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Maternal supplementation of omega 3 fatty acids to micronutrient-imbalanced diet improves lactation in rat

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ABSTRACT

The present study aims to examine the effect of maternal supplementation of omega 3 fatty acids to a micronutrient (folic acid and vitamin B_{12})-imbalanced diet on gastric milk volume and long-chain polyunsaturated fatty acid composition. Pregnant female rats were divided into 6 groups at 2 levels of folic acid in both the presence and absence of vitamin B_{12}. Both vitamin B_{12}–deficient groups were supplemented with omega 3 fatty acid. Gastric milk volume and long-chain polyunsaturated fatty acids were analyzed. Our results for the first time indicate that imbalance in maternal micronutrients reduces gastric milk volume and milk docosahexaenoic acid levels ($P < .01$ for both) as compared with control. Supplementation with omega 3 fatty acids to this diet imbalanced in micronutrients increases ($P < .01$) milk docosahexaenoic acid level as compared with control. Imbalance in maternal micronutrients during pregnancy can alter milk fatty acid composition, which may ultimately affect infant growth and development.

1. Introduction

Pregnancy is recognized as a vulnerable period for the health and well-being of both the fetus and the mother. During pregnancy, maternal, placental, and fetal requirements for long-chain polyunsaturated fatty acids (LCPUFAs) are high. A series of our earlier studies in pregnancy have highlighted the importance of omega 3 fatty acids especially docosahexaenoic acid (DHA) during pregnancy [1,2]. These fatty acids are found in high proportions in the structural lipids of cell membranes, particularly those of the central nervous system; and their accretion primarily occurs during the last trimester of pregnancy and the first year of life [3].

During pregnancy, DHA and arachidonic acid (AA) cross the placenta to the fetus. Postnatally, these fatty acids are supplied through breast milk, which contains a full complement of all PUFAs including precursors and metabolites. It has been reported that LCPUFAs especially DHA affect the development of visual and cognitive functions during early life [4,5]. A large number of studies have shown that the neurodevelopmental outcome of children is determined by breastfeeding [6]. Furthermore, it has also been reported that high levels of PUFAs in breast milk are important for cognitive development [7]. Studies suggest that maternal vitamin B_{12} status in pregnancy also influences the cognitive function in offspring [8]. Studies also suggest that neural maturation of breastfed infants is linked to breast milk LCPUFA concentrations [9].

In the lactating mammary gland, there is a high demand for LCPUFAs like DHA and AA to incorporate them into the milk. These LCPUFAs are essential and crucial for the normal growth and development of the brain and retina of the

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newborn [9]. Docosahexaenoic acid and AA are the important LCPUFAs in human breast milk and play an important role in the neurological development of the newborn because they are potent neurobiological agents that affect neuronal membrane structure, synaptogenesis, and myelination [10–12]. The synthesis of DHA and AA from precursor fatty acids is limited in human infants; and breast milk is, therefore, an important source of these fatty acids [13].

The lipid content of breast milk is known to be influenced by several factors, such as the mother's diet and nutritional status during pregnancy and lactation [14]. Changing maternal dietary fat intake has been shown to alter fatty acid content of breast milk [15]. We have recently reported altered LCPUFA levels in breast milk in women with pregnancy complications [1,2]. However, there are no studies that have examined the effect of maternal micronutrients like folic acid and vitamin B_{12} on breast milk volume and LCPUFA composition. These 2 vitamins play a crucial role in nucleic acid synthesis and 1-carbon metabolism. Low maternal folate status has been implicated in the etiology of neural tube defects [16], leading to a policy of supplementation with folic acid before and during pregnancy and fortification of flour with folic acid in some countries [17,18]. In India, the policy is to provide iron and folic acid (60 mg/d and 500 μg/d) to all pregnant mothers [19] without any consideration for vitamin B_{12} that is deficient in the Indian population [20], which primarily consumes a vegetarian diet. Recent studies have raised concerns that folic acid fortification may mask symptoms as well as worsen the vitamin B_{12} deficiency and lead to adverse neurologic consequences [21,22].

We hypothesize that imbalance of maternal micronutrients (folic acid, vitamin B_{12}) during pregnancy will (1) decrease gastric milk volume and (2) alter the composition of LCPUFAs. The present study therefore examines the effects of maternal folic acid supplementation at normal and excess levels in both the presence and absence of vitamin B_{12} on gastric milk LCPUFAs. Furthermore, because our earlier study [23] had indicated that altered folic acid and vitamin B_{12} metabolism was also associated with altered DHA metabolism, this study also examined the effects of omega 3 fatty acid supplementation in ameliorating the effects of maternal micronutrient imbalance using a rat model.

2. Materials and methods

All experimental procedures were in accordance with guidelines of Institutional Animal Ethics Committee. The institute is recognized to undertake experiments on animals as per the Committee for the Purpose of Control and Supervision of Experimental Animals, Government of India (No. 258/CPCSEA).

2.1. Animals

Wistar albino rats (60 females, 20 males) with an average weight of 35 to 40 g were obtained from the National Toxicology Centre, Pune. They were maintained at 22°C on a controlled 12-hour light and 12-hour dark cycle with appropriate ventilation system. Animals were marked with picric acid as H (head), back (B), tail (T), etc, for identification.

2.2. Breeding

The animals were bred at 3 months of age. Males were housed individually before mating to acquire cage dominance. Virgin female rats were bred (sex ratio, 1 male to 3 females). On the following morning, vaginal smears were taken to confirm mating. Vaginal smears were taken on a clean microscope slide using a cotton bud dipped in saline. The slides were examined under a microscope at 40× magnification using a Zeiss microscope (USA). The sperm-positive smear was considered a result of successful mating and considered day 0 of gestation. The pregnant dams were housed individually (in polypropylene cages with dimensions of 29 × 22 × 14 cm containing rice husk as bedding material) and allowed to deliver normally. Out of 60 females, 48 females became pregnant. After confirmation of pregnancy, these pregnant female rats were allotted randomly to the following 6 (1 control and 5 experimental) diets (8 on each). All dams delivered by cesarean birth on day 20 of gestation. Two pups from each group were randomly selected and analyzed for brain weights and for levels and expression of neurotrophins.

2.3. Diets

The composition of the control and the treatment diets (Table 1) was as per the American Institute of Nutrition (AIN) 93 purified diets for laboratory rodents [24]. Protein level in the control and treatment diets was 18%. A total of 6 isocaloric diets were formulated and have been described by us recently [25]. Four diets were formulated for examining the effects of 2 different levels of folic acid (ie, 2 and 8 mg folic acid per kilogram diet) during pregnancy in both the presence and absence of vitamin B_{12}. In addition, 2 more diets were formulated to examine the effects of omega 3 fatty acid (DHA + EPA) supplementation on both vitamin B_{12}–deficient groups. Vitamin B_{12} deficiency was obtained exclusively through dietary means. Animals were kept in special cages to prevent coprophagy. Vitamin-free casein was used for all treatment diets.

Thus, there were a total of 6 groups: normal folic acid (control), normal vitamin B_{12} (NFB); normal folic acid, vitamin B_{12} deficient (NFBD); normal folic acid, vitamin B_{12} deficient, omega 3 fatty acid supplemented (NFBDO); excess folic acid, normal vitamin B_{12} (EFB), excess folic acid, vitamin B_{12} deficient (EFBD); excess folic acid, vitamin B_{12} deficient, omega 3 fatty acid supplemented (EFBDO).

The lowest level, that is, 2 mg/kg, represents the normal level of folic acid used in the control diet as per the current AIN 93 guidelines, whereas 8 mg/kg is roughly 4 times the requirement of a normal rat. This is in accordance with the fact that folic acid requirement for Indian pregnant woman is set at 400 μg/d, which is 4 times the requirement of a nonpregnant woman. The level of omega 3 fatty acid supplementation was chosen to have an omega 6 to omega 3 ratio of 1:1, which is considered to be the ideal ratio [26]. All diets contained tertiary butyl hydroquinone to prevent oxidation [24,27,28]. Diets were stored at refrigeration temperatures,

Table 1 – Composition of the diets

Diets (g/kg)	NFB	NFBD	EFB	EFBD	NFBDO	EFBDO
Corn starch	398	398	398	398	398	398
Casein (> 85% protein)	200	200	200	200	200	200
Dextrinized starch	132	132	132	132	132	132
Sucrose	100	100	100	100	100	100
Soya bean oil	70	70	70	70	25	25
Fish oil	0	0	0	0	45	45
Fiber	50	50	50	50	50	50
Mineral mixture [a]	35	35	35	35	35	35
Vitamin mixture [b]	10	10	10	10	10	10
Folic acid (mg)	2	2	8	8	2	8
B_{12} (in 0.1% mannitol) (μg)	2.5	0	2.5	0	0	0
Cystine	3	3	3	3	3	3
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5
Tertiary butyl	0.014	0.014	0.014	0.014	0.014	0.014
Total energy (kJ)	1.57	1.57	1.57	1.57	1.57	1.57

[a] Mineral mixture (in grams per kilogram mixture):calcium carbonate, 357; potassium phosphate, 196; potassium citrate, 70.78; sodium chloride, 78; potassium sulfate, 46.6; magnesium oxide, 24; ferric citrate, 6.06; zinc carbonate, 1.65; manganous carbonate, 0.63; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenate, 0.01; ammonium paramolybdate, 0.007; sodium metasilicate, 1.45; chromium potassium sulfate, 0.275; lithium chloride, 0.01; boric acid, 0.08; sodium fluoride, 0.06; nickel carbonate, 0.03; ammonium vanadate, 0.006; sucrose, 221.02.

[b] Vitamin mixture (in grams per kilogram mixture): nicotinic acid, 3; calcium pantothenate, 1.6; pyridoxine-HCl, 0.7; thiamin-HCl, 0.6; riboflavin, 0.6; D-biotin, 0.02; vitamin B_{12} (in 0.1% mannitol), 2.5; vitamin E, 15; vitamin A, 0.8; vitamin D-3, 0.25; vitamin K, 0.075; folic acid, 0.2 (control); and sucrose, 974.655 (used to make total weight of the vitamin mixture 1 kg).

and fresh diet was provided daily. The ingredients were weighed on a Shimadzu electronic balance (Japan) with least count of 0.001 g, mixed thoroughly, and molded into cylindrical pellets. All animals had free access to food and water.

2.4. Observations recorded

During pregnancy, dam weights were recorded on 0, 7, 14, and 20 days to obtain weight gains. The litter weights and size were recorded at birth and subsequently at 5, 7, 12, 14, and 21 days of age.

2.5. Gastric milk volume

Gastric milk was obtained from the pups after being separated from their dams. Before collection of milk, they were exposed to anesthetic ether; and then milk was obtained from the stomach with the help of a syringe, snap frozen in liquid nitrogen, and then stored frozen at –80°C until analysis. Milk volume was determined as per the method of Sampson and Jansen [29] in which the weight of animals from the fifth to the 12th day after birth was recorded. The milk production (grams per pup per day) was calculated using the equation [(0.0332 + (weight of pups on day 12)) + weight gain of pups (day 12 – day 5)], where weight of pups on day 12 is the weight on the last day of the period and weight gain is the difference between the initial weight, that is, day 5, and the weight on the last day, that is, day 12 of the period.

2.6. Analysis of gastric milk fatty acids

One hundred micrograms of gastric milk was used for estimation of fatty acids. Fatty acids were analyzed as methyl esters by the method recently described by us [30]. Briefly, transesterification of the phospholipid fraction was carried out using HCl-methanol. Methyl esters were separated and quantified using a Perkin-Elmer gas chromatograph (SD 2330, 30-m capillary column; Supelco, PA). Helium was used as carrier gas at 1 mL/min. Oven temperature was held at 150°C for 10 minutes programmed to rise from 150°C to 220°C at 10°C/min and at 220°C for 10 minutes. The detector temperature was 275°C, and the injector temperature was 240°C. Retention times and peak areas were automatically computed. Individual fatty acids were identified by comparison of peaks with peaks of standard fatty acid methyl esters (Sigma, St Louis, MO). The data included are only on critically important LCPUFAs and major fatty acids. The omega 3 fatty acids included α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and DHA, whereas omega 6 fatty acids included linoleic acid (LA), γ-linolenic acid, dihomo-γ-linolenic acid, docosapentaenoic acid, and AA. Monounsaturated fatty acids included myristoleic acid, palmitoleic acid, oleic acid, and nervonic acid. Saturated fatty acids included myristic acid, palmitic acid, and stearic acid. Fatty acids were expressed in grams per 100 g fatty acid, that is, percentage of total fatty acids as 100%.

2.7. Statistical methods

Litter means were used as the unit of analysis. Values are mean ± SD. The data were analyzed using SPSS/PC+ package (Version 11.0; Chicago, IL). Mean values of the estimates of various parameters for the treatment groups were compared with those of control group at conventional levels of significance using least significance difference estimated from 1-way analysis of variance.

3. Results

3.1. Reproductive performance

Weight gain of dams during pregnancy was similar in groups, indicating that alteration in maternal micronutrients did not influence weight gain. In addition, it also had no effect on both litter size and litter weight at birth. Furthermore, omega 3 fatty acid supplementation too showed no effect on weight gains

during pregnancy and on litter size and litter weights at birth, as has been reported by us recently [25].

3.1.1. *Weight gain of pups during lactation*

The pup weight gain was lower in the NFBD group as compared with the NFB group only on day 5 ($P < .05$) and day 7 ($P < .01$) of lactation. In contrast, excess folic acid supplementation to a vitamin B_{12} (EFBD)-deficient diet increased ($P < .05$) the pup weight gain as compared with that in the NFBD group only on day 7 of lactation. The pup weight gain from the EFB group was comparable to that of the NFB group throughout the period of lactation (Fig. 1). The pup weight gain was lower in the NFBDO group as compared with the NFB group only on day 5 ($P < .05$) and day 7 ($P < .01$) of lactation. Weight gain of pups in the EFBDO group was similar to that in the NFB group at all time points.

3.2. *Gastric milk volume*

Gastric milk volume in the folic acid–supplemented group (EFB) was similar to that of control. However, there was a reduction in total gastric milk volume in both vitamin B_{12}–deficient groups, that is, NFBD ($P < .05$) and EFBD ($P < .01$), as compared with control. Further supplementation with omega 3 fatty acid to the B_{12}-deficient improved the milk volume only in the EFBDO group ($P < .05$) as compared with the EFB group (Fig. 2).

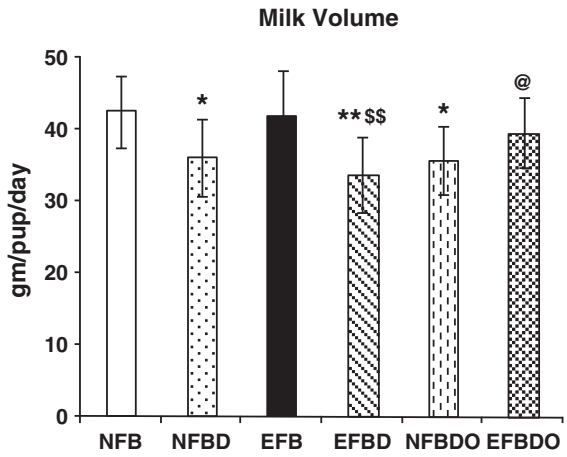

Fig. 2 – Gastric milk volume. $^{*}P < .05$ and $^{**}P < .01$, compared with control; $^{\$\$}P < .01$, compared with EFB; $^{@}P < .05$, compared with EFBD.

3.3. *Gastric milk fatty acids*

Folic acid supplementation (EFB group) increased all fatty acids including ALA, LA, DHA, and AA levels ($P < .01$) as compared with those in control. In contrast, folic acid supplementation in the absence of vitamin B_{12} (EFBD) reduced ($P < .05$) DHA and AA, the critical fatty acids, as compared with those in the EFB group, although they still remained higher ($P < .01$) as compared with those in control. A similar trend was also seen in case of saturated fatty acids. Omega 3 fatty acids supplementation to both the NFBD and the EFBD groups as expected increased ($P < .01$) levels of DHA ($P < .05$), although it reduced levels of AA (Table 2). Omega 3 fatty acid supplementation to both the NFBD and the EFBD groups increased ($P < .01$) levels of palmitoleic (NFBDO, 4.39 ± 0.71 g/100 g fatty acids; EFBDO, 3.95 ± 0.86 g/100 g) and oleic acid concentrations

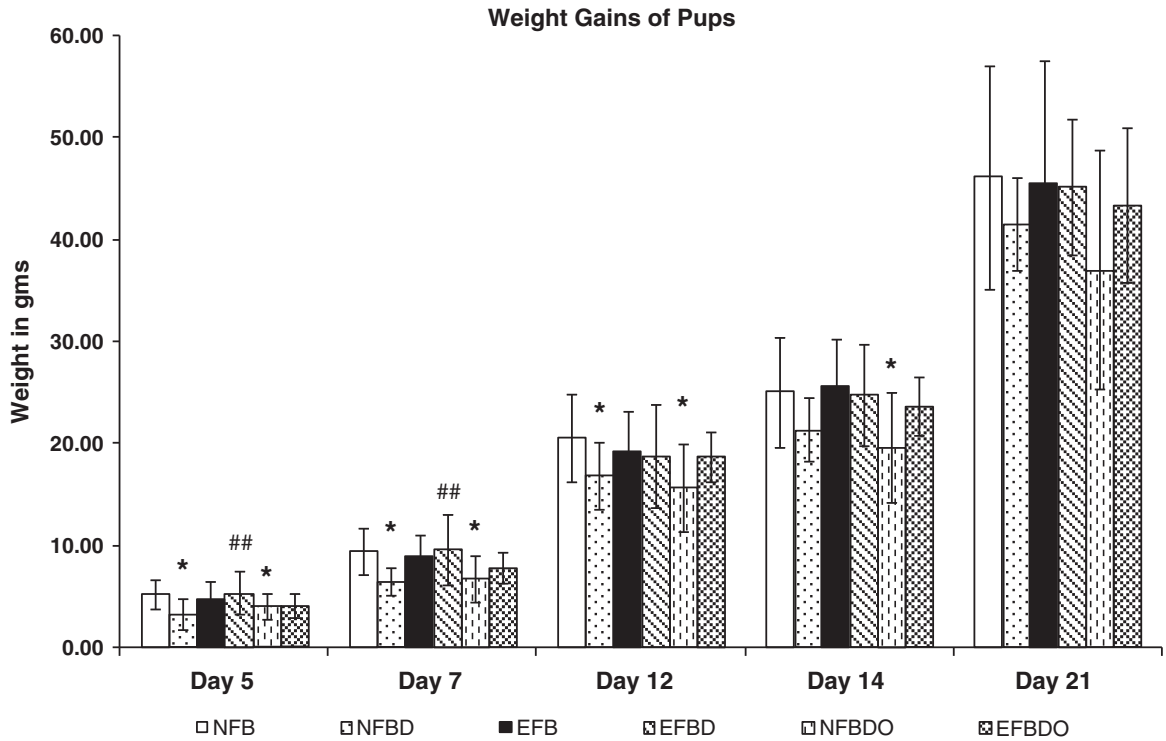

Fig. 1 – Weight gain of pups during lactation. $^{*}P < .05$, compared with control; $^{\#\#}P < .01$, compared with NFBD.

Table 2 – Milk fatty acids between different groups (in grams per 100 g fatty acids)

	NFB (n = 8)	NFBD (n = 8)	EFB (n = 8)	EFBD (n = 8)	NFBDO (n = 8)	EFBDO (n = 8)
DHA [22:6(n-3)]	0.72 ± 0.09	0.60 ± 0.13*	1.15 ± 0.17**	0.98 ± 0.11**## $	4.51 ± 0.76** ##	4.72 ± 0.56** $ @@
AA [20:4(n-6)]	4.13 ± 1.20	4.06 ± 1.26	6.11 ± 1.05**	5.57 ± 0.94**##	2.99 ± 0.69*#	2.61 ± 0.55**$ @@
LA [18:2(n-6)]	20.77 ± 4.38	23.50 ± 2.58	25.39 ± 2.29**	25.79 ± 2.56**	14.24 ± 2.52**##	18.17 ± 1.84$ @@
ALA [18:3(n-3)]	1.41 ± 0.28	1.67 ± 0.42	1.87 ± 0.26**	2.10 ± 0.38**##	0.71 ± 0.27**##	0.97 ± 0.17** $ @@
Omega 3	2.50 ± 0.46	2.63 ± 0.58	3.56 ± 0.37**	3.65 ± 0.45**##	7.94 ± 1.46**##	8.53 ± 1.14**$@@
Omega 6	6.31 ± 1.51	6.36 ± 1.64	9.09 ± 1.49**	8.46 ± 1.26 **##	21.52 ± 2.29**##	25.23 ± 2.21 $ @@
Saturated fatty acids	41.26 ± 7.42	35.68 ± 4.14**	29.50 ± 2.31**	30.20 ± 1.12**#	36.6 ± 4.38*	34.26 ± 3.58**$
Monounsaturated	22.54 ± 3.96	23.75 ± 2.63	24.56 ± 2.29	24.64 ± 3.22	31.59 ± 3.22**##	29.4 ± 2.84**$@@
AA:DHA ratio	5.67 ± 1.23	6.63 ± 0.80*	5.44 ± 1.26	5.73 ± 1.09	0.68 ± 0.20**##	0.56 ± 0.14**$@@

Omega 3: (ALA + EPA + DHA); omega 6: (LA + γ-linolenic acid + dihomo-γ-LA + AA + docosapentaenoic acid); monounsaturated: (myristoleate + palmitoleate + oleic acid + nervonic acid); saturated: (myristic acid + palmitic acid + stearic acid).
*P < .05 and **P < .01, compared with control.
#P < .05 and ##P < .01, compared with NFBD.
$P < .05 and $P < .01, compared with EFB.
@P < .05 and @@P < .01, compared with EFBD.
ωP < .05 and ωωP < .01, compared with NFBDO.

(NFBDO, 26.80 ± 2.70 g/100 g fatty acids: EFBDO, 25.11 ± 2.58 g/100 g) as compared with those in the control group (palmitoleic acid, 2.31 ± 1.41 g/100 g fatty acids; oleic acid, 19.60 ± 3.22 g/100 g fatty acids).

4. Discussion

To our knowledge, this is the first report that has examined the effect of maternal folic acid supplementation in the presence and absence of vitamin B_{12} on gastric milk volume and essential polyunsaturated fatty acid levels. Our results indicate that (1) maternal vitamin B_{12} deficiency at both normal and excess folic acid levels reduced milk volume, (2) gastric milk fatty acids (ALA, LA, DHA, and AA) were reduced when maternal folic acid was given in the absence of vitamin B_{12}, and (3) omega 3 fatty acid supplementation to vitamin B_{12}–deficient groups at both normal and excess levels of folic acid increased both milk volume and DHA but reduced AA levels.

As a national policy in developing countries like India, folic acid is routinely given to all pregnant women; or many countries have implemented the flour fortification program. However, there are no studies that have examined the effect of this supplementation on breast milk volume and LCPUFA composition in milk. In addition, there are no studies that have examined the effect of maternal vitamin B_{12} deficiency on milk volume.

In our study, excess maternal folic acid supplementation to a vitamin B_{12}–deficient diet increased milk DHA levels as compared with those in control, suggesting that smaller milk yield may maintain quality of milk as suggested by earlier reports [31]. This reduced milk volume probably results in the lower weight gain of pups in these groups through the initial period of lactation. A study in mice indicates that milk is the only source of nutrients for suckling pups and that reduced milk fat concentration might affect growth and survival of the offspring during the suckling period [32]. It has been suggested that production of milk is closely linked to nutritional state especially vitamin B_{12} deficiency, although the mechanisms by which changes in nutritional state are signaled to the mammary glands are poorly understood [33,34]. It has been reported that methylmalonyl-CoA mutase, a vitamin B_{12}-dependent enzyme that plays a part in the conversion of propionate to succinyl-CoA, is required for the large amount of energy and glucose needed during lactation and also to sustain milk production [35]. When omega 3 fatty acids were supplemented to a maternal diet imbalanced in folic acid and vitamin B_{12}, there was an improvement in gastric milk volume.

In our study, gastric milk fatty acids (ALA, LA, DHA, and AA) were reduced when maternal folic acid was given in the absence of vitamin B_{12}. The supplementation of omega 3 fatty acids to these diets led to an increase in DHA levels in gastric milk but decreased AA concentrations. It may be possible that the decrease in AA may be compensated by the increase in milk volume. Further benefits to the growth of the offspring could also be possible because the relative ratio of AA to DHA was reduced in the omega 3 fatty acids–supplemented group. Omega 3 and omega 6 LCPUFAs are important for infant growth and brain development. Docosahexaenoic acid plays an important role in neurodevelopment, whereas both AA and DHA are important for infant growth and overall development. In adult humans, although the recommended daily level is 500 mg, supplementation is often done at 1 or 2 g, which is high and may lead to a relative reduction in AA levels. It is therefore important to have a balance of both DHA and AA (AA:DHA ratios, 1:1 to 1:10).

Gastric milk of dams fed omega 3 fatty acid are reported to increase levels of all omega 3 fatty acids (ALA, EPA, n-3 DPA, and DHA) [36]. A number of human studies also report increases in breast milk DHA content after DHA supplementation to lactating women [36-38]. In our study, supplementation of omega 3 fatty acids increased both palmitoleic and oleic acid. It has been reported that EPA increased the ability of glucose to suppress fatty acid oxidation and regulated pathways involved in carbohydrate metabolism [39].

It is known that maternal status or intake of the B vitamins (except folate), vitamin A, selenium, and iodine strongly affects the amount of these nutrients secreted in breast milk [40]. Our results suggest that maternal micronutrients like folate and vitamin B_{12} and omega 3 fatty acids also play a key role in determining both the quantity and quality of milk, although the mechanisms need to be understood.

It is known that Δ6 desaturase (Δ^6) and Δ5 desaturase (Δ^5) are required for the synthesis of highly unsaturated fatty acids such as EPA and DHA (synthesized from ALA) and AA (synthesized from LA). It has been reported that there is an adaptive mechanism to synthesize LCPUFA through an increase in the expression of sterol regulatory element binding protein 1 in mammary gland that in turn increases the expression of Δ^5 and Δ^6 [41]. Studies in our department are therefore being undertaken to examine the effect of imbalance in maternal micronutrients on the expression of Δ^5 and Δ^6 genes.

Early postnatal interplay between nutrition, growth patterns, and metabolic and epigenetic phenomena is crucial in determining subsequent health [42]. Human breast milk is universally recognized as the optimal food for infants during early postnatal life. Fat is a critical component of breast milk, which provides energy and nutrients like DHA and AA that are key to the development of the central nervous system but cannot be synthesized de novo by the infant [43]. It is possible that variation in the amounts of micronutrients consumed during pregnancy can ultimately affect infant growth and development. It has been reported that DHA, uridine, and choline found in mother's milk are part of a regulatory mechanism through which plasma composition influences brain development [44]. We have earlier reported that maternal folic acid supplementation at marginal protein levels decreased brain DHA levels [45].

Our results suggest that maternal micronutrients like folate and vitamin B_{12} play a key role in determining both the quantity and quality of milk. Thus, because of the complex nature of interrelationships between single-carbon metabolism and other vital cell biological systems and functions, further studies need to examine the cellular and molecular mechanism underlying the effects of micronutrients such as folic acid and vitamin B_{12} on the essential polyunsaturated fatty acid composition in breast milk.

In conclusion, our findings for the first time demonstrate that maternal vitamin B_{12} deficiency reduces milk volume and DHA levels. Supplementation with omega 3 fatty acids to the vitamin B_{12}–deficient diet improves both milk volume and DHA levels. Our findings are of significance in the Indian population primarily consuming a vegetarian diet that is deficient in vitamin B_{12} and highlights the interactions of these micronutrients with milk production as well as the composition of LCPUFAs and mammary gland development.

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REFERENCES

[1] Dangat K, Mehendale S, Yadav H, et al. Long chain polyunsaturated fatty acid composition of breast milk in pre-eclamptic mothers. Neonatology 2010;97:190-4.
[2] Kilari A, Mehendale S, Dangat K, et al. Long chain polyunsaturated fatty acids in mothers and term babies. J Perinat Med 2009;37:513-8.
[3] Clandinin MT, Chappell JE, Leong S, et al. Intrauterine fatty acid accretion rates in infant brain: implications for fatty acid requirements. Early Hum Dev 1980;4:121-9.
[4] Crawford MA. Commentary on the workshop statement. Essentiality of and recommended dietary intakes for omega-3 and omega-6 fatty acids. Prostaglandins Leukot Essent Fatty Acids 2000;63:131-4.
[5] Uauy R, Mena P, Wegher B, et al. Essential fatty acids in early life: structural and functional role. Proc Nutr Soc 2000;59:3-15.
[6] Vestergaard M, Obel C, Henriksen TB, et al. Duration of breastfeeding and developmental milestones during the latter half of infancy. Acta Pediatr 1999;88:1327-32.
[7] Gustafsson PA, Duchén K, Birberg U, et al. Breastfeeding, very long polyunsaturated fatty acids (PUFA) and IQ at 6/4 years of age. Acta Paediatr 2004;93:1280-7.
[8] Bhate V, Deshpande S, Bhat D, et al. Vitamin B12 status of pregnant Indian women and cognitive function in their 9-year-old children. Food Nutr Bull 2008;29:249-54.
[9] Brenna JT, Varamini B, Jensen RG, et al. Am J Clin Nutr 2007;85:1457-64.
[10] Agostoni C, Giovannini M. Cognitive and visual development: influence of differences in breast and formula fed infants. Nutr Health 2001;15:183-8.
[11] Birch EE, Hoffman DR, Uauy R, et al. Visual acuity and essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatr Res 1998;44:201-8.
[12] Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr 2007;85:614S-20S.
[13] Innis SM. Fatty acids and early human development. Early Hum Dev 2007;83:761-6.
[14] Rocquelin G, Tapsoba S, Dop MC, et al. Lipid content and essential fatty acid (EFA) composition of mature Congolese breast milk are influenced by mothers' nutritional status: impact on infants' EFA supply. Eur J Clin Nutr 1998;52:164-71.
[15] Nasser R, Stephen AM, Goh YK, et al. The effect of a controlled manipulation of maternal dietary fat intake on medium and long chain fatty acids in human breast milk in Saskatoon, Canada. Int Breastfeed J 2010;19:3.
[16] Rasmussen MM, Clemmensen D. Folic acid supplementation in pregnant women. Dan Med Bull 2010;57:A413-4.
[17] Freire WB, Hertrampf E, Cortes F. Effect of folic acid fortification in Chile: preliminary results. Eur J Pediatr Surg 2000;10:42-3.
[18] Metz J, Sikaris KA, Maxwell EL, et al. Changes in serum folate concentrations following voluntary food fortification in Australia. Med J Aust 2002;76:90-1.
[19] Indian Council of Medical Research. Evaluation of the National Nutritional Anaemia Prophylaxis Programme, an ICMR Task Force study. New Delhi: Central Electric; 1989.
[20] Yajnik CS, Deshpande SS, Lubree HG, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India 2006;54:775-82.
[21] Carmel R. Efficacy and safety of fortification and supplementation with vitamin B12: biochemical and physiological effects. Food Nutr Bull 2008;29:S177-87.
[22] Smith SE, Kinney HC, Swoboda KJ, et al. Subacute combined degeneration of the spinal cord in cblC disorder despite treatment with B12. Mol Genet Metab 2006;88:138-45.
[23] Kale A, Napahde N, Sapkale S, et al. Reduced folic acid, vitamin B12 and docosahexaenoic acid and increased homocysteine and cortisol in never-medicated schizophrenia patients. Psychiatry Res 2010;175:47-53.
[24] Reeves P, Nielsen F, Fahey G. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-51.

[25] Roy S, Kale A, Dangat K, et al. Maternal micronutrients (folic acid and vitamin B_{12}) and omega 3 fatty acids: implications for neurodevelopmental risk in the rat offspring. Brain Dev 2011 [Epub ahead of print].
[26] Simopoulos AP. Essential fatty acids in health and chronic disease. Am J Clin Nutr 1999;70:560S-9S.
[27] Fritsche KL, Johnston PV. Rapid autoxidation of fish oil in diets without added antioxidants. J Nutr 1988;118:425-6.
[28] Gonzalez MJ, Gray JI, Schemmel RA, et al. Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants. J Nutr 1992;122:2190-5.
[29] Sampson DA, Jansen GR. Measurement of milk yield in the lactating rat from pup weight and weight gain. J Pediatr Gastroenterol Nutr 1984;3:613-7.
[30] Ranjekar P, Hinge M, Hegde M, et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry Res 2003;121:109-12.
[31] Weisstaub AR, Zeni S, Maria L, et al. Influence of maternal dietary calcium levels on milk zinc, calcium and phosphorus contents and milk production in rats. J Trace Elem Med Biol 2006;20:41-7.
[32] Robert R, Daniela S, Andre' M, et al. Dietary conjugated linoleic acids lower the triacylglycerol concentration in the milk of lactating rats and impair the growth and increase the mortality of their suckling pups. J Nutr 2004;134:3327-34.
[33] Graulet B, Matte J, Desrochers A, et al. Effects of dietary supplements of folic acid and vitamin B12 on metabolism of dairy cows in early lactation. J Dairy Sci 2007;90:3442-55.
[34] Stewart KW, Cooper GJ, Davis SR. Coordination of mammary metabolism and blood flow after refeeding in rats. J Dairy Sci 2009;92:1543-53.
[35] Girard CL, Lapierre H, Matte JJ, et al. Effects of dietary supplements of folic acid and rumen protected methionine on lactational performance and folate metabolism of dairy cows. J Dairy Sci 2005;88:660-70.
[36] Destaillats F, Joffre C, Acar N, et al. Differential effect of maternal diet supplementation with alpha-linolenic acid or n-3 long-chain polyunsaturated fatty acids on glial cell phosphatidylethanolamine and phosphatidylserine fatty acid profile in neonate rat brains. Nutr Metab 2010;14:2.
[37] Gibson RA, Neumann MA, Makrides M, et al. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr 1997;51:578-84.
[38] Henderson RA, Jensen RG, Lammi-Keefe CJ, et al. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:863-9.
[39] Hessvik N, Bakke S, Fredriksson K, et al. Metabolic switching of human myotubes is improved by n-3 fatty acids. J Lipid Res 2010;51:2090-104.
[40] Jensen CL, Maude M, Anderson RE, et al. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr 2000;71:292S-9S.
[41] Maricela R, Armando R, Tovar, et al. Synthesis of long-chain polyunsaturated fatty acids in lactating mammary gland: role of D5 and D6 desaturases, SREBP-1, PPARα, and PGC-1. J Lipid Res 2008;47:554-60.
[42] Allen LH. Multiple micronutrients in pregnancy and lactation: an overview. Am J Clin Nutr 2005;81:1206S-12S.
[43] Neu J, Hauser N, Douglas-Escobar M. Postnatal nutrition and adult health programming. Semin Fetal Neonatal Med 2007;12:78-86.
[44] Wurtman R. Synapse formation and cognitive brain development: effect of docosahexaenoic acid and other dietary constituents. Metab Clin Exp 2008;57:S6-S10.
[45] Rao S, Joshi S, Kale A, et al. Maternal folic acid supplementation to dams on marginal protein level alters brain fatty acid levels of their adult offspring. Metab Clin Exp 2006;55:628-34.