effect towards child development. Six randomized controlled trials on 1,280 breastfeeding mothers showed no significant differences in child visual acuity (two trials, n = 349), language development (two trials, n = 349), problem solving ability (two trials, n = 817), psychomotor development (two trials, n = 279), and motoric development (two trials, n = 349). Otherwise, DHA supplementation in breastfeeding mothers yielded significant outcome for the effects on child's attention (one trial), and body length (two trials, n = 834). Study also showed a significant risk ratio reduction of allergy manifestation in allergic infants (risk ratio, RR = 0.12). Another review by Lo et al. in 2012 showed that DHA supplementation in pregnancy does not improve infants' visual maturity and neurobehavioral outcome[50]. The review recorded that in nine studies, most studies showed no significant increase in infant visual maturity and infant cognitive abilities. Three studies showed no significant visual development differences in infant born from mothers supplemented with additional DHA. The other six studies focused on neurobehavioral development, which also showed no significant differences compared to infants whose mothers did not supplemented with additional DHA.

CONCLUSION

This quick review showed an obvious result discrepancy between animal models and clinical studies on DHA fortification for infant formula milk. There is still a deeply rooted concept of infant being seen as a quickly developing human who needs optimum nutrition and stimulation to achieve best growth and development. Studies on PUFA, especially DHA, are inevitably falling in this concept. However, decades of trials in both pregnant mothers and infants showed that this is not the case for DHA supplementation. This further showed that more needs to be known to give DHA supplementation to achieve its clinical significance, rather than giving DHA or PUFA as if, or as an isolated nutrition.

As far as the neurobehavioral effect of DHA in animal and human models are concerned, it is found in this review that there is still a massive gap between taking the results of animal model experiments and applying them to humans. It seemed that although study protocols in animal models are qualified for drawing pre-clinical conclusions, the emphasis on how DHA targets brain neurons thus contributing to its significance remains to be first thing that must explored. Moreover, many of the aforementioned animal studies did not distinguish clearly between the expected post-DHA supplementation effect and the expected result due to learning stimuli. Hence, blunder between both operational definitions may affect the conclusion-drawing process in animal studies. More careful conclusion-drawing for the applicable findings of DHA studies can be achieved by improving our knowledge and comparing DHA neurobiochemistry in animal models with its human counterpart.

Perhaps the biggest rhetoric question raised in the writing of this review is, whether it is the DHA – or as viewed by most clinical trials, LC-PUFA – that is the most important factor in the anthropometric growth, neurobehavioral development, and visual development of children? While the authors of most reviews agreed that the answer is negative, it must be realised that proper child stimulation following DHA-supplementation was never assessed in the reviewed trials. It must be emphasized that when viewing an infant as a growing and developing human, nutrition is only one side of the coin. Balanced stimulation to achieve optimal motoric and social development is the other side of the coin in this situation.

Conflict of interest

The authors do not declare any financial or personal links to other persons or organisations that could adversely affect the content of this publication or claim rights thereto.

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