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Prenatal omega 3 fatty acid supplementation to a micronutrient imbalanced diet protects brain neurotrophins in both the cortex and hippocampus in the adult rat offspring

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ABSTRACT

Objective. Our earlier studies show that maternal diets imbalanced in micronutrients like folic acid and vitamin B_{12} reduced brain docosahexaenoic acid (DHA) and brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the offspring at birth and postnatal d21. This study followed the offspring till 3 months to examine the hypothesis that impaired brain neurotrophins at birth and d21 due to altered maternal micronutrients can be reversed by prenatal omega 3 fatty acid but not a postnatal control diet leading to altered cognition in adult life.

Materials and Methods. Pregnant rats were divided into control and five treatment groups at two levels of folic acid (normal and excess folate) in the presence and absence of vitamin B_{12} (NFBD, EFB and EFBD). Omega 3 fatty acid supplementation was given to the vitamin B_{12} deficient groups (NFBDO and EFBDO). Following delivery, 8 dams from each group were shifted to control and remaining continued on same diet.

Results. Imbalance in maternal micronutrients up to 3 months decreased DHA, BDNF and NGF in cortex and only BDNF in the hippocampus and impaired cognitive performance. Postnatal control diet normalized BDNF in the cortex but not the hippocampus and also altered cognitive performance. Prenatal omega 3 fatty acid supplementation normalized DHA, BDNF and NGF while long term supplementation was not beneficial only when micronutrients were imbalanced.

Conclusion. Patterns established at birth are not totally reversible by postnatal diets and give clues for planning intervention studies for improving brain functioning and cognitive abilities.

1. Introduction

Maternal nutrition plays an important role in determining risk for diabetes and cardiovascular diseases in adult life [1–3]. Recent studies indicate that it can also play an important role in brain development [4]. It is known that maternal micronutrients, which are part of the one carbon cycle, can influence the susceptibility of the offspring to diseases [5–7]. Particularly, low maternal plasma vitamin B_{12} levels have been shown to be associated with poor cognitive development

Abbreviations: AA, Arachidonic Acid; BDNF, Brain derived neurotrophic factor; DHA, Docosahexaenoic Acid; DoHad, Developmental Origins of Health and Disease; NGF, Nerve Growth Factor.

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in the child [8]. However the mechanisms leading to these adverse neurodevelopmental outcomes are not clear.

We have demonstrated earlier that maternal micronutrients can influence fatty acid metabolism [9]. Fatty acids are vital for the development and functioning of the brain and influence cognitive development [10,11]. Apart from fatty acids, the other mediators of normal cognitive development are neurotrophins like the brain derived neurotrophic factor (BDNF) [12] and the nerve growth factor (NGF) [13]. BDNF stimulates cell proliferation and improves cognition by activation of pathways like PI3-Akt, MAP kinase and STAT-3 [14,15]. On similar lines, neuroprotective actions of NGF have also been reported through activation of such pathways [16]. It has been suggested that the BDNF and NGF gene may play a vital role in mediating processes linking early life environment and adult brain health [17,18].

Recent studies suggest that the nature of such changes occurring in the offspring is dependent upon the timing and duration of the insult [19,20]. Our earlier studies have shown that maternal micronutrients during pregnancy play an important role in regulating protein and mRNA levels of neurotrophins in the offspring at birth [21]. Further we have also demonstrated that a postnatal control diet which normalizes the one carbon metabolism does not have the ability to normalize neurotrophin levels in the pup brain [22]. On the other hand supplementation of DHA to the imbalanced diet starting from pregnancy and continuing through the postnatal period can protect levels of both BDNF and NGF suggesting that DHA influences levels of neurotrophins [22]. In the current study we hypothesize that impaired brain neurotrophins at birth and d21 due to altered maternal micronutrients can be reversed by prenatal omega 3 fatty acid but not long term postnatal supplementation thereby leading to altered cognition in adult life. The current study followed these offspring longitudinally till 3 months to examine this hypothesis and to also examine whether the beneficial effects of omega 3 fatty acid supplementation persisted into young adulthood.

2. Materials and methods

2.1. Ethical approval

All experimental procedures were in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC). The institute is recognized to undertake experiments on animals as per the committee for the purpose of control and supervision of experimental animals, Govt. of India (No. 258/CPCSEA).

2.2. Animals

The protocol for the study has been described by us in detail earlier [9,21,23]. Briefly, 6 dietary groups (control and five experimental) were designed based on AIN93 guidelines and pregnant dams were randomly allocated to them. Diet composition was as described by us earlier [9,21]. There were a total of 6 groups at two levels of folic acid: Control: Normal folic acid, normal vitamin B_{12}, NFBD: normal folic acid, vitamin B_{12} deficient, NFBDO: normal folic acid, vitamin B_{12} deficient, omega-3 fatty acid supplemented, EFB: Excess folic acid, normal vitamin B_{12}, EFBD: Excess folic acid, vitamin B_{12} deficient, EFBDO: Excess folic acid, vitamin B_{12} deficient, omega-3 fatty acid supplemented. Immediately after delivery, randomly 8 dams from each group were shifted back to control and the remaining 8 continued on the same treatment diet (Fig. 1). In the case of control, all the animals that delivered continued on control until 3 months of age. All dams were allowed to deliver normally and the litter size was culled to 8 thereafter.

2.3. Analysis of fatty acids

Fatty acid analysis was performed using gas chromatography (Perkin-Elmer gas chromatograph; SD 2330, 30 m capillary column, Supelco, USA) as per the method described by us earlier [9,22]. A total of 15 fatty acids were identified by comparison of sample peaks with the fatty acids present in the standard fatty acid methyl esters (purchased from Sigma Chemicals) and were expressed as g/100 g fatty acids.

2.4. Analysis of plasma micronutrients and homocysteine

Plasma folate, vitamin B_{12} and homocysteine were estimated using the chemiluminescent microparticle immunoassay (CMIA) method. Values of plasma folate were expressed as ng/ml, plasma vitamin B_{12} levels were expressed as pg/ml and plasma homocysteine levels were expressed as μmol/L.

2.5. Pup brain BDNF and NGF protein levels

BDNF and NGF protein levels were measured from pup brain homogenates with a conventional sandwich ELISA using the BDNF and NGF Emax immunoassay system (Promega, Madison, WI, USA) respectively and have been described by us earlier [21]. Protein measurements from the samples were performed by the Lowry method. Values of BDNF and NGF were expressed as pg/mg protein.

2.6. Morris water maze

The protocol described here is similar to our earlier reported study [24]. Offspring from all groups were tested for cognitive performance by using a conventional method that uses a circular tank (115 cm in diameter and 62 cm high) made of opaque plastic. On the first day, the rats were required to locate the hidden platform (22 cm in diameter and 36 cm high) situated 1 cm below the surface of the water. On each trial, the rat was placed facing the wall in one of the four quadrants in the tank, and the time taken to locate the platform was recorded. The rats were returned to the cage after being appropriately warmed. This was done for 5 consecutive days, with the first day being considered the training day.

2.7. Statistical analysis

Values were expressed as mean ± SD. The data were analyzed using SPSS/PC + package (Version 20, Chicago, IL). The treatment groups were compared with the control at conventional level of significance using least significant difference estimate