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Research report

Different concentrations of docosahexanoic acid supplement during lactation result in different outcomes in preterm Sprague-Dawley rats

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ABSTRACT

Propose: In this study, we evaluated the effects of different concentrations of docosahexanoic acid (DHA) supplement on preterm Sprague-Dawley rat pups, and in parallel, measured the phosphorylation activity of the mTOR pathway in the hippocampal CA1 area.

Methods: Preterm Sprague-Dawley rat pups were randomly assigned to experimental groups which included; a sufficient DHA group (100 mg/kg/day); an enriched DHA group (300 mg/kg/day); an excess DHA group (800 mg/kg/day); and a deficient DHA group (normal saline gavage 0.1 ml/10 g). Body weight (g) was measured at days 1/7/14/21/28/42, respectively. Spatial learning and memory were also tested using the Morris water maze at week 6 (day 42). Finally, activation of the mTOR signaling pathway in hippocampal CA1 area were evaluated by western blotting.

Results: Postnatal sufficient/enriched docosahexanoic acid supplement ameliorated body weight restriction, spatial learning and memory restriction, and decreased phosphorylation of AKT, mTOR, P70S6K1, and 4EBP1 in hippocampal CA1 area. Furthermore, excess docosahexanoic acid supplement impeded weight gain and spatial learning and memory, perturbed serum unsaturated fatty acid, and downregulated phosphorylation of AKT, mTOR, P70S6K1, and 4EBP1 in hippocampal CA1 area.

Conclusion: Postnatal sufficient/enriched DHA supplement ameliorated growth and spatial learning and memory impairment and upregulated the mTOR pathway in preterm pups, although excessive DHA supplement did not have any beneficial effects.

1. Introduction

Docosahexanoic acid (DHA), an omega-3 long chain polyunsaturated fatty acid (LCPUFA), is as a neurotrophic factor and associated with neurodevelopment across various species. Research has demonstrated that DHA modulates blood-brain barrier functions, neuronal membrane fluidity and neurotransmission systems (Fontani et al., 2005; Kidd, 2007). Due to these essential functions, along with excessive advertising from companies selling LCPUFAs, parents gradually regarded these fatty acids, especially DHA, as indispensable supplements for infants. However, to date, meta-analysis and multi-center randomized control trials have failed to show any improvement for full-term infants taking these supplements (Makrides et al., 2010; Qawasmi et al., 2012). On the contrary, two studies indicated that high doses of LCPUFAs were harmful to hearing ability of rat pups (Church et al., 2009, 2010). It is well established that during the last gestational trimester, there is rapid and significant DHA-mediated development of the brain, and preterm infants seem to be vulnerable to DHA deficiency (Clandinin et al., 1989; Rombaldi Bernardi et al., 2012). Therefore, we speculated that different concentrations of DHA supplement would lead to distinct outcomes, that is, appropriate doses of DHA would ameliorate congenital DHA deficiency-induced growth and spatial learning and memory retardation caused by preterm birth, while these effects would be reduced or even abolished by excess DHA treatment.

Abbreviations: DHA, Docosahexanoic acid; LCPUFA, long chain polyunsaturated fatty acid; PKB, protein kinase B; mTORC1, mammalian target of rapamycin complex 1; Sug, sufficient DHA Group; Eng, enriched DHA Group; Exg, excess DHA Group; DeG, deficient DHA Group; PND, postnatal day; AA, arachidonic acid; EPA, eicosapentaenoic acid; ALA, linolenic acid; LA, linoleic acid; MDI, mental development index; BSID-II, Bayley scales of infant development, second edition; BSA, body surface area.

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Phosphorylation of the AKT-mTOR-p70S6K1/4E-BP1 pathway is associated with cell growth and proliferation, mRNA translation and ribosome biogenesis of expressed tissue (Hannan et al., 2003; Meijer and Codogno, 2004; Peng et al., 2002; Richter and Sonenberg, 2005; Sarbassov et al., 2005; Volarevic and Thomas, 2001; Wullschleger et al., 2006). In addition, the mTOR pathway is a critical regulator of translational activity and plays important roles in regulating neuronal polarity and neurite elongation (Feng et al., 2012; Kidd, 2007; Mita et al., 2016; Yang et al., 2014; Zhang et al., 2015), all of which are downregulated by preterm birth (Su et al., 2015). Based on these studies, we evaluated whether appropriate DHA-intake would ameliorate DHA-deficient induced spatial learning and memory delay caused by preterm birth. Additionally, whether excess DHA-intake would cause adverse neurocognitive behavior or growth retardation in preterm pups was also tested. We also hypothesized phosphorylation of the mTOR pathway in hippocampus would positively correlate with the spatial learning and memory and growth of the pups.

2. Results

2.1. Sufficient and enriched, but not excess DHA supplement ameliorated body weight restrictions

Body weights on postnatal days (PND) 1/7/14/21/28/42 are displayed in Fig. 1. No significant differences were found between any of the groups on PND 1/7/14/21. Pups in the EnG (110.134 g ± 2.07 g) had higher body weights compared to those in the ExG (101.681 g ± 1.728 g) on PND 28. Furthermore, pups in the EnG and SuG (232.460 g ± 4.488 g and 228.411 g ± 4.261 g, respectively) had higher body weights compared to the ExG and DeG (203.025 g ± 4.942 g and 206.171 g ± 3.903 g, respectively) on PND 42. Finally, there were no significant differences between the DeG and ExG.

2.2. Excess DHA supplement perturbed serum unsaturated fatty acid

Results are displayed in Fig. 2. LCPUFAs levels related including DHA, AA, EPA, ALA, LA were expressed as a percentage of the total fatty acids. Pups in the ExG had imbalanced concentrations of DHA and AA. There's no significant difference of concentration of EPA, ALA, LA among each group.

2.3. Sufficient and enriched, but not excess DHA supplement ameliorated learning and memory deficiencies

Results are displayed in Fig. 3. No significant differences were found between any of the groups during the first two days of the Morris water maze test. However, pups in the SuG (12.022 s ± 1.012 s) were significantly faster at reaching the platform on the third day of the test compared to pups in the ExG and DeG (16.895 s ± 0.827 s and 17.939 s ± 1.198 s, respectively). Likewise, on the fourth

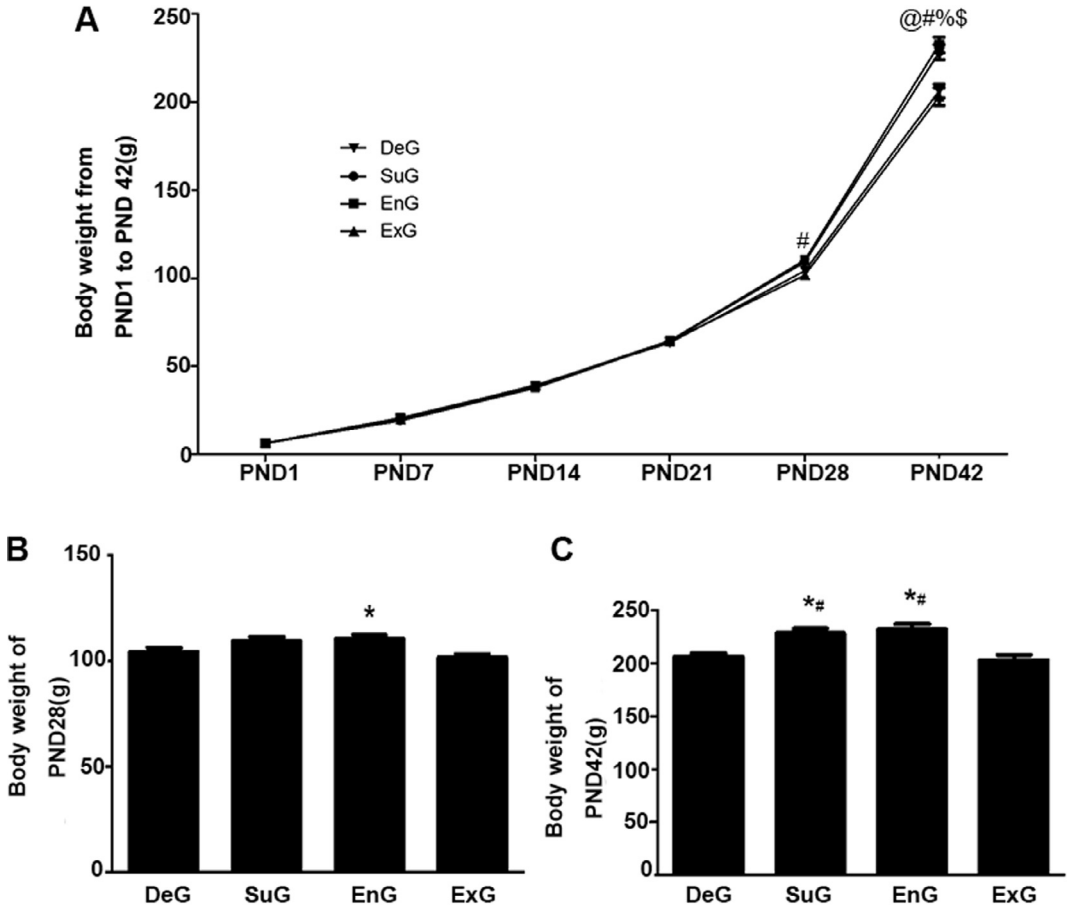

Fig. 1. The effect of DHA supplement on body weight. A, the body weight of DeG, SuG, EnG and ExG were tested on postnatal day (PND) 1, 7, 14, 21, 28 and 42. There are no significant differences among the groups on PND 1, 7, 14 and 21 except on 28 and 42. @: P < .05, SuG versus ExG;%: P < .05, SuG versus DeG; #: P < .05, EnG versus ExG; $: P < .05, EnG versus DeG. B, Pups in the EnG had higher body weights compared to pups in the ExG on PND 28. *: P < .05 versus ExG. C, Pups in the EnG and SuG have higher body weights than those in the ExG and DeG on PND 42. *: P < .05 versus ExG; #: P < .05 versus DeG. Data are expressed as mean ± SEM.