

The history of arachidonic acid biology essay

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Abstract

Objective: Our earlier studies show that maternal diets imbalanced in micronutrients like folic acid and vitamin B12 reduced brain docosahexaenoic acid (DHA) and e 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 caused 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 B12 (NFBD, EFB and EFBD). Omega 3 fatty acid supplementation was given to the vitamin B12 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. Keywords: Brain Derived Neurotrophic Factor, Cognition, Developmental Origins of Health and Disease, Docosahexaenoic Acid, Nerve Growth Factor

Abbreviations

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

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 B12 levels have been shown to be associated with poor cognitive development 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 caused 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.

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 B12, NFBDO: normal folic acid, vitamin B12 deficient, NFBDO: normal folic acid, vitamin B12 deficient, omega-3 fatty acid supplemented, EFB: Excess folic acid, normal vitamin B12, EFBDO: Excess folic acid, vitamin B12 deficient, EFBDO: Excess folic acid, vitamin B12 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 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.. 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/100g fatty acids.

2. 4. Analysis of Plasma Micronutrients and Homocysteine Plasma folate, vitamin B12 and homocysteine were estimated using the chemiluminescent microparticle immunoassay (CMIA) method. Values of plasma folate were expressed as ng/ml, plasma vitamin B12 levels were expressed as pg/ml and plasma homocysteine levels were expressed as $\mu\text{mol/L}$.

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 has been described by us earlier [21]. Protein measurements from the samples were performed by 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 \pm SD. The data was 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 using one way analysis of variance (ANOVA) and the post-hoc least significant difference test.

Results

3. 1. Growth Curves A maternal vitamin B12 deficiency at both normal (NFBD-NFBD) and excess folate levels (EFBD-EFBD) did not affect the growth curves at 3 months of age. Neither did maternal omega 3 fatty acid

supplementation or a postnatal control diet affect growth patterns (Fig. 2). 3.

2. Brain Weights The absolute cortex weights were lower in EFBD-EFBD and NFBD-NFBD as compared to EFBD-C and NFBD-C respectively ($p < 0.05$ for both). Relative cortex weights were higher in EFBD-EFBD as compared to EFBD-EFBD ($p < 0.05$). Absolute and relative hippocampal weights were similar amongst all groups (Table 1). 3. 3. Folate, Vitamin B12 and Homocysteine levels in Pup Plasma

1) Levels of folate were comparable to control in vitamin B12 deficient groups at normal folic acid (NFBD-C and NFBD-NFBD). Whereas, plasma vitamin B12 levels were reduced in both NFBD-C ($p < 0.05$) and NFBD-NFBD ($p < 0.01$), as compared to control.

Vitamin B12 deficiency (NFBD-NFBD) at normal levels of folic acid showed higher ($p < 0.05$) levels of homocysteine in the plasma 2) However excess folic acid supplementation both in the presence (EFB-EFB) ($p < 0.05$) and absence (EFBD-EFBD) ($p < 0.01$) of vitamin B12 increased the levels of plasma folate as compared to control but reduced levels of plasma vitamin B12 ($p < 0.01$ for both). Similarly folic acid supplementation to a vitamin B12 deficiency (EFBD-EFBD) also displayed significantly ($p < 0.01$) higher levels

of homocysteine as compared to control 3) Omega 3 fatty acid supplementation to these vitamin B12 deficient diets increased the levels of folic acid only in the EFBDO-EBFDO group as compared to control ($p < 0.05$) while omega-3 supplementation at normal and excess folic acid levels (NFBDO-NFBDO and EFBDO-EBFDO) had no effect on vitamin B12 levels as the vitamin B12 remained low ($p < 0.01$ for both) in both these groups. Omega-3 supplementation at normal and excess folic acid levels (NFBDO-NFBDO and EFBDO-EBFDO) had higher levels of homocysteine ($p < 0.01$ for both) in both these groups while shifting back to a control diet (NFBDO-C and EFBDO-C) could maintain homocysteine levels close to that of control (Table 2).

3. 4. Fatty Acids from the Cortex

1) Vitamin B12 deficiency at normal levels of folic acid (NFBDO-NFBDO) reduced ($p < 0.01$) levels of AA, while shifting these groups back to a control diet (NFBDO-C) normalised their levels to those of control. In contrast, there was no significant differences observed in the levels of DHA in both NFBDO-NFBDO and NFBDO-C

2) Vitamin B12 deficiency at excess levels of folic acid i. e. EFBDO-EBFDO reduced levels of AA ($p < 0.01$) and DHA ($p < 0.05$), while shifting this group back to a control diet i. e. EFBDO-C normalised their levels to those of control.

3) Excess folic acid supplementation in the presence of vitamin B12 i. e. EFBDO-EBFDO group had reduced ($p < 0.01$) levels of AA. Shifting back to a control diet could not normalise the levels. There was however no significant differences in the levels of DHA in the EFBDO-EBFDO and EFBDO-C groups

4) Omega 3 fatty acid supplementation to the vitamin B12 deficient groups i. e. NFBDO-NFBDO and EFBDO-EBFDO showed lower ($p < 0.01$) levels of AA. But higher ($p < 0.01$ for all) DHA was observed in EFBDO-EBFDO as compared to control, EFBDO-EBFDO

and EFB-EFB and in NFBDO-NFBDO compared to NFBD-NFBD. In the NFBDO-C group levels of AA were lower ($p < 0.05$) while DHA was comparable to that of control. In the EFBDO-C group levels of AA and DHA were comparable to that of control (Table 3).

3. 5. Fatty Acid Levels in the Hippocampus¹) There was no change in AA and DHA levels in the groups given vitamin B12 deficient diet at normal levels of folic acid (NFBD-NFBD), however in the NFBD-C group DHA levels were lower ($p < 0.05$) as compared to control 2) Vitamin B12 deficiency at excess levels of folic acid (EFBD-EFBD) reduced levels of AA ($p < 0.01$) and DHA ($p < 0.05$), while shifting these groups back to a control diet (EFBD-C) normalised their levels to those of control. 3) There was no difference in AA and DHA levels in the groups given excess folic acid supplementation i. e. EFB-EFB and EFB-C 4) Omega 3 fatty acid supplementation to the vitamin B12 deficient groups i. e. NFBDO-NFBDO and EFBDO-EFBDO groups had lower levels ($p < 0.05$) of AA. In the NFBDO-C and EFBDO-C groups levels of AA and DHA were comparable to that of control (Table 3).

3. 6. BDNF protein Levels in the Cortex The levels of BDNF in the cortex are shown in Figure 3. The results observed were as follows: 1) Vitamin B12 deficiency at normal levels of folic acid i. e. (NFBD-NFBD) led to reduced ($p < 0.05$) BDNF levels which were normalised if the group was shifted back to a control diet (NFBD-C). 2) Vitamin B12 deficiency at excess levels of folic acid i. e. (EFBD-EFBD) reduced ($p < 0.01$) levels of BDNF, while shifting these groups back to a control diet (EFBD-C) normalised their levels 3) Excess folic acid supplementation (EFB-EFB) led to significantly reduced ($p < 0.01$) BDNF levels which remained low ($p < 0.05$) when the group was shifted back to control after delivery (EFB-C). 4) Omega 3 fatty acid

supplementation to vitamin B12 deficient groups at excess levels of folic acid i. e. (EFBDO-EFBDO) led to lower levels ($p < 0.05$) of BDNF as compared to control. In the NFBDO-C, NFBDO-NFBDO and EFBDO-C the levels of BDNF were comparable to those of control (Fig. 3).

3. 7. NGF protein Levels in the Cortex

The levels of NGF in the cortex are shown in Figure 4. The results observed were as follows:

- 1) Vitamin B12 deficiency at normal levels of folic acid (NFBDO-NFBDO) led to reduced ($p < 0.01$) NGF levels which were normalised if the group was shifted back to control after delivery (NFBDO-C).
- 2) Vitamin B12 deficiency at excess levels of folic acid (EFBDO-EFBDO) reduced ($p < 0.01$) levels of NGF, while shifting these groups back to a control diet (EFBDO-C) did not normalise their levels.
- 3) Excess folic acid supplementation (EFBDO-EFBDO) led to significantly reduced ($p < 0.01$) NGF levels which remained low ($p < 0.05$) when the group was shifted back to control after delivery (EFBDO-C).
- 4) Omega 3 fatty acid supplementation to vitamin B12 deficient groups i. e. (NFBDO-NFBDO and EFBDO-EFBDO groups) normalised the levels of NGF. Shifting to control i. e. (NFBDO-C and EFBDO-C) also normalised the levels of NGF to that of control (Fig. 4).

3. 8. BDNF protein Levels in the Hippocampus

The levels of BDNF in the hippocampus are shown in Figure 5. The results observed were as follows:

- 1) Vitamin B12 deficiency at normal levels of folic acid (NFBDO-NFBDO) led to reduced ($p < 0.01$) BDNF levels which were not normalised when the group was shifted back to a control diet after delivery (NFBDO-C).
- 2) Vitamin B12 deficiency at excess levels of folic acid (EFBDO-EFBDO) led to reduced ($p < 0.01$) BDNF levels which were not normalised when the group was shifted back control after delivery (EFBDO-C)
- 3) Excess folic acid supplementation (EFBDO-EFBDO) reduced ($p < 0.01$)

BDNF levels which remained low ($p < 0.01$) when the group was shifted back to control after delivery (EFB-C). 4) Omega 3 fatty acid supplementation to the vitamin B12 deficient groups i. e. (NFBDO-NFBDO and EFBDO-EFBDO groups) also led to lower levels ($p < 0.01$ for NFBDO-NFBDO and $p < 0.05$ for EFBDO-EFBDO) of BDNF as compared to control. Shifting to a control diet i. e. (NFBDO-C) also could not normalise the levels of BDNF. Levels of EFBDO-C were comparable to those of control (Fig. 5).

3. 9. NGF protein Levels in the Hippocampus
The levels of NGF in the hippocampus are shown in Figure 6. There was no difference in any of the groups except EFBDO-C which showed higher levels ($p < 0.05$) as compared to control (Fig. 6).

3. 10. Morris Water Maze
The time taken to reach the platform is expressed as graphs in Figure 7. The results observed were as follows: 1) There was no change in the time taken to reach the platform in the animals fed a vitamin B12 deficiency at normal levels of folic acid (NFBDO-NFBDO, NFBDO-C) 2) Animals in the vitamin B12 deficient diet at excess levels of folic acid (EFBDO-EFBDO) took a longer time ($p < 0.01$) in locating the platform. Shifting to a control diet (EFBDO-C) could not normalise the time 3) There was no difference in the time taken in the group fed excess folic acid levels as compared to control 4) Omega 3 fatty acid supplementation to vitamin B12 deficient groups i. e. (NFBDO-NFBDO and EFBDO-EFBDO groups) were similar to that control. Shifting to control i. e. (NFBDO-C and EFBDO-C) also could normalise the time to that of control (Fig. 7).

Discussion

Our data demonstrates that among the various treatment groups studied, the adverse effects in the offspring were observed if the maternal and

postnatal diet was imbalanced in micronutrients (excess folic acid and vitamin B12 deficiency). This group showed a reduction in DHA, AA, BDNF and NGF levels in the cortex while in the hippocampus DHA, AA and BDNF were reduced but not NGF. Further, offspring's in these imbalanced groups also showed cognitive impairment at adult age. The current study for the first time also tries to identify modifiable periods of metabolic plasticity (possibly during early postnatal life) in the life course during which it may be possible to intervene with specific nutrients like omega 3 fatty acids to influence cognition in adult life. The current data also suggests that 1) Shifting to a control diet normalised levels of plasma folate, homocysteine and vitamin B12. In addition, in the cortex levels of AA, DHA and BDNF could be normalised but not NGF. while in the hippocampus shifting to a postnatal control diet could normalise levels of AA and DHA but not BDNF. 2) Prenatal omega 3 supplementation i. e. during pregnancy could normalise DHA, BDNF and NGF in both the cortex and hippocampus 3) Long term omega-3 supplementation i. e. throughout life normalised DHA but not BDNF in both, the cortex and hippocampus 4) Impaired cognition could not be reversed by a postnatal control diet although omega 3 fatty acid supplementation both prenatal and postnatal could improve cognition. Studies have adequately demonstrated that the maternal one carbon cycle plays an important role in fetal programming [7, 25]. To the best of our knowledge there are no studies examining the effects of these altered maternal micronutrients on the key molecules involved in brain function in an adult offspring. We have earlier demonstrated the adverse effects of a maternal diet imbalanced with micronutrients on BDNF and NGF at birth and d21 of life [21, 22]. The current

study followed these offspring till adult age and our findings suggest that a lifelong imbalance in maternal micronutrients affects long chain polyunsaturated fatty acid composition especially DHA and neurotrophins in both the cortex and hippocampus, regions which play a vital role in storage and processing of different types of information and are vital for cognition. Recent animal studies have shown that maternal B deficient diets lead to accumulation of homocysteine and reduced levels of plasma DHA [26]. Vitamin B12 is known to play an important role in regulating the balance of the network of cytokines and growth factors in the central nervous system of the rat [27]. Studies have shown that intervention with folic acid and leptin during particular windows in postnatal life can improve the adverse effects on metabolic syndrome variables in the offspring induced due to maternal protein restriction and undernutrition [28, 29]. Similarly methyl supplementation has also been shown to reverse epigenetic changes in the glucocorticoid receptors in the rat brain offspring [30]. The current study for the first time examines whether the long term effects of a postnatal diet can modify or ameliorate the negative effects of a maternal diet imbalanced with micronutrients. The current study highlights that a postnatal shift to a control diet was able to normalise the plasma levels of folate, vitamin B12 and brain DHA levels. However it was seen that amongst the two brain regions a postnatal control diet was able to normalise the levels of BDNF in the cortex but not the hippocampus. It is known that brain development takes place during critical windows when neural connections are made and any insult during these phases alters the development of the brain [31, 32]. A study examining the effect of depletion and recovery of DHA found that the diet

reversal for 12 weeks resulted in complete DHA recovery in almost all regions of the brain [33]. It is well known that like folic acid and vitamin B12 are involved in the formation of the methyl donor, S-adenosylmethionine (SAM) which maintains methyl group supply for deoxyribonucleic acid (DNA), neurotransmitters, proteins and membrane phospholipids. Studies have demonstrated that the conversion of phospholipid from PE to PC (via S-adenosylmethionine and Phosphatidylethanolamine Methyl Transferase) is critical for DHA mobilization from the liver into the plasma. When this reaction does not occur normally it could reduce DHA mobilization from the liver into the plasma, causing tissue depletion of DHA [34]. Recent studies in our lab have already demonstrated alterations in the fatty acid metabolism as a consequence of altered maternal micronutrients in the liver [35]. It is likely that an imbalance in maternal micronutrients results in reduced DHA levels in the pup brain possibly due to the impaired conversion of PE-DHA to PC-DHA. Nevertheless, further studies need to be conducted to examine the relative contribution of PEMT activity to total plasma DHA concentration. Alternatively it is also possible that the levels of DHA and AA may be regulated by desaturases and transport proteins as a consequence of altered maternal micronutrients as we have demonstrated in placenta [36]. Desaturases are an important class of proteins which play a role in mediating the unsaturation of fatty acids thereby controlling their function and metabolism [37]. We have also discussed the possible mechanisms for the observed results which include down regulation of nuclear transcriptional factors like SREBP and PPAR- γ which control expression of genes involved in fatty acid metabolism or reduced placental global methylation ultimately

causing reduced $\Delta 5$ deaturase expression [35]. Thus it is likely that the B12 and folic acid supplementation will influences DHA metabolism in various regions of the brain. In addition to the effect of an imbalanced diet (EFBD-EFBD) on levels of DHA, significant reductions were also found in AA levels in both, the cortex and hippocampus. Long term omega-3 supplementation to the micronutrient imbalanced diet also resulted in a reduction of AA in both the cortex and hippocampus. Results from our study, are in accordance with other studies, which demonstrate that changes in levels of DHA are accompanied by a compensatory alteration in AA metabolism [38]. Studies have shown that omega-3 deficiency for a 15 week period can lead to upregulation of AA dependent phospholipases (PL) like cytosolic phospholipase A2 (PLA2), secretory PLA2, and cyclooxygenase (COX-2), which can facilitate neuroinflammatory and excitotoxic processes [39]. In contrast, a prenatal omega 3 fatty acids supplemented diet could improve the levels of both DHA and BDNF. Omega 3 is known to play a role in the regulation of neurotrophins like BDNF and its receptor Trk B which play a role in spatial learning and memory [40]. Several studies have reported enhanced hippocampal neurogenesis along with increased levels of BDNF levels following omega-3 PUFA treatment [41-43]. However a long term postnatal supplementation with omega 3 fatty acids could not offer any benefit to BDNF only at excess folate supplementation. This may possibly be due to the fact that DHA exerts both oxidative and antioxidant properties which are majorly dependent upon its dose [44]. It is possible that long term omega 3 fatty acid supplementation on a micronutrient imbalanced diet increases oxidative stress and leads to reduced BDNF levels. We have earlier

reported increased oxidative stress in both dams and offspring at birth fed a micronutrient imbalanced diet [9]. Increased oxidative stress has been shown to reduce the levels of neurotrophins [45, 46]. We have recently demonstrated that maternal micronutrient imbalance leads to oxidative stress in the mother [9] and in the offspring brain at d21 [47]. In the current study an imbalance in micronutrients resulted in increased levels of homocysteine. A recent study demonstrates that homocysteine leads to an increase in reactive oxygen species and thiobarbituric acid reactive substances both in the cortex and plasma [48]. Our studies in animals at the end of pregnancy have also demonstrated that a deficiency of maternal micronutrients leads to higher levels of malondialdehyde and reduced DHA levels in the pup brain [9] and lower protein and mRNA levels of neurotrophins (BDNF and NGF) at birth [21]. Human studies carried out in our lab have also demonstrated a negative association between oxidative stress and plasma DHA levels in preeclampsia [49]. Thus in the current study there are 2 different scenarios where omega 3 fatty acid supplementation (short term and long term) was given 1) vitamin B12 deficiency in the presence of normal levels of folic acid 2) vitamin B12 deficiency in the presence of excess folic acid. Our data suggests that both short term and long term omega 3 fatty acid supplementation could normalize the levels of BDNF in the cortex but not in the hippocampus when there was vitamin B12 deficiency in the presence of normal levels of folic acid. In contrast, when there was an imbalance in folic acid and vitamin B12 (vitamin B12 deficiency in the presence of excess folic acid) only short term omega 3 fatty acid was able to normalize the levels of BDNF both in the cortex and hippocampus.

When long term omega 3 fatty acid was given it was not able to normalize the BDNF levels. Our data therefore indicates that the effects omega 3 fatty acid supplementation is determined not only by the duration of supplement but also by the presence/ absence of other micronutrients like folic acid and vitamin B12. In the current study the offspring from the micronutrient imbalanced group showed impaired cognition on the water maze. This may possibly be attributed to the low DHA levels which are known to be associated with a decline in cognitive abilities [50, 51]. Our results are in accordance with studies in elders where high serum folate along with a vitamin B12 deficiency is associated with cognitive decline [52]. It has been proposed that the adverse effects of a maternal vitamin B12 deficiency on cognition in the offspring could be due to a reduction in total brain volume or improper myelination [53, 54]. The cognitive impairment observed in our study was not reversible when the rats were shifted on to a control diet after delivery. This could possibly be due to the fact that a postnatal control diet to a maternal imbalanced diet was unable to normalize BDNF levels in the hippocampus. In the imbalanced group (EFBD-EFBD and EFBD-C) where the cognition of animals was significantly impaired, hippocampal BDNF levels were also low. In contrast to this, BDNF levels in the cortex improved in the EFBD-C cognition remained impaired. Our findings are in line with other reported studies suggesting the importance of hippocampus in learning and memory [55-57]. An increase in hippocampal BDNF has also been shown to be associated with improved spatial memory [58]. Studies have shown that the hippocampus is a vital organ and any impairment in learning and memory tasks have frequently been associated with abnormalities in the

hippocampus [47-49]. Although, omega 3 fatty acid supplementation improves the performance to that of the control group. These findings are of relevance since DHA is known to play a key role in brain cell maturation and function [59]. One limitation of our study is that we used only the Morris water maze to assess cognition. Reports suggest that more than one test should be used to assess spatial memory since performance by rats may differ within the radial arm maze and the Morris Water Maze [60-62]. Nevertheless this is the first study which examines the changes in cognition with respect alterations in neurotrophin protein levels in the cortex and the hippocampus as a consequence of altered maternal micronutrients. Future studies need to also measure levels of various antioxidants in the brain. In conclusion, our results suggest that a maternal and postnatal diet imbalanced in micronutrients (folic acid and vitamin B12) leads to reduced levels of DHA and BDNF in the adult offspring both in the cortex and hippocampus. These changes were not totally reversible with a control diet in the postnatal period and could lead to cognitive decline in later life. It is thus possible that multiple (oxidative breakdown, altered desaturases, and mobilization of plasma DHA in membrane phospholipids PE-PC by PEMT) mechanisms leading to changes in PUFA and BDNF levels play a role. The current study demonstrates the importance of prenatal fish oil supplementation to a micronutrient imbalanced diet for long term beneficial effects on brain neurotrophins and cognition as compared to a long term postnatal omega 3 fatty acid supplementation. The findings of this study are of significance since brain development has been shown to be sensitive towards changes in gene expression through altered methylation [63] and

epigenetic modifications of genes like BDNF are suggested to underlie some of observed effects of an early life environment on behaviour in later life [18]. Future studies need to examine changes in the global and gene specific methylation patterns in the brain and folic acid and vitamin B12 signalling neuron using cell culture (in vitro) to further understand the effect of maternal micronutrients on brain development and function. Since both folic acid and vitamin B12 are reported to influence hippocampus neuronal differentiation. As demonstrated, that folic acid can stimulate hippocampal neurogenesis in the rat brain [64] while folic acid deficiency in embryonic stem cells derived from rhesus monkey, inhibits neuronal differentiation [65]. Other studies in the ND-7 cell line show that folic acid plays a role only in proliferation and not differentiation [66]. Further in a model of rat sciatic nerve injury, it has been demonstrated that methylcobalamin promotes neurite outgrowth and survival through the methylation cycle [67]. Cell culture studies report that a vitamin B12 deficiency leads to reduced cell proliferation but increased differentiation [68]. This may lead to the development of new treatment approaches for prevention of neurodevelopmental disorders.

Author Contribution

SRJ and AAK designed and conducted the study; AAK and PSS collected and analysed the data. All authors contributed to writing the manuscript

Acknowledgments

. We thank Mr. Vinayak Dhawale for his assistance at the animal house.

Funding Agency

We thank the Department of Biotechnology, New Delhi, India for partially funding this project
Conflict of Interest: None declared