J Nutr Health. 2023 Dec;56(6):655-666
https://doi.org/10.4163/jnh.2023.56.6.655
eISSN 2288-3959

JNH Journal of Nutrition and Health

Research Article

Check for updates

Efficacy of the combined supplementation of choline and docosahexaenoic acid during gestation on developmental outcomes of rat pups

Rajarethnem Huban Thomas [1], **Kumar Megur Ramakrishna Bhat** [2], **Sivakumar Gopalkrishnan** [3], **and Kiranmai Sesappa Rai** [4]

[1]Department of Anatomy, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India
[2]Department of Anatomy, Ras Al Khaimah College of Medical Sciences, RAK Medical & Health Sciences University, Ras Al Khaimah 11172, United Arab Emirates
[3]Department of Physiology, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India
[4]Division of Physiology, Department of Basic Medical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India

Received: Jan 25, 2023
Revised: Jul 15, 2023
Accepted: Sep 22, 2023
Published online: Nov 15, 2023

Correspondence to
Kiranmai Sesappa Rai
Division of Physiology, Department of Basic Medical Sciences, Manipal Academy of Higher Education, Madhav Nagar, Manipal Karnataka 576104, India.
Tel: +91-8202922327
Email: hod.physio@gmail.com
kiranmai.rai@manipal.edu

ORCID iDs
Rajarethnem Huban Thomas
https://orcid.org/0000-0003-0235-1181
Kumar Megur Ramakrishna Bhat
https://orcid.org/0000-0003-1805-3453
Sivakumar Gopalkrishnan
https://orcid.org/0000-0003-0322-4512
Kiranmai Sesappa Rai
https://orcid.org/0000-0002-4888-7056

ABSTRACT

Purpose: Gestational nutrition has an impact on the growth and development of the fetus. Choline (C) and docosahexaenoic acid (DHA) are important and essential nutrients for humans that play a role in the structural integrity of the membranes as well as signalling. C is used in the synthesis of phosphatidylcholine, and cell membranes are highly enriched with DHA. The dietary intake of C or DHA during pregnancy directly influences fetal development. Currently, there is no evidence to prove the effectiveness of the combined dietary supplementation of both C and DHA during gestation on developmental outcomes in the offspring.
Methods: The current study was designed to assess the physical, sensory, and motor development of rat pups born to mothers supplemented with C and/or DHA during the entire gestational period. Pregnant rat dams were divided into the following five groups: Normal control (NC), Saline control (SC), Choline (C), DHA, and Choline+DHA (C+DHA). The NC dams did not receive any supplementation during the entire gestation period. The experimental groups were supplemented with Saline, C, and/or DHA, respectively, during the entire gestation (E0 to delivery).
Results: Rat pups (n = 6/group) exposed to combined C and DHA showed significant improvement in birth weight, fur development, eye-opening as well as weight gain on the 7th, 14th, and 21st postnatal day and pinnae detachment (assessed from birth to postnatal day 21) when compared with age-matched NC, SC or C or DHA pups. Further, significant reflex responses were observed in visual placing and bar holding of pups exposed to both C and DHA, whereas the differences in surface righting, negative geotaxis, and grasping reflexes were not significant between the groups.
Conclusion: Gestational supplementation of both C and DHA rather than either of them alone is better in enhancing developmental outcomes in rat pups.

Keywords: choline; docosahexaenoic acid; fetal development

Conflict of Interest
There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Gestational nutritional status has a considerable role in the physical, sensory, and motor development of the embryo as growth and nutrition are having a direct relationship with each other. The process of multiplication of the cells and their growth in size requires an adequate supply of essential nutrition [1]. In addition, nutrition levels during fetal development can unfavourably affect cognition including spatial memory, and also influences the growth and development of the offspring [2]. Maternal malnutrition leads to poor intrauterine growth and low birthweight (LBW) which leads to irreversible, cognitive, motor, and health impairment, which includes a possible predisposition to chronic disease in adult life. LBW is also one of the causes of neonatal deaths, which is strongly linked with perinatal morbidity and can lead to the risk of long-term disability [3]. There is an association between cardiovascular disease, hypertension, type 2 diabetes, and intrauterine growth and development [4]. Micronutrient deficiency results in physical, and mental growth retardation, a deficit in motor coordination in animal models, and behavioural and cognitive impairments [5].

Choline (C) is one of the essential nutrients which is an important precursor for the production of the neurotransmitter acetylcholine and also essential for the synthesis of phospholipids of the cell membrane and other signalling molecules. C has a vital role in fetal development [6]. Many studies have shown the association between placental weight and docosahexaenoic acid (DHA) concentration, which is correlated between placental weight and infant growth and development [7]. Previous studies executed in adult animal models have shown few positive effects of C or DHA alone on central nervous system (CNS) development. Combined supplementation of C and DHA during the prenatal period enhances neural cell development of the hippocampus in rat offspring compared to the normal control group rather than supplementation of these nutrients separately [8]. In addition, most of the previous studies reveal the consequences of the deficiencies of C and DHA, rather than their effect on the physical, sensory, and motor development of the fetus. Moreover, the efficacy of combined dietary supplementation of both C and DHA during gestation on developmental outcomes in offspring was seldom found in the literature. The outcomes of this combined therapy in the physical, sensory, and motor development of the fetus should be examined since C is an essential component used to synthesize maximum neuronal membrane phospholipids, and the DHA is the most preferred omega-3 fatty acid than any other polyunsaturated fatty acids during the synthesis. Therefore, the current study was designed to assess the physical, sensory, and motor development of rat pups born to mothers supplemented with C and or DHA during the entire gestational period.

METHODS

The current experimental study was conducted after obtaining approval of the Animal Ethics Committee (IAEC/KMC/32/12) from the Manipal Academy of Higher Education (MAHE), and also following guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals, Indian Government.

Animals

Wistar strain laboratory rats (three months old), weighing approximately between 200 to 250 grams were housed in an animal house research facility, MAHE, Manipal, Udupi District. Rats were encased in cages made of polypropylene, with paddy husk as bedding material, and maintained under standard lab conditions with temperatures ranging from 23 ± 2°C, humidity (50 ± 5%), and a 12-hour light-dark cycle. These rates were provided with pellet feed procured from VRK Nutritional Solutions (VRK's "Scientist's Choice" Laboratory Animal Diets Pune, India) (with C content 1 mg/kg) and these animals were free to access ad libitum water. Female rats during the estrus cycle were separated and permitted for mating with male rats. Upon identification of sperms in the vaginal smear, the first day of gestation was decided. Proper care was provided to the pregnant female rats which are maintained in separate polypropylene cages.

Study design

Pregnant dams (n = 6) were split into the following five groups: normal control (NC), saline control (SC), C, DHA, and C + DHA. NC dams were not disturbed, during the entire gestation period and were fed with normal animal feed. Pregnant dams of the SC group were supplemented orally with saline using a feeding needle from E0 to delivery. Dams from the C group were supplemented orally with C from E0 to delivery using a feeding needle. Pregnant dams from the DHA group were supplemented orally with DHA from E0 to delivery using a feeding needle. Pregnant dams from C + DHA group were supplemented orally with C **+** DHA from E0 to delivery using a feeding needle. After delivery one pup from each dam (A total of six pups from each group of dams) were collected. Postnatal physical landmarks of rat pup development such as birth weight, body weight on the 7th, 14th and 21st days, pinnae detachment, fur development, incisor eruption, eye-opening, and postnatal sensory and motor development of the rat pups like surfacing righting, negative geotaxis, visual placing response, grasping reflex, bar holding, were assessed. Test animals were handled with care during the entire procedure. The marking of each animal was made with a unique identification using a nontoxic, indelible marker. The pups are separated from their mothers only for the short time (less than 5 minutes) to avoid the maternal separation stress.

Oral supplementation of C and DHA

Extra Pure C chloride (98%) was purchased from (Loba Chemie Laboratory Reagents; Loba Chemie Pvt. Ltd., Mumbai, India) and dissolved in distilled water (4.6 mmol/kg/day of C) was supplemented. Soft gelatin capsules containing 300 mg DHA capsules were procured from Nouveau Medicament (P) Ltd., Chennai, India and the capsules (400 mg/day of DHA) were supplemented [1].

Physical landmarks of rat's development

Birth weight and body weight on the 7th, 14th and 21st days were assessed using the digital weighing machine. The pinna detachment of pups was examined individually when the tip of the ear is separated from the head and the minimum days required for pinna detachment were recorded. Upper incisor eruptions were examined until the first appearance of upper incisors and days required for the incipience of incisors were recorded. Fur development was monitored every day until the fur appeared on the dorsal surface of pups and days required for the same were recorded. The number of days required for both eyes to fully open in the pups was recorded. During the entire process, test animals were handled with care [9].

Sensory and motor development

Surface righting

The righting reflex was evaluated to estimate motor function and coordination. The pup was placed on its back and the time it took for the pup to turn over onto its belly was noted. If the pup spends more than 60 seconds, the test was stopped and the time was recorded as 60 seconds [10].

Negative geotaxis

Negative geotaxis was conducted to diagnose the vestibular and proprioceptive function, it is an automatic stimulus where animals show a reaction in their postural by turning upright. Each of the pups was placed on an inclined plane (typically 30° from horizontal) with its head facing downwards. The latency period (in seconds) was recorded for the pup to change its direction so that its head faces up in the inclined plane. If the pup spends more than 60 seconds, the test was stopped and the time was recorded as 60 seconds. The newborn pups were well protected to avoid damage.

Visual placing response

The visual placing response is used to assess the animal's proprioceptive ability. The pups were hanged by their tails and scowled towards a hard surface. If the pup lifts its head and properly extends its limbs, the observation was recorded as a 'Yes' or else as a 'No'.

Grasping reflex

This test is to assess the neuromuscular strength of the rat pup since this reflex is controlled by the spinal reflex mechanism, with the help of motor areas of the brain through their interneurons. The paw of the pups was stroked with a blunt instrument and observation was recorded whether the animal grasped the instrument or not.

Bar holding

The function of the cerebellar and vestibular system is reflected in bar holding. Pups were lifted by clasping the trunk and brought near the thin metal bar which is ≤ 4 to 7 mm in diameter. The animal was allowed to grasp with its front paws and hang by its front paws. The duration of the animal's grasp was recorded, up to a maximum of 10 seconds. Care was taken to prevent the animal from jumping or falling from the bar.

Statistical analysis

Data were analysed using one-way analysis of variance followed by Bonferroni's test (post hoc) and expressed as mean ± SEM with the significance level at $p < 0.05$ using statistical software Graph pad prism version 5.03.

RESULTS

Physical and developmental landmarks

Rat pups exposed to combined C and DHA during gestation showed a significant increase in birth weight ($^{c}p < 0.05$) when compared with the NC and SC groups of rat pups. Rat pups supplemented prenatally with C and DHA alone did not show any significant difference in birth weight when compared with the NC and SC group of rat pups. Additionally, a significant increase in birth weight ($^{d}p < 0.05$) was observed when compared with groups of rats supplemented with DHA alone (**Fig. 1**).

Rat pups supplemented prenatally with the combination of C and DHA showed significant weight gain ($^{cc}p < 0.01$) when compared with the NC and SC group of rat pups on the 7th day. Rat pups supplemented prenatally with C and DHA alone did not show significant weight gain when compared with the NC and SC control group of rat pups on the 7th day. Additionally, a significant increase in weight gain ($^{e}p < 0.05$) was observed when compared with groups of rats supplemented with C alone. Rat pups exposed to C ($^{a}p < 0.05$) and DHA

https://doi.org/10.4163/jnh.2023.56.6.655

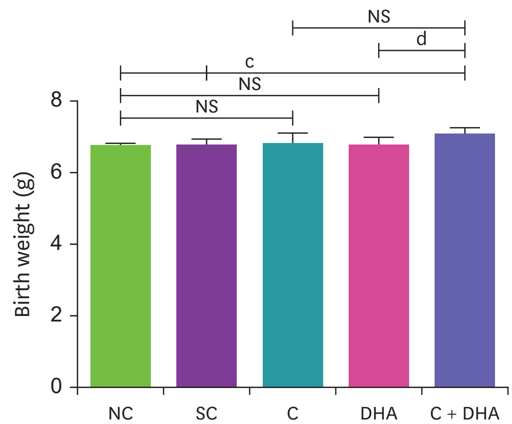

Fig. 1. Comparison of birth weight among rat pup groups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letters within a variable are different as below: c, NC and SC vs. C + DHA ($^{c}p < 0.05$). d, DHA supplemented group vs. C + DHA group.
NS, not significant; NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

($^{b}p < 0.05$) alone showed significant weight gain when compared with the NC and SC group of rat pups on the 14th day. However, rat pups supplemented prenatally with combined C, and DHA showed significantly more weight gain ($^{cc}p < 0.01$) when compared with the NC and SC group of rat pups on the 14th day. Additionally, a significant increase in weight gain ($^{d}p < 0.05$) was observed when compared with the groups of rats supplemented with DHA alone. Rat pups supplemented prenatally with C ($^{aa}p < 0.01$) and DHA ($^{b}p < 0.05$) alone showed significant weight gain when compared with the NC and SC group of rat pups on the 21st day. However, rat pups supplemented prenatally with the combination of C and DHA showed more significant weight gain ($^{ccc}p < 0.001$) when compared with the NC and SC group of rat pups on the 21st day. Additionally, a significant increase in weight gain ($^{d}p < 0.05$) was observed when compared with the group of rats supplemented with DHA alone (**Fig. 2**).

Rat pups exposed to combined C and DHA showed significantly earlier pinnae detachment ($^{cc}p < 0.01$) when compared with the NC and SC group of rat pups. Rat pups supplemented prenatally with C and DHA alone did not show a significant difference in pinnae detachment when compared with the NC and SC group of rat pups (**Fig. 3**).

Rat pups exposed to combined C and DHA showed significantly earlier fur development ($^{cc}p < 0.01$) when compared with the NC and SC group of rat pups. Rat pups supplemented prenatally with C and DHA alone did not show a significant difference in fur development when compared with the NC and SC group of rat pups (**Fig. 4**). There was no significant difference in the day of upper incisor teeth eruption between the groups (**Fig. 5**).

Rat pups exposed to combined C and DHA showed the opening of both eyes significantly earlier ($^{cc}p < 0.01$) when compared with the NC and SC group of rat pups. Rat pups supplemented prenatally with C and DHA alone did not show a significant difference in days of eye-opening when compared with the NC and SC group of rat pups (**Fig. 6**).

Sensory and motor development

Rat pups exposed to C ($^{aa}p < 0.01$) and DHA ($^{b}p < 0.05$) alone showed significant enhancement in visual placing response when compared to the NC and SC group of rat

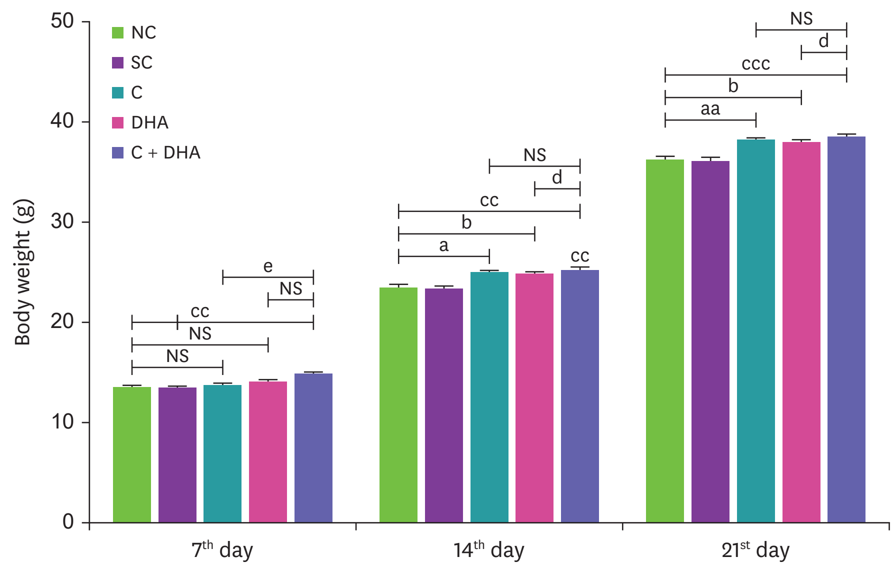

Fig. 2. Comparison of weight gain in rat pups among the groups tested on 7th day.
Data are shown as mean ± SE (n = 6 per group) for rat pups in each group. Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letters within a variable are different as below: On 7th day NC and SC vs. C + DHA: $^{cc}p < 0.01$, C vs C+DHA: $^{e}p < 0.05$. On the 14th day NC and SC vs. C: $^{a}p < 0.05$, NC and SC vs. DHA: $^{b}p < 0.05$ and NC vs. C + DHA: $^{cc}p < 0.01$ and DHA vs C+DHA: $^{d}p < 0.05$. On the 21st day NC and SC vs. C: $^{aa}p < 0.01$, NC and SC vs. DHA: $^{b}p < 0.05$, NC and SC vs. C + DHA: $^{ccc}p < 0.001$ and DHA vs C+DHA: $^{d}p < 0.05$.
NS, not significant; NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

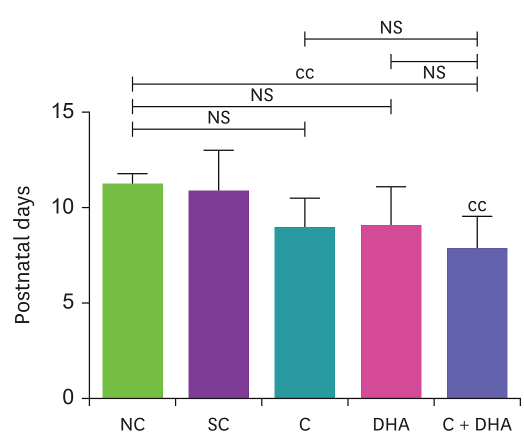

Fig. 3. Comparison of days required for pinnae detachment in rat pups.
Data are shown as mean ± SE (n = 6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letter within a variable is different as below: NC and SC vs. C + DHA, $^{cc}p < 0.01$.
NS, not significant; NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

pups. Rat pups supplemented prenatally with combined C and DHA also showed significant enhancement in visual placing response ($^{cc}p < 0.01$) when compared to the NC and SC group of rat pups (**Fig. 7**).

Rat pups exposed to C and DHA alone did not show a significant difference in bar-holding response when compared to the NC and SC groups of rat pups. However, rat pups supplemented prenatally with combined C and DHA showed significant enhancement in a

https://doi.org/10.4163/jnh.2023.56.6.655

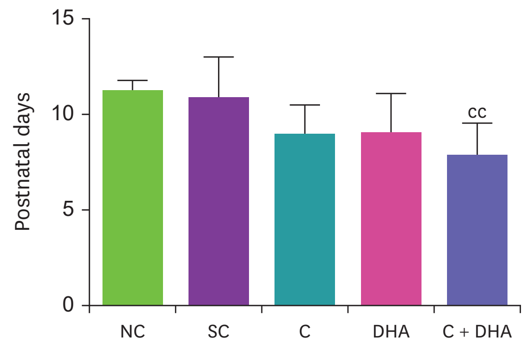

Fig. 4. Comparison of days required for fur development in rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letter within a variable is different as below: NC and SC vs. C + DHA: $^{cc}p < 0.01$.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

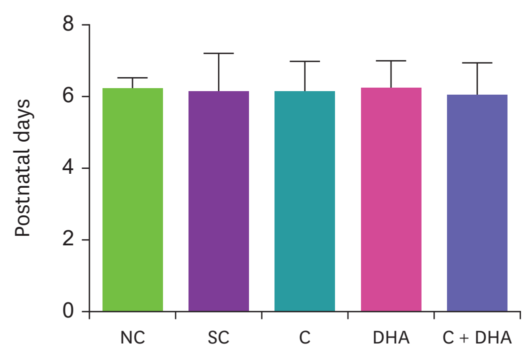

Fig. 5. Comparison of days required for incisor teeth eruption in rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

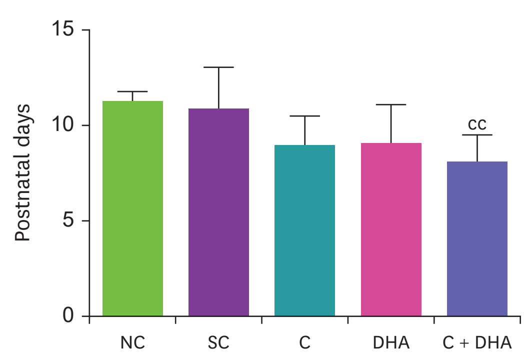

Fig. 6. Comparison of days required for eye-opening in rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letter within a variable is different as below: NC and SC vs. C + DHA: $^{cc}p < 0.01$.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

bar-holding response ($^{c}p < 0.05$) when compared to the NC and SC group of rat pups (**Fig. 8**). There was no significant difference in the findings of surface righting reflex and negative geotaxis reflex between the groups (**Figs. 9** and **10**).

JNH Journal of Nutrition and Health

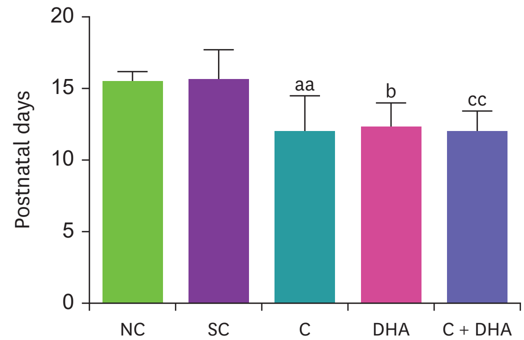

Fig. 7. Comparison of days required for correct visual placing response in rat pups.
Data are shown as mean ± SD (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letters within a variable are different as below: NC and SC vs. C: $^{aa}p < 0.01$, NC and SC vs. DHA: $^{b}p < 0.05$, and NC and SC vs. C + DHA: $^{cc}p < 0.01$.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

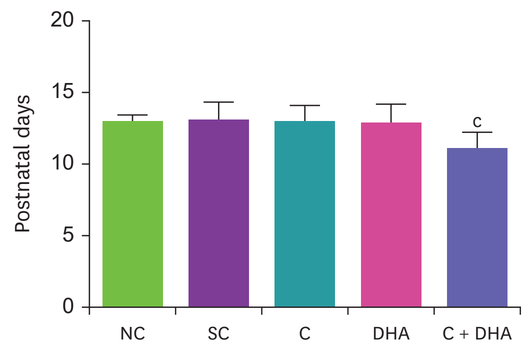

Fig. 8. Comparison of days required for correct bar holding among rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test. Different letter within a variable is different as below: NC and SC vs. C + DHA: $^{c}p < 0.05$.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

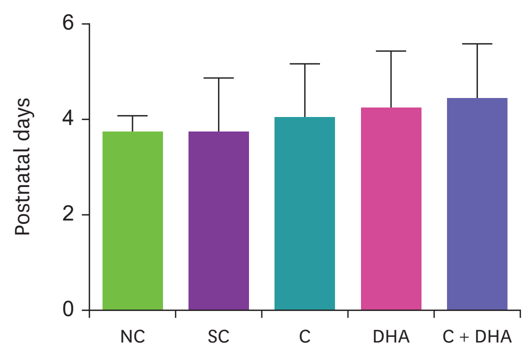

Fig. 9. Comparison of days required for correct surface righting among rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

DISCUSSION

In the present study, prenatal supplementation of a combination of C and DHA during normal pregnancy in rats caused a significant increase in birth weight, weight gain on the 7th, 14th and 21st days, significantly earlier pinnae detachment, fur development, and eye-opening.

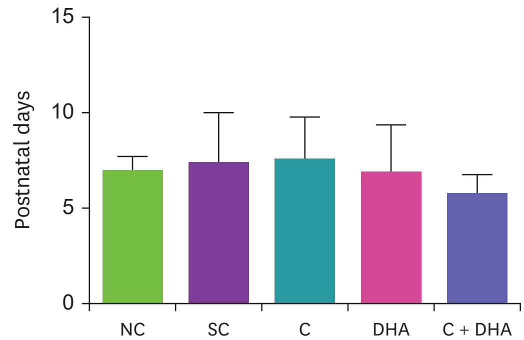

Fig. 10. Comparison of days required for showing negative geotaxis among rat pups.
Data are shown as mean ± SE (n=6 per group). Data were analysed using one-way analysis of variance followed by Bonferroni's test.
NC, normal control; SC, saline control; C, choline; DHA, docosahexaenoic acid; C + DHA, choline + docosahexaenoic acid.

Also, prenatal supplementation of C or DHA alone showed significant weight gain on the 14th and 21st days when compared to the same in rat pups from NC and SC groups.

C is an essential component in the diet for the normal function of all cells and critical to general health and cognition. C also forms complementary to B vitamins and omega-3 fatty acids [11]. C is a precursor for acetylcholine, which functions as phosphatidylcholine, and forms the major component of all cell membranes. C is vital for the integrity of the communication for all cells in the body including the CNS. C availability for the fetus brings enduring significance during adolescence [12]. C availability ensures the formation of membranes of the CNS [13]. Prenatally supplementation of C improves cognitive function until adulthood in animals [14].

During pregnancy, DHA has a vital role in CNS development and function [15]. DHA status of the pregnant mother is linked with fetal development including birth weight, head circumference, and the weight of the placenta [16]. DHA is preferably conveyed to the fetus during the last stages of gestation which is related to brain and retinal development [17,18]. Many authors have reported that DHA exposure to animals during gestation has a positive effect on, neural differentiation, neurotransmitter release, and synaptogenesis. Specifically, DHA is also essential for the development of dopaminergic the signalling system in the body [19-21].

Normal rat fetuses/pups supplemented prenatally with a combination of C and DHA was observed to show significant enhancement in visual placing response and bar holding compared to normal control rat pups postnatally. Additionally, rat pups supplemented prenatally with either C or DHA alone also showed enhancement although to a lesser significant level in visual placing response when compared to NC and SC groups of rat pups.

Negative geotaxis, an automatic, stimulus-bound, orientation, and movement directionally against gravitational cues are often conducted to assess the behaviour of infant rodents. Negative geotaxis was conducted to examine motor coordination and the motor performance of the rat pups [22]. Negative geotaxis, bar holding, and surface righting are reflecting the function of the cerebellar and vestibular system and are concerned with vestibular and proprioceptive function [23,24]. Visual placing response is to assess the proprioceptive abilities of the animals. If this response is impaired, it may be due to the motor deficit or damage to the sensory pathway for proprioception, or damage to the centers of the cerebellum which integrate this response [25]. The grasping reflex is controlled by the spinal reflex mechanism, and under the control of motor areas of the brain through the interneurons [26].

C is essential for the development of CNS during the perinatal period. The fetus receives C from the mother which transfers through the placenta, necessary for neural tube closure and CNS development [27]. C deficiency during gestation affects the development of cortical structure and function by abnormal estimated glomerular filtration rate (EGFR) and signalling [28]. EGFR knockout mice also show neurodegeneration in the olfactory bulbs and frontal cortex even during postnatal development and a decrease in the volume of the hippocampus [29].

DHA status of the fetus and infant is positively related to movement quality, enhanced visual activity, better mental and psychomotor development, and visual system function [30,31]. Oral supplementation of fish oil increases the DHA content of breast milk and is a positive effect on Griffith's experiment which include the coordination of the eye and hand of the infant [32]. Various studies show that prenatal and postnatal concentration of DHA influences infant cognitive and motor development [33]. DHA is the primary component of the nerve cell membranes and has a structural and neuroprotective role which are essential for cerebral and visual performances by influencing the development and maintenance [34].

Lipid-based nutrients (C and DHA) are essential for the growth and development of the fetus. It has been observed that there was a potential interaction between the metabolism of C and DHA, especially during its combined supplementation. The availability of DHA allows increased C uptake by the cells of the nervous system when compared to the supplementation of C alone [35]. The combined efficiency of C and DHA were demonstrated by the previous study in the $Pemt^{-/-}$ mouse model. Phosphatidylethanolamine-N-methyltransferase (PEMT) acts as a link between the metabolism of C and DHA. PEMT influences endogenous production of C. C influences phosphatidylcholine synthesis in the (cytidine diphosphate-choline) CDP-choline pathway and PEMT pathway. The PEMT pathway is most important since it catalyzes *de novo* biosynthesis of phosphatidylcholine even during the absence of dietary C [36]. PEMT enzyme prefers PtdEtn which contains DHA which is the long-chain polyunsaturated fatty acid [37] that facilitates the formation of DHA-enriched PtdCho in cell membranes, importantly in the brain. Thus, the availability of C and DHA during development facilitates better laying down of cell membranes which is an important prerequisite for increased formation of the tissues of the body. This may be the reason why combined supplementation of C and DHA shows enhanced physical, sensory and motor development.

SUMMARY

The aim of this study was to explore the potential benefits of gestational supplementation of C and DHA individually or in combined form on fetal developmental parameters. The findings of the study provide evidence of the importance of combined supplementation of C and DHA during gestation in rats for enhancing postnatal sensory, motor, and development of reflexes as well as their physical growth milestones. Furthermore, the findings show that pups exposed to a combination of C and DHA during gestation attain physical milestones and reflexes much more quickly than pups exposed to either nutrient alone.

REFERENCES

1. Thomas RH, Bhat KM, Gopalkrishnan SK, Jetti R, Thangarajan R, Indujaa M, et al. Neurocognitive developmental outcomes in early adolescent rats prenatally exposed to choline and docosahexaenoic acid. J Clin Diagn Res 2018; 12(1): KC01-KC05.
CROSSREF
2. Kriengwatana B, Farrell TM, Aitken SD, Garcia L, MacDougall-Shackleton SA. Early-life nutritional stress affects associative learning and spatial memory but not performance on a novel object test. Behaviour 2015; 152(2): 195-218.
CROSSREF
3. Bhutta ZA, Darmstadt GL, Hasan BS, Haws RA. Community-based interventions for improving perinatal and neonatal health outcomes in developing countries: a review of the evidence. Pediatrics 2005; 115(2 Suppl): 519-617.
PUBMED | CROSSREF
4. Jaddoe VW. Fetal nutritional origins of adult diseases: challenges for epidemiological research. Eur J Epidemiol 2008; 23(12): 767-771.
PUBMED | CROSSREF
5. Mhillaj E, Morgese MG, Trabace L. Early life and oxidative stress in psychiatric disorders: what can we learn from animal models? Curr Pharm Des 2015; 21(11): 1396-1403.
PUBMED | CROSSREF
6. Zeisel SH, Niculescu MD. Perinatal choline influences brain structure and function. Nutr Rev 2006; 64(4): 197-203.
PUBMED | CROSSREF
7. Rogers LK, Valentine CJ, Keim SA. DHA supplementation: current implications in pregnancy and childhood. Pharmacol Res 2013; 70(1): 13-19.
PUBMED | CROSSREF
8. Thomas Rajarethnem H, Megur Ramakrishna Bhat K, Jc M, Kumar Gopalkrishnan S, Mugundhu Gopalram RB, Rai KS, et al.. Combined supplementation of choline and docosahexaenoic acid during pregnancy enhances neurodevelopment of fetal hippocampus. Neurol Res Int 2017: 2017: 8748706.
PUBMED | CROSSREF
9. Soujanya M, Reddy PR, Reddy PS Perinatal exposure of biochanin-A induced abnormalities in offspring of rats. J Infertil Reprod Biol 2014; 2(4): 115-123.
10. Kvarik T, Mammel B, Reglodi D, Antonelli MC, Farkas J, Tamas A, et al. Effects of maternal stress during different periods of pregnancy on the early neurobehavioral response of rats. J Neurol Neurosci 2016; 7(2): 1-8.
CROSSREF
11. Zeisel SH, Blusztajn JK. Choline and human nutrition. Annu Rev Nutr 1994; 14(1): 269-296.
PUBMED | CROSSREF
12. Meck WH, Williams CL, Cermak JM, Blusztajn JK. Developmental periods of choline sensitivity provide an ontogenetic mechanism for regulating memory capacity and age-related dementia. Front Integr Nuerosci 2008; 1: 7.
PUBMED | CROSSREF
13. Cansev M. Synaptogenesis: Modulation by availability of membrane phospholipid precursors. Neuromolecular Med 2016; 18(3): 426-440.
PUBMED | CROSSREF
14. Rudy JW, Stadler-Morris S, Albert P. Ontogeny of spatial navigation behaviors in the rat: dissociation of “proximal”- and “distal”-cue-based behaviors. Behav Neurosci 1987; 101(1): 62-73.
PUBMED | CROSSREF
15. Calder PC. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukot Essent Fatty Acids 2008; 79(3-5): 101-108.
PUBMED | CROSSREF
16. Crawford MA, Doyle W, Drury P, Lennon A, Costeloe K, Leighfield M. n-6 and n-3 fatty acids during early human development. J Intern Med Suppl 1989; 731: 159-169.
PUBMED | CROSSREF
17. Carlson SE. Docosahexaenoic acid and arachidonic acid in infant development. Semin Neonatol 2001; 6(5): 437-449.
PUBMED | CROSSREF
18. Innis SM. Essential fatty acid transfer and fetal development. Placenta 2005; 26 Suppl A: S70-S75.
PUBMED | CROSSREF
19. Innis SM, de La Presa Owens S. Dietary fatty acid composition in pregnancy alters neurite membrane fatty acids and dopamine in newborn rat brain. J Nutr 2001; 131(1): 118-122.
PUBMED | CROSSREF

20. McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. Prostaglandins Leukot Essent Fatty Acids 2006; 75(4-5): 329-349.
PUBMED | CROSSREF

21. Carlson SE. Docosahexaenoic acid supplementation in pregnancy and lactation. Am J Clin Nutr 2009; 89(2): 678S-684S.
PUBMED | CROSSREF

22. Ramirez RL, Spear LP. Ontogeny of ethanol-induced motor impairment following acute ethanol: assessment via the negative geotaxis reflex in adolescent and adult rats. Pharmacol Biochem Behav 2010; 95(2): 242-248.
PUBMED | CROSSREF

23. Secher T, Novitskaia V, Berezin V, Bock E, Glenthøj B, Klementiev B. A neural cell adhesion molecule-derived fibroblast growth factor receptor agonist, the FGL-peptide, promotes early postnatal sensorimotor development and enhances social memory retention. Neuroscience 2006; 141(3): 1289-1299.
PUBMED | CROSSREF

24. Motz BA, Alberts JR. The validity and utility of geotaxis in young rodents. Neurotoxicol Teratol 2005; 27(4): 529-533.
PUBMED | CROSSREF

25. Palmer AC. Introduction to animal neurology. Oxford: Blackwell Scientific Publications; 1976.

26. Futagi Y, Toribe Y, Suzuki Y. The grasp reflex and moro reflex in infants: hierarchy of primitive reflex responses. Int J Pediatr 2012; 2012: 191562.
PUBMED | CROSSREF

27. Sweiry JH, Yudilevich DL. Characterization of choline transport at maternal and fetal interfaces of the perfused guinea-pig placenta. J Physiol 1985; 366(1): 251-266.
PUBMED | CROSSREF

28. Wang Y, Surzenko N, Friday WB, Zeisel SH. Maternal dietary intake of choline in mice regulates development of the cerebral cortex in the offspring. FASEB J 2016; 30(4): 1566-1578.
PUBMED | CROSSREF

29. Sibilia M, Steinbach JP, Stingl L, Aguzzi A, Wagner EF. A strain-independent postnatal neurodegeneration in mice lacking the EGF receptor. EMBO J 1998; 17(3): 719-731.
PUBMED | CROSSREF

30. Bakker EC, Hornstra G, Blanco CE, Vles JS. Relationship between long-chain polyunsaturated fatty acids at birth and motor function at 7 years of age. Eur J Clin Nutr 2009; 63(4): 499-504.
PUBMED | CROSSREF

31. Krabbendam L, Bakker E, Hornstra G, van Os J. Relationship between DHA status at birth and child problem behaviour at 7 years of age. Prostaglandins Leukot Essent Fatty Acids 2007; 76(1): 29-34.
PUBMED | CROSSREF

32. Dunstan JA, Mitoulas LR, Dixon G, Doherty DA, Hartmann PE, Simmer K, et al. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: a randomized controlled trial. Pediatr Res 2007; 62(6): 689-694.
PUBMED | CROSSREF

33. Morse NL. Benefits of docosahexaenoic acid, folic acid, vitamin D and iodine on foetal and infant brain development and function following maternal supplementation during pregnancy and lactation. Nutrients 2012; 4(7): 799-840.
PUBMED | CROSSREF

34. Alessandri JM, Guesnet P, Vancassel S, Astorg P, Denis I, Langelier B, et al. Polyunsaturated fatty acids in the central nervous system: evolution of concepts and nutritional implications throughout life. Reprod Nutr Dev 2004; 44(6): 509-538.
PUBMED | CROSSREF

35. Treen M, Uauy RD, Jameson DM, Thomas VL, Hoffman DR. Effect of docosahexaenoic acid on membrane fluidity and function in intact cultured Y-79 retinoblastoma cells. Arch Biochem Biophys 1992; 294(2): 564-570.
PUBMED | CROSSREF

36. da Costa KA, Rai KS, Craciunescu CN, Parikh K, Mehedint MG, Sanders LM, et al. Dietary docosahexaenoic acid supplementation modulates hippocampal development in the Pemt-/- mouse. J Biol Chem 2010; 285(2): 1008-1015.
PUBMED | CROSSREF

37. Ridgway ND, Vance DE. Phosphatidylethanolamine N-methyltransferase from rat liver. Methods Enzymol 1992; 209: 366-374.
PUBMED | CROSSREF