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Maternal folic acid supplementation to dams on marginal protein level alters brain fatty acid levels of their adult offspring

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Abstract

Studies on fetal programming of adult diseases have highlighted the importance of maternal nutrition during pregnancy. Folic acid and long-chain essential polyunsaturated fatty acids (LC-PUFAs) have independent effects on fetal growth. However, folic acid effects may also involve alteration of LC-PUFA metabolism. Because marginal deficiency of LC-PUFAs during critical periods of brain growth and development is associated with risks for adult diseases, it is highly relevant to investigate how maternal supplementation of such nutrients can alter brain fatty acid levels. We examined the impact of folic acid supplementation, conventionally used in maternal intervention, on brain essential fatty acid levels and plasma corticosterone concentrations in adult offspring at 11 months of age. Pregnant female rats from 4 groups (6 in each) were fed with casein diets either with 18 g protein/100 g diet (control diet) or treatment diets that were marginal in protein (MP), such as 12 g protein/100 g diet supplemented with 8 mg folic acid (FAS/MP), 12 g protein/100 g diet without folic acid (FAD/MP), or 12 g protein/100 g diet (MP) with 2 mg folic acid. Pups were weaned to a standard laboratory diet with 18 g protein/100 g diet. All male adult offspring in the FAS/MP group showed lower docosahexaenoic acid ($P < .05$) as compared with control adult offspring (6.04 $\pm$ 2.28 vs 10.33 $\pm$ 0.86 g/100 g fatty acids) and higher n-6/n-3 ratio ($P < .05$). Docosahexaenoic acid levels in FAS/MP adult offspring were also lower ($P < .05$) when compared with the MP group. Plasma corticosterone concentrations were higher ($P < .05$) in male adult offspring from the FAS/MP group compared with control as well as the MP adult offspring. Results suggest that maternal folic acid supplementation at MP intake decreased brain docosahexaenoic acid levels probably involving corticosterone increase.

1. Introduction

The fact that low birth weight (LBW) increases the risk for adult diseases has underscored the importance of maternal nutrition during gestation. Recent epidemiologic studies suggest that LBW babies have a higher prevalence of noncommunicable diseases such as insulin resistance, type 2 diabetes mellitus, and ischemic heart disease during their adult life [1]. In many developing countries such as India, LBW continues to be a major public health problem, and maternal undernutrition during pregnancy is identified as one of the major factors responsible for LBW.

Short-term measures through nutrition interventions during pregnancy are undertaken in many populations to ameliorate the adverse effects of maternal undernutrition on birth weight. Studies with maternal supplementation of macronutrients, that is, energy and protein, have not yielded consistent results [2]. Recently, it has been shown that when maternal diets are limited in energy, micronutrients play important role in determining birth size [3]. One of the critical issues is to investigate the role of micronutrients in fetal programming of adult diseases. In particular, it remains to be investigated whether micronutrient supplementation during gestation can have beneficial effect in reducing the risk associated with noncommunicable diseases in adult offspring. Among the micronutrients, beneficial effects for iron and folic acid supplementation to pregnant undernourished women are well documented [4,5]. Particularly, maternal folate supplementation is the most popular intervention given during

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pregnancy to improve birth weight and has been extensively studied for its effect on neural tube formation and fetal growth [4,6].

There is also growing interest in the role of long-chain essential polyunsaturated fatty acids (LC-PUFAs), especially n-3 fatty acids, in promoting fetal growth [7]. In Faroese women, an estimated 104-g increase of birth weight was attributed to both a 4-day increase in gestational length and rapid fetal growth due to high n-3 fatty acid intake [8]. In another randomized control study, gestation was also observed to prolong by 4 days when pregnant women consumed a fish oil supplement [9]. Thus, maternal essential fatty acid status during pregnancy may be critical in fetal growth. In fact, subtle deficiencies in accretion of essential fatty acids into cell membranes are believed to be a mechanism making a child susceptible to metabolic derangements such as insulin resistance [10].

Although both folic acid and LC-PUFAs have independent effects on fetal growth, folic acid may also modify LC-PUFA metabolism [11]. It would therefore be worthwhile to investigate whether folic acid supplementation during gestation has any impact on brain fatty acid metabolism as brain development is vital during fetal adaptation to maternal undernutrition.

Maternal diets in developing countries are often inadequate in protein, and hence it would also be relevant to investigate whether folic acid supplementation at low or marginal protein levels during gestation can have impact on the brain LC-PUFA and subsequently on risk of noncommunicable diseases in adult offspring. Maternal dietary protein restriction during pregnancy has been shown to activate the hypothalamic-pituitary-adrenal axis (HPAA) [12]. This greater activation of the HPAA could translate into an elevated risk for cardiovascular diseases, diabetes, and hypertension [13] that ultimately leads to adult disease in offspring. Such effects of maternal malnutrition in offspring could be related to disturbances in the maternal and/or fetal hormonal environment. One of the indicators suggestive of subtle abnormalities in the programming of the HPAA is increased corticosterone concentrations [14]. Thus, the mechanisms of long-term pathophysiologic consequences of folic acid supplementation during gestation are not known and need to be studied.

The present study therefore examines the effect folic acid supplementation at marginal level of protein during pregnancy on the brain fatty acid profile and corticosterone concentrations in the Wistar rat adult offspring at 11 months of age.

2. Methods

All experimental procedures were in accordance with the guidelines of the institutional animal ethics committee. Animal maintenance and handling were in accordance with the National Institute of Nutrition, India, guidelines [15].

2.1. Diets

The composition of control diet was as per AIN 93–purified diets for laboratory rodents [16] and contained 18 g protein/100 g diet (Table 1). Three treatment diets that were marginal in protein (MP) were formulated to observe the effects of folic acid supplementation during pregnancy, that is, one with folic acid deficiency (FAD/MP) and the other with folic acid supplementation (FAS/MP). A group with 12% protein (MP) diet was included to observe the effects of folic acid supplementation at marginal protein level (12%). This would allow us to attribute the observed effects to folic acid supplementation and would rule out that they are not due to lower protein level in the treatment diets. All treatment diets were isoenergetic and their composition is given in Table 1. Because of the lower protein level in treatment diets, the amount of cornstarch was increased to 45.75 g/100 g diet. Vitamin-free casein was used for treatment groups. The composition of soya bean oil is given in Table 2.

Control rats received 2 mg folic acid per kilogram of diet [16]. Folic acid deficiency was obtained exclusively through dietary means rather than using folic acid antagonists or antibiotics. This more closely resembles the overall effects of folic acid deprivation upon pregnancy as it might occur in

Table 1
Composition of diets

Diets	g/100 g diet			
	Control	FAD/MP	FAS/MP	MP
Cornstarch	39.8	45.8	45.8	45.8
Casein (>85% protein)	20.0	14.0	14.0	14.0
Dextrinized starch	13.2	13.2	13.2	13.2
Sucrose	10.0	10.0	10.0	10.0
Soya bean oil	7.0	7.0	7.0	7.0
Fiber	5.0	5.0	5.0	5.0
Mineral mix[a]	3.5	3.5	3.5	3.5
Vitamin mix[b]	1.0	1.0	1.0	1.0
L-Cystine	0.3	0.3	0.3	0.3
Choline bitartate	0.25	0.25	0.25	0.25
Tertiary butyl hydroquinone	0.0014	0.0014	0.0014	0.0014
Total energy (kJ)	1.57	1.57	1.57	1.57

Control diet, 18 g protein/100 g diet; FAS/MP, 12 g protein/100 g diet supplemented with 8 mg folic acid; FAD/MP, 12 g protein/100 g diet without folic acid; MP, 12 g protein/100 g diet with 2 mg folic acid.

[a] Mineral mixture (g/kg mix): 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 (g/kg mix): nicotinic acid, 3, Ca pantothenate, 1.6; pyridoxine-HCl, 0.7; Thiamin-HCl, 0.6; riboflavin 0.6; D-biotin, 0.02; vitamin B_{12} (0.1% in mannitol), 2.5; vitamin E, 15; vitamin A, 0.8; vitamin D_3, 0.25; vitamin K, 0.075; folic acid: control, 0.2; FAD, 0; FAS, 0.8; MP, 0.2. Sucrose (974.655) was used to make total weight of the vitamin mixture to 1 kg.

Table 2
Fatty acid composition of soybean oil fed to dams throughout pregnancy

Fatty acid (g/100 g fatty acids)	Soybean oil
Docosahexaenoic acid [22:6(n-3)]	0
Eicosapentaenoic acid [20:5(n-3)]	0
Arachidonic acid [20:4(n-6)]	0
Linoleic acid [18:2(n-6)]	54.0
γ-Linoleic acid [18:3(n-6)]	0
α-Linolenic acid [18:3(n-3)]	7.5
Oleic acid [18:1(n-9)]	22.0
Stearic acid [18:0]	4.1
Palmitic acid [16:0]	11.0
Myristic acid [14:0]	Trace

humans rather than using a drug to produce deficiency. Folic acid–supplemented diet was enriched with 8 mg folic acid per kilogram of diet. This is roughly 4 times the requirement of a normal rat. This is in accordance with the fact that folic acid requirement for an Indian pregnant woman is 400 μg/d, which is 4 times the requirement of a nonpregnant woman.

2.2. Experimental design

Virgin female Wistar rats weighing 200 to 250 g were obtained from the animal house of Agharkar Research Institute, Pune, India, and bred with a female-male ratio of 3:1. Pups born were separated according to sex on day 21 and 24 female pups were randomly selected. The temperature was maintained at 22°C on a controlled 12-hour light and 12-hour dark cycle with appropriate ventilation system. They were given control diet for 3 months and kept for breeding. On confirmation of pregnancy (through sperm-positive vaginal smears), that is, day 0 of pregnancy, they were randomly allocated to 1 of the 4 groups (n = 6 per group). After delivery the litters in each group were culled to 8 pups each. The treatment dams received the control diet (18 g protein/100 g diet) after delivery and the pups were also weaned onto the same diet. Daily food intake during pregnancy and lactation and weekly body weights were recorded. The adult offspring from each group were killed by cardiac puncture at 11 months of age to study the effects of dietary treatments in utero on the brain LC-PUFA in the offspring.

2.3. Body and organ weights

Weekly weights of all animals were recorded. The weights of 2 important vital organs such as brain, which is rich in n-3 fatty acids, and liver were also recorded. Whole brain and liver were weighed on a Mettler balance (Afcoset, Mumbai, India) with a sensitivity of 0.001 g. Brain and liver weights were expressed as absolute as well as relative to body weight.

2.4. Blood samples

The adult offspring were food deprived overnight before dissection, and blood samples were taken the next morning via heart puncture under anesthesia with diethyl ether. Blood samples were centrifuged, plasma separated, and corticosterone estimated by the radioimmunoassay method [17].

2.5. Brain fatty acid analysis

A vertical section was made, and one half of the fresh brain was minced and homogenized in phosphate-buffered saline (pH 7.5) using a Teflon glass homogenizer on ice. The homogenate was centrifuged at 10 000 rpm at 4°C for 20 minutes. The membranes were diluted in 5-mL phosphate-buffered saline and stored at −20°C. Methyl esters were prepared by the method of Manku et al [18]. Briefly, trans-esterification of the phospholipid fraction was carried out using HCl-methanol. These were separated and quantified using a Shimadzu (GC-17A) gas chromatograph (SP-2330, 30-m capillary column, Supelco, Bellefonte, PA). Nitrogen was used as carrier gas at 1 mL/min. Oven temperature was maintained at 175°C for 15 minutes and programmed to rise from 175°C to 220°C at 10°C/min and at 220°C for 10 min. The detector temperature was 275°C and the injector temperature was 240°C. Retention times and peak areas were automatically computed. Peaks were identified by comparison with standard fatty acid methyl esters (Sigma, St Louis, MO).

2.6. Statistical methods

Values are expressed as mean ± SD. The data were analyzed using SPSS/PC+ package (version 11.0, SPSS, Chicago, IL). The FAD/MP and FAS/MP treatment groups were compared with the control group as well as MP group by analysis of variance and the post hoc least significant difference test. These comparisons were done separately for both the sexes. Differences were considered significant at $P < .05$.

3. Results

3.1. Feed intake

Female rats consumed around 10 to 13 g food per day before breeding. Feed intake increased to 15 to 16 g/d during pregnancy. Despite differences in protein levels, there was no significant difference in mean feed intake of dams between different groups both during pregnancy and lactation.

3.2. Weight gain during pregnancy

Dams in the FAD group had a significantly ($P = .006$) lower weight gain (92.8 ± 9.9 g) as compared with those in control group (117.4 ± 12.6 g). However, dams from the FAS/MP group had a mean weight gain of 113.9 ± 22.1 g, almost similar to that observed in the control group. Dams from the MP group also had comparable weight gain (116.2 ± 19.5 g). However, there was no significant difference in the duration of gestation or litter size among different groups. Mean litter weights for the FAD/MP and FAS/MP groups were comparable to the control group.

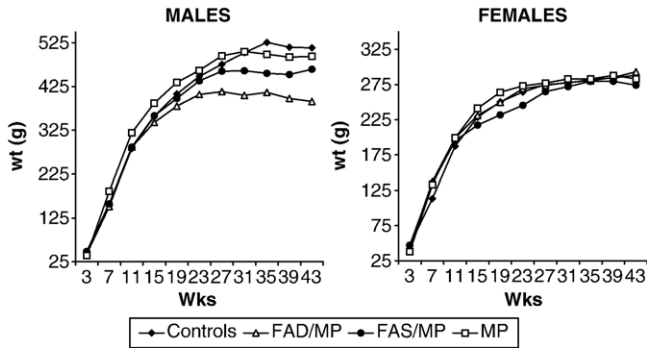

Fig. 1. Growth curve of animals.

3.3. Litter weights

The litter weights in all the treatment groups were comparable to the control group. The mean litter weight in the FAD/MP group was 59.1 ± 7.9 g compared with 61.9 ± 8.3 g in the control group. The FAS/MP group had a mean litter weight of 62.2 ± 6.3 g, whereas the offspring from MP group had litter weights of 61.4 ± 6.6 g.

3.4. Body weights

In the postweaning period, although the pups were given control diet, male adult offspring from the FAD/MP group had lower mean weights throughout ($P < .05$), but more so from 4 months onward (Fig. 1). The male adult offspring from the FAS/MP group had comparable growth, up to the seventh month, but were lower ($P < .05$) thereafter. Adult offspring in the MP group had comparable mean body weights compared with those of the control adult offspring. On the other hand, female adult offspring from all the groups had similar growth curves (Fig. 1).

3.5. Brain and liver weights

Absolute brain weights in both male and female adult offspring were significantly ($P < .05$) lower for adult offspring in the FAD/MP group (1.74 ± 0.05 and 1.71 ± 0.06 g for males and females, respectively) as compared with the control adult offspring (1.9 ± 0.06 and 1.80 ± 0.05 g for males and females, respectively). In contrast, the absolute brain weights in the FAS/MP and MP groups were comparable to the control group in both males and females (Table 3). The relative brain weights for adult offspring in all treatment groups in both sexes were comparable to the control group, indicating brain sparing. In the case of liver, the absolute weights of male adult offspring in the FAD/MP group were significantly lower ($P < .05$) (11.32 ± 1.99 g) as compared with the control adult offspring (14.25 ± 2.82 g). However, the absolute liver weights of males in the FAS/MP and MP groups were comparable to the control group. There was no change in absolute liver weights in females between groups. The relative liver weights for both males and females were also comparable to the control group.

3.6. Plasma corticosterone

Mean levels of plasma corticosterone for adult offspring in various groups are given in Table 3. Male adult offspring from the FAS/MP group had significantly ($P < .05$) higher concentrations of plasma corticosterone as compared with both the control and MP groups. Female adult offspring from the FAD/MP and FAS/MP groups had lower ($P < .05$) corticosterone level as compared with the MP group.

3.7. Brain fatty acid profile

Mean values of brain fatty acids of adult offspring in the 4 groups are shown in Table 4. The mean level of

Table 3
Absolute, relative weights of organs and plasma corticosterone levels in adult offspring at 11 months of age in different groups

	Control	FAD/MP	FAS/MP	MP
Males (n)	6	6	5	6
Brain				
Absolute weight (g)	1.90 ± 0.06	1.74 ± 0.05*	1.89 ± 0.08	1.90 ± 0.10
% Relative weights	0.39 ± 0.08	0.47 ± 0.07	0.46 ± 0.10	0.44 ± 0.05
Liver				
Absolute weight (g)	14.25 ± 2.82	11.32 ± 1.99*	13.16 ± 2.04	12.48 ± 4.11
% Relative weights	2.83 ± 0.15	2.99 ± 0.35	3.11 ± 0.25	2.65 ± 0.86
Females (n)	6	6	6	6
Brain				
Absolute weight (g)	1.80 ± 0.05	1.71 ± 0.06*	1.82 ± 0.05	1.78 ± 0.06
% Relative weights	0.65 ± 0.11	0.62 ± 0.03	0.64 ± 0.05	0.66 ± 0.05
Liver				
Absolute Wt (g)	7.92 ± 1.29	8.03 ± 1.12	8.11 ± 0.52	8.41 ± 1.56
% Relative weights	2.78 ± 0.23	2.89 ± 0.29	2.87 ± 0.25	3.11 ± 0.40
Males (n)	6	6	5	6
Corticosterone (μg/dL)	2.35 ± 0.58	1.90 ± 0.22*	3.34 ± 0.46* **	1.73 ± 0.66
Females (n)	6	6	6	6
Corticosterone (μg/dL)	1.25 ± 0.64	2.00 ± 0.21**	1.52 ± 0.77**	2.97± 0.81

* $P < .05$ for differences between control and treatment groups.
** $P < .05$ for differences between MP and treatment groups.

Table 4
Brain fatty acid profile of adult offspring from different groups (g/100 g fatty acids)

	Control	FAD/MP	FAS/MP	MP
Males				
n	6	6	5	6
Docosahexaenoic acid [22:6(n-3)]	10.33 ± 0.86	10.42 ± 1.21	6.04 ± 2.28* **	11.31 ± 1.79
Arachidonic acid [20:4(n-6)]	10.44 ± 1.88	11.00 ± 2.28	11.29 ± 3.81	9.26 ± 0.89
n-3 PUFA	12.53 ± 2.0	12.00 ± 1.34	7.43 ± 2.29* **	11.31 ± 1.79
n-6 PUFA	13.97 ± 1.81	14.58 ± 2.97	14.79 ± 3.79**	10.15 ± 1.09*
Oleic acid [18:1(n-9)]	17.07 ± 8.00	21.84 ± 3.94	32.18 ± 3.26*	23.49 ± 2.02*
Saturated fatty acids	39.37 ± 6.08	37.17 ± 5.03	41.42 ± 1.57	39.51 ± 1.19
n-6/n-3 ratio	1.14 ± 0.23	1.23 ± 0.27	2.10 ± 0.82* **	0.92 ± 0.19
Females				
n	6	6	6	6
Docosahexaenoic acid [22:6(n-3)]	10.15 ± 1.15	10.23 ± 0.94	11.01 ± 0.62	10.99 ± 2.54
Arachidonic acid [20:4(n-6)]	12.29 ± 4.14	10.27 ± 0.56**	10.33 ± 1.51**	8.56 ± 0.31*
Oleic acid [18:1(n-9)]	21.77 ± 2.31	23.13 ± 5.46	19.90 ± 1.41	23.04 ± 1.95
n-3 PUFA	10.88 ± 2.14	11.59 ± 0.74	11.79 ± 0.66	11.57 ± 2.22
n6 PUFA	15.87 ± 3.97	13.86 ±1.45**	14.47 ± 2.41**	10.01 ± 0.96
Saturated fatty acids	37.92 ± 2.51	36.52 ± 3.0	38.98 ± 2.62	37.99 ± 3.49
n-6/n-3 ratio	1.50 ± 0.47	1.20 ± 0.15**	1.23 ± 0.25**	0.90 ± 0.25*

* $P < .05$ for differences between control and treatment groups.
** $P < .05$ for differences between MP and treatment groups.

docosahexaenoic acid (DHA) (6.04 ± 2.28 g/100 g fatty acids) in the FAS/MP male adult offspring showed significant ($P < .05$) reduction when compared with the adult offspring from the control (10.33 ± 0.86 g/100 g fatty acids) as well as the MP group (11.31± 1.79 g/100 g fatty acids). Adult offspring from the other treatment groups, namely, MP and FAD/MP, did not show such reduction in DHA levels. In the case of female adult offspring, no reduction in DHA levels was observed.

Mean arachidonic acid levels in male adult offspring in treatment groups were not statistically significant compared with the control group. However, in female adult offspring, arachidonic acid levels (8.56 ± 0.31 g/100 g fatty acids) in the MP group were significantly ($P < .05$) reduced as compared with the control group (12.29± 4.14 g/100 g fatty acids) as well as the FAD/MP (10.27 ± 0.56 g/100 g fatty acids) and FAS/MP (10.33 ± 1.51) groups.

There was a significant increase in oleic acid levels ($P < .05$) in the male adult offspring from both the FAS/MP and MP groups, but similar increase was not seen in female adult offspring. The brain n-6/n-3 ratio in male adult offspring from the FAS/MP group was significantly ($P < .05$) higher compared with the control and MP groups, whereas among female adult offspring, n-6/n-3 ratio decreased in the FAS/MP group, but was not statistically significant.

4. Discussion

We have recently reported that folic acid deficiency during pregnancy resulted in insulin resistance in adult offspring [19]. Furthermore, this study showed that at marginal protein levels, despite folic acid supplementation, higher serum concentrations of glucose, low-density lipoprotein cholesterol, and triglycerides were seen in adult offspring, whereas this was not true with fish oil supplementation [19]. Data indicated that at MP, folic acid supplementation increased the insulin resistance and altered the lipid metabolism, whereas fish oil supplementation prevented insulin resistance, suggesting a role for ω-3 fatty acids in fetal growth. The present study showed that compared with FAD/MP, maternal folic acid supplementation at MP was comparable to the control or MP group in terms of litter weights, the postnatal growth of male (up to the seventh month) and female adult offspring, and brain and liver weights. However, male adult offspring showed a significant reduction in body weights after the seventh month and the brain fatty acids, especially DHA concentrations ($P < .05$). This was not observed in the control (MP, 12% protein diet) adult offspring, indicating that the decrease in DHA is not due to protein reduction, but due to folic acid supplementation at marginal protein level. As a result, the n-6/n-3 ratio in the brain was significantly higher in the FAS/MP group. This is in confirmation with reports that the ratio of membrane ω-3 to ω-6 PUFAs can be modulated by dietary intake [20]. Furthermore, the plasma corticosterone levels were increased ($P < .05$) only in the male FAS/MP adult offspring at 11 months of age. Our observations thus indicate that excess folic acid supplementation at marginal protein level during gestation results in adverse effects such as decline in brain DHA, drop in growth beyond the seventh month, and raised corticosterone levels, and these are a cause of concern.

Although folic acid is known to alter LC-PUFA metabolism [11], the effect of folic acid supplementation on the brain fatty acid profile is hardly examined. The mechanisms associated with effects of folic acid supplementation at MP may be complex. However, it is likely that supplementing folic acid to a low-protein diet may have interfered in the steps involved in the conversion of α-linolenic acid to eicosapentaenoic acid and to DHA, thus creating its deficiency. There was however no change in the brain fatty acid composition in females in this group. One of the possibilities is that sex hormones may be involved, that is, negative impact of testosterone [21] in LC-PUFA metabolism. It is well known that environmental exposures in prenatal life may imprint the rodent HPAA resulting in permanent modifications of the neuroendocrine responses to stress [22]. It has been shown that the hippocampus responds to adrenal steroids and is associated with changes in brain structure and neurochemistry [23]. It is possible that increased corticosterone levels may lead to a reduced expression of desaturases, which leads to reduced synthesis of DHA. Adrenal corticotrophin hormone is known to depress Δ^6 and Δ^5 desaturation activity in the rat liver [24].

Sex differences in relation to growth and development of organs have been reported earlier [25], which are attributed often to the fact that nutritional sensitivity in the male relates to the faster growth of tissues [26]. This observation is partially supported by the fact that, in our study, we observe a negative correlation ($r = -0.545$, $P < .05$) between weight at the time of weaning (day 22) and adult offspring brain (11 months) DHA content only in males, but weight gains were not correlated at any postnatal time point. There was an increase ($P < .05$) in the brain oleic acid levels in both the folic acid–supplemented and 12%-protein-diet–fed adult offspring. High blood oleic acid levels under conditions of undernutrition are proposed to be an adaptation to conserve glucose in the form of glycogen [27]. Female adult offspring from the MP group showed lower amino acid levels. Offspring from dams fed with 9% protein diet during pregnancy have been shown to have lower DHA and arachidonic acid levels after lactation. Our results confirm that marginal protein intakes during pregnancy result in alterations in arachidonic acid levels even at adult age.

Our result show, for the first time, that folic acid supplementation at marginal maternal protein levels was not able to maintain brain fatty acid profile and plasma corticosterone concentrations in male adult offspring. The significant reduction in the DHA levels in adult offspring from the folic acid–supplemented group was however not seen at 6 months [28]. Therefore, these findings call for future research to study optimum levels of folic acid supplementation during pregnancy for getting long-term benefits. In addition, it would be necessary to examine the effect of folic acid supplementation when dams are continued on marginal protein (12%) even during lactation and the adult offspring too continue on marginal protein throughout their life, which depicts the real-life conditions in rural poor communities in India. Because iron folic acid supplementation program is in operation over last 20 years in India, it may be worthwhile to examine plasma fatty acid profile in young rural adults and their mothers who had taken such supplementation during their pregnancy.

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References

[1] Godfrey KM, Barker DJ. Fetal nutrition and adult disease. Am J Clin Nutr 2000;71:1344S-52S.
[2] Kramer M. Effects of energy and protein intakes on pregnancy outcome: an overview of the research evidence from controlled clinical trials. Am J Clin Nutr 1993;58:627-35.
[3] Rao S, Yajnik CS, Kanade AN, Fall CHD. Intake of micronutrient rich foods in rural Indian mothers is associated with the size of their babies at birth: Pune Maternal Nutrition Study. J Nutr 2001;131:1217-24.
[4] Wagner C. Symposium on the subcellular compartmentation of folate metabolism. J Nutr 1996;126:1228S.
[5] O'Scholl T, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71:1295S-303S.
[6] Iyengar L, Rajalakshmi K. Effect of folic acid supplement on birth weight of infants. Am J Obstet Gynecol 1975;122:332-6.
[7] Saldeen P, Saldeen T. Women and omega-3 fatty acids. Obstet Gynecol Surv 2004;59:722-30.
[8] Olsen SF, Hansen HS, Sorensen TIA, Jensen B, Secher NJ, Sommer S, et al. Intake of marine fat, rich in (n3) polyunsaturated fatty acids may increase birth weight by prolonging gestation. Lancet 1986;2:367-9.
[9] Olson SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS, et al. Randomised controlled trial of effect of fish oil supplementation on pregnancy duration. Lancet 1992;339:1003-7.
[10] Sattar N, Berry C, Greer IA. Essential fatty acids in relation to pregnancy complications and foetal development. Br J Obstet Gynaecol 1998;105:1248-55.
[11] Pita ML, Delgado MJ. Folate administration increases n-3 polyunsaturated fatty acids in rat plasma and tissue lipids. Thromb Haemost 2000;84:420-3.
[12] Langley Evans SC, Welhan SJM, Sherman SC, Jackson AA. Weanling rats exposed to maternal low protein diets during discrete periods of gestation exhibit differing severity of hypertension. Clin Sci 1996;91:607-15.
[13] Traustadottir T, Bosch PR, Matt KS. Gender differences in cardiovascular and hypothalamic-pituitary-adrenal axis responses to psychological stress in healthy older adult men and women. Stress 2003;6:133-40.
[14] Phillips DIW, Barker DJP, Fall CHD, Seckl JR, Whorwood CB, Wood PJ, et al. Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? J Clin Endocrinol Metab 1998;83:757-60.
[15] Raghuramulu N, Nair KM, Kalyansundaram S. A manual of laboratory techniques. Hyderabad (India): National Institute of Nutrition; 1983. p. 277.

[16] Reeves PG, Nielsen FH, Fahey GC. 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-76 rodent diet. J Nutr 1993;123:1939-51.

[17] Ruder HJ, Guy RL, Lipsett MB. A radioimmunoassay for cortisol in plasma and urine. J Clin Endocrinol Metab 1972;35:219-24.

[18] Manku MS, Horrobin DF, Huang S, Morse N. Fatty acids in plasma and red cell membranes. Lipids 1983;18:906-8.

[19] Joshi S, Rao S, Golwilkar A, Patwardhan M, Bhonde R. Fish oil supplementation of rats during pregnancy reduces adult disease risks in their offspring. J Nutr 2003;133:3170-4.

[20] Haag M. Essential fatty acids and the brain. Can J Psychiatry 2003;48:195-203.

[21] Maria CA, De Alianz MJT. Influence of testosterone administration on the biosynthesis of unsaturated fatty acids in male and female rats. Lipids 1989;24:1014-9.

[22] Barbazanges A, Piazza PV, Le Moal M, Maccari S, et al. Maternal glucocorticoid secretion mediates long term effects of prenatal stress. J Neurosci 1996;16:3943-9.

[23] McEwen BS. Steroid hormones and brain development: some guidelines for understanding actions of pseudo hormones and other toxic agents. Environ Health Perspect 1987;74:177-84.

[24] Elisabet CM, Dumm IN, de Alanfz M, Marra CA, Brenner RR. ACTH depresses Δ^6 and Δ^5 desaturation activity in rat adrenal gland and liver. J Lipid Res 1987;28:1377-83.

[25] Desai M, Cowther NJ, Lucas A, Hales CN. Organ selective growth changes in the offspring of protein restricted mothers. Br J Nutr 1996;76:591-608.

[26] Lucas A, Baker BA, Desai M, Hales CN. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. J Nutr 1996;76:605-12.

[27] Tichelaar HY. Possible relevance of abnormal fatty acid metabolism in undernutrition: the relationship between oleic acid and growth. Med Hypotheses 2000;54:708-11.

[28] Joshi S, Rao S, Girigosavi S, Daware M, Kale A, Hegde M. Differential effects of fish oil and folate supplementation during pregnancy in rats on cognitive performance and serum glucose in their offspring. Nutrition 2004;20:465-72.