Differential Effects of Fish Oil and Folic Acid Supplementation During Pregnancy in Rats on Cognitive Performance and Serum Glucose in Their Offspring

Sadhana Joshi, PhD, Shobha Rao, PhD, Subhash Girigosavi, SSC, Mahadeo Daware, MSc, Anvita Kale, MSc, and Mahabaleshwar Hegde, PhD

From the Biometry and Nutrition Unit, Agharkar Research Institute, Pune, India; and the Interactive Research School for Health Affairs, Bharati Vidyapeeth, Pune, India

OBJECTIVE: We studied the effect of folic acid and polyunsaturated fatty acid supplementation during pregnancy in Wistar albino rats on cognitive performance and serum glucose concentrations in their pups.

METHODS: Pregnant female rats from four groups ($n = 6$/group) were fed casein diets with 18% protein and 2 mg of folic acid/kg of diet (group I), 12% protein and no folic acid (group II), 12% protein and 8 mg of folic acid/kg of diet (group III), or 12% protein and 70 g of cod liver oil/kg of diet (group IV). All pups were weaned on standard control diet with 18% protein. Cognitive performance, brain fatty acid profile, and serum glucose concentrations were studied in offspring at age 6 mo.

RESULTS: There was no significant difference in length of gestation or litter size, but the litter weight for group IV was lower ($P = 0.047$) than that for group I. After weaning, males in group II had lower ($P < 0.05$) body weights, but those in group III had weights comparable to those in group I for both sexes. In group IV, body weights were lower beyond 15 wk ($P < 0.05$). Relative brain weight and cognitive performance were significantly higher ($P < 0.05$) in group IV males and showed higher levels of brain γ-linolenic acid. Further, these animals had serum glucose levels comparable to those of control animals at age 6 mo, whereas serum glucose levels were higher in males from groups II ($P = 0.01$) and III ($P = 0.01$).

CONCLUSION: Fish oil supplementation during pregnancy improved cognitive performance and maintained glucose levels into adulthood, unlike folic acid supplementation, which supported only fetal growth and did not maintain glucose levels. *Nutrition* 2004;20:465–472. ©Elsevier Inc. 2004

KEY WORDS: folic acid, cognition, glucose, docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, γ-linolenic acid

INTRODUCTION

In developing countries, low birth weight is a major public health problem and is attributed to maternal undernutrition. Worldwide supplementary programs to pregnant women have concentrated on macronutrients[1] rather than on micronutrients. Perinatal malnutrition resulting in low birth weight and early growth retardation are speculated to be important risk factors for syndrome X in later life.[2–4] Barker reported evidence in support of fetal programming in utero leading to increased risks of adult diseases.[5] In view of this finding, it is essential to understand the role of maternal nutrition in fetal adaptation, especially with respect to the long-term risks associated with adult diseases.

The role of folic acid in fetal adaptation is well documented. Deficiency of folic acid during pregnancy results in babies with low birth weight and neural tube defects,[6] whereas its supplementation during pregnancy increases birth weight of infants.[7] However, aside from its effect on weight at birth, its long-term benefits or risks remain to be investigated. It is speculated that folic acid deficiency during the fetal stage predisposes toward thrombotic episodes during adulthood, possibly due to increased plasma homocysteine levels.[8]

Recent studies in humans have shown that increased intake of long-chain polyunsaturated fatty acids (LC PUFAs) during pregnancy increase the length of gestation and birth size,[9] suggesting that maternal LC PUFA status during pregnancy is critical in determining the development of the fetus. Adequate fetal accretion of LC fatty acids is important for the neurologic development of the fetus.[10] Further, protein deprivation during gestation may complicate the inadequate supply of LC PUFAs to the fetus because maternal protein malnutrition may render the child susceptible to metabolic derangement such as insulin resistance. Although folic acid and LC PUFA have independent effects on fetal growth, the former is known to modify LC PUFA metabolism and thus may play a role in fetal adaptations. LC PUFAs also affect brain growth, development, and function.[11]

Maternal undernutrition is a major factor responsible for fetal growth retardation in developing countries. Therefore, it is essential to examine whether maternal supplementation of specific micronutrients, especially at low or marginal protein levels, can offer beneficial effects in this aspect. In the present study, we examined the effect of supplementation of folic acid and ω-3 fatty acids, namely docosahexaenoic acid (DHA) and eicosapentaenoic acid

Sadhana Joshi was a Research Associate at the Indian Council of Medical Research, New Delhi during the tenure of the study.

Correspondence to: Shobha Rao, PhD, Director, Animal Science Division, Department of Biometry and Nutrition, Agharkar Research Institute, Pune 411004, India. E-mail: raoari@yahoo.com

0899-9007/04/$30.00
doi:10.1016/j.nut.2004.01.012

TABLE I.

COMPOSITION OF DIETS

Composition (g/kg diet)	Group I	Group II	Group III	Group IV
Corn starch	398	458	458	458
Casein (85% protein)	200	140	140	140
Dextrinized starch	132	132	132	132
Sucrose	100	100	100	100
Cod liver oil	0	0	0	70
Soybean oil	70	70	70	0
Fiber	50	50	50	50
Mineral mix*	35	35	35	35
Vitamin mix†	10	10	10	10
L-cystine	3	3	3	3
Choline bitartarate	2.5	2.5	2.5	2.5
Tertiary butyl hydroquinone	0.014	0.014	0.014	0.014
Total energy (kcal)	3766	3766	3766	3766

* 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 meta-silicate, 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.

† Vitamin mixture (g/kg mix): nicotinic acid, 3; calcium pantothenate, 1.6; pyridoxine-HCl, 0.7; thiamin-HCl, 0.6; riboflavin, 0.6; D-biotin, 0.02; vitamin B12 (0.1% in mannitol), 2.5; vitamin E, 15; vitamin A, 0.8; vitamin D3, 0.25; vitamin K, 0.075; sucrose, 974.655; folic acid, 0.2 in group I, 0 in group II, 0.8 in group III, 0.2 in group IV.

(EPA), at marginal levels of protein during pregnancy on birth size, cognitive performance, and blood glucose.

MATERIALS AND METHODS

All experimental procedures were in accordance with guidelines of the Institutional Animal Ethics Committee. Animal maintenance and handling were in accordance with the guidelines of the Indian National Institute of Nutrition.[12]

Diets

The composition of the diet (Table I) was according to the American Institute of Nutrition (AIN 93) purified diets for laboratory rodents[13] and contained 18 g of protein/100 g of diet. Three other isocaloric treatment diets were formulated. One was deficient in folic acid, the other was supplemented with folic acid, and the third was supplemented with fish oil. In treatment diets, protein level was reduced to 12 g/100 g of diet to satisfy the optimum requirement during pregnancy, and the amount of corn starch was 45.75 g/100 g of diet. This composition allowed us to study the role of micronutrients at marginal levels of protein during gestation.

Control animals received 2 mg of folic acid/kg of diet. Animals deficient in folic acid received no folic acid. Folic acid deficiency was obtained exclusively through dietary means rather than using folate antagonists or antibiotics because it is a more rational way of showing the overall effects of folic acid deprivation on pregnancy as it might occur in humans rather than using a drug to produce deficiency. The diet supplemented with folic acid was enriched with 8 mg of folic acid/kg of diet. This is roughly four times the requirement of a normal rat. This amount also is in accordance with the folic acid requirement of Indian pregnant women being 400 μg/d, which is four times the requirement of a non-pregnant women.[14] The diet supplemented with fish oil used cod liver oil (Seven Seas, Mumbai, India) as the source of DHA and EPA (Table II). Thus, the diet in group IV was composed of 12% protein and contained 2 mg of folic acid and cod liver oil.

TABLE II.

FATTY ACID COMPOSITION OF SOYBEAN OIL AND COD LIVER OIL FED TO DAMS THROUGHOUT PREGNANCY

Composition (g/100 g fatty acids)	Soybean oil	Cod liver oil
Docosahexaenoic acid (22:6 w-3)	0	12.46
Eicosapentaenoic acid (20:5 w-3)	0	18.4
Arachidonic acid (20:4 w-6)	0	0
Linoleic acid (18:2 w-6)	54.0	4.52
γ-Linolenic acid (18:3 w-6)	0	3.48
α-Linolenic acid (18:3 w-3)	7.5	2.8
Oleic acid (18:1 w-9)	22.0	10.19
Stearic acid (18:0)	4.1	12.48
Palmitic acid (16:0)	11.0	17.27
Myristic acid (14:0)	Trace	12.5

Experimental Design

Virgin female Wistar rats weighing 200 to 250 g were obtained from the animal facility of Agharkar Research Institute, Pune, India and were bred in a male:female ratio 1:3. Pups were separated on day 21 according to sex, and 24 female pups were randomly selected. They were housed in a facility maintained at 22°C on a controlled 12-h light and 12-h dark cycle with an appropriate ventilation system. They were given the control diet for about 3 mo and kept for breeding. On confirmation of pregnancy (through sperm-positive vaginal smears), i.e., 0 d of pregnancy, they were randomly allocated to one of the four groups (n = 6/group; Figure 1). Thus, dams in group I had the control diet, those in group II were given the diet deficient in folic acid, those in group III were given the diet supplemented with folic acid (8 mg/kg of diet), and those in group IV received the diet supplemented with ω-3 fatty acid (70 g of cod liver oil/kg of diet) throughout pregnancy.

Rats on the diet deficient in folic acid were placed in cages with plastic wire bottoms to reduce access to folic acid through coprophagy. One day before the expected delivery day, the dams were placed in plastic solid bottom cages with rice husk as bedding material. After delivery, the litters in each group were culled to eight pups per dam. The treatment dams received the control diet from the day of delivery, and the pups were weaned onto a control diet. At age 6 mo, five to six randomly selected males and females from each group were studied for cognitive performance and dissected for studying relative brain weight, brain fatty acid profile, and serum glucose concentrations. This method enabled us to study the exclusive effects of dietary treatments in utero on the long-term health outcomes of the progeny.

Measurements

On confirmation of pregnancy, weights were recorded on day 0 (i.e., the day when sperm-positive vaginal smears were noted) and then weekly (7, 14, and 21 d) to obtain weight gains. The litter weights were recorded at birth and weekly until age 6 mo. Daily

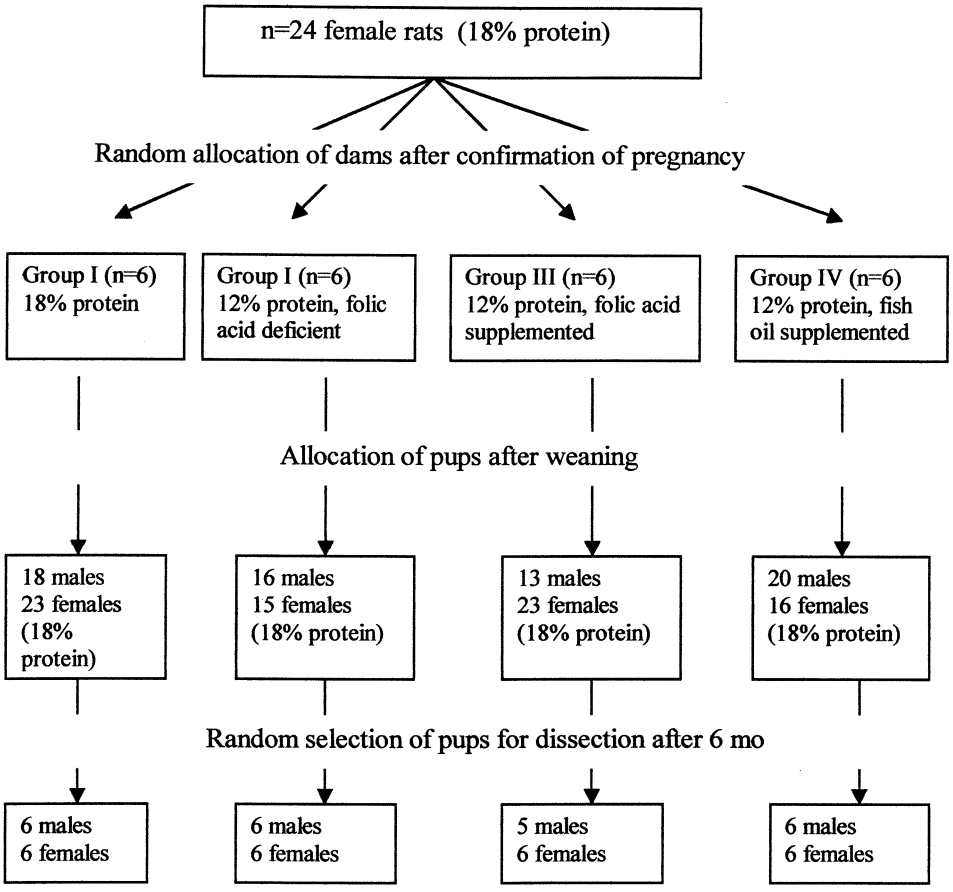

FIG. 1. Random allocation of dams after confirmation of pregnancy.

feed intake was recorded before conception and during gestation and lactation.

Water Maze Testing

Offspring of all four groups were tested for cognitive performance by using a conventional method[15] that uses a circular tank (70 cm in diameter and 32 cm high) made of opaque plastic. On the first day, the rats were required to locate the hidden platform (17 cm in diameter and 19.5 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.

Brain Weight and Fatty Acid Analysis

The offspring were weighed and killed at age 6 mo. Whole brains were weighed on a Mettler balance (Afcoset, Mumbai, India) with a sensitivity of 0.001 g. A vertical section was made, and 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 min. The membranes were diluted in 5 mL of phosphate buffered saline and stored at −20°C. Methyl esters were prepared according to the method of Manku et al.[16] Briefly, transesterification of the phospholipid fraction was carried out using HCl and methanol. These were separated and quantified with a Shimadzu (GC-17A Tokyo, Japan) gas chromatograph (SD 2330, 30-m capillary column; Supleco Bellfonte, PA, USA). Nitrogen at 1 mL/min was used as the carrier gas. Oven temperature was held at 175°C for 15 min 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 computed automatically. Peaks were identified by comparison with standard fatty acid methyl esters (Sigma, St. Louis, MO, USA).

Blood Samples

At age 6 mo, offspring were fasted overnight before dissection and blood samples were taken the next morning by heart puncture under anesthesia with diethyl ether. Blood samples were centrifuged, and serum was separated and analyzed for glucose with a diagnostic kit for glucose using the glucose oxidase method[17] and then stored at −20°C for further biochemical estimations.

Statistical Analysis

Values are mean ± standard deviation. The data were analyzed using SPSS/PC+ 11.0 (Chicago, IL, USA). Mean values of the estimates of weekly weights, brain fatty acid profile, and glucose for the treatment groups were compared with those of the control group at conventional levels of significance (i.e., 5% and 1%) by using least significance difference estimated from one-way analysis of variance.

TABLE III.

WEIGHT GAIN DURING PREGNANCY AND BIRTH OUTCOME*

	Group I	Group II	Group III	Group IV
Dams (*n*)	6	6	6	6
Weight gain (g)	117.4 ± 12.6	92.8 ± 9.9†	113.9 ± 22.1	102.6 ± 4.6
Litter weight (g)	61.9 ± 9.1	59.1 ± 8.6	62.2 ± 6.9	50.8 ± 11.3†
Litter size	11.3 ± 1.6	11.3 ± 1.8	11.7 ± 1.4	10.8 ± 3.4
Birth weight (g)	5.5 ± 0.2	5.3 ± 0.3	5.3 ± 0.1	4.8 ± 0.6
Pup weight (g)				
7 d	14.6 ± 0.9	12.9 ± 0.8†	13.8 ± 1.0	14.1 ± 1.6
14 d	30.1 ± 1.1	28.5 ± 0.8	28.8 ± 0.8	29.5 ± 3.0
21 d	48.7 ± 2.7	43.7 ± 1.3†	46.9 ± 1.7	46.6 ± 2.5

* Mean ± standard deviation.

† $P < 0.05$, control versus treatment groups.

Group I, casein plus 18% protein (control); group II, 12% protein without folic acid supplementation; group III, 12% protein plus 8 mg of folic acid; group IV, 12% protein plus ω-3 fatty acid (cod liver oil).

RESULTS

Feed Intake

Female rats consumed approximately 10 to 13 g of 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 across groups. Feed intake increased to approximately 27 to 29 g/d during lactation. Between-group differences in feed intake were not significant during lactation.

Weight Gain During Pregnancy

There was maximum weight gain in the third week of gestation in all groups. Dams in group II had a significantly ($P = 0.006$) lower weight gain (92.8 ± 9.9 g) than did those in group I (control; 117.4 ± 12.6 g; Table III). However, dams from group III had a mean weight gain of 113.9 ± 22.1 g, similar to that observed in the control group. Dams from group IV had lower weight gain (102.6 ± 4.6 g) than did dams on the control diet, but the difference was not statistically significant.

Reproductive Performance

There was no significant difference in the duration of gestation or litter size across groups (Table III). Mean litter weight in group IV was lower ($P = 0.047$) than that in the control group. However, there was no difference in mean litter weights for groups II and III compared with the control group.

Body Weights

Although dams received the control diet during lactation, the mean litter weights of the pups from group II were lower before weaning compared with those of the control group (Table III) and may be attributed to the effect of folic acid deficiency during pregnancy. After weaning, even though the pups were given the control diet, male pups from group II had lower mean weights throughout, but more so from week 15 (Table IV). In contrast, females in this group had mean body weights comparable to those in the control group. Pups from group III had comparable growth throughout the 6-mo period for both the sexes, indicating beneficial effect of folic acid supplementation. In group IV, males showed slower growth throughout the 6-mo period, whereas females had weights comparable to those in the control group except at week 19.

Brain Weights

Absolute brain weights were significantly lower ($P = 0.016$) for males in group II, but the relative brain weights (relative to body weight at age 6 mo) were comparable to those in the control group (Table V). In group III, there was no significant difference in absolute or relative brain weights, indicating beneficial effect of supplementation. In group IV, absolute brain weights for both sexes were lower than those in the control group, but the difference was significant only for males ($P = 0.033$). Relative brain weights of males in this group were higher ($P = 0.036$) when compared with those of the control animals.

Cognitive Function

All animals took longer (>120 s) to locate the platform on the first day, but the location task took much less time on the second day and even less time on subsequent days (Figure 2). Animals in group II took a longer time than did those in control group on all 4 d, with the difference being significant on day 2. Time taken by animals in group III was similar to that in those in the control group. In contrast, animals in group IV had shorter times on all days, and the differences were significant ($P < 0.05$) only for males. Thus, fish oil supplementation at marginal protein levels during gestation seemed to have a beneficial effect on cognitive performance in the offspring.

Brain Fatty Acid Profile

In view of the differential effect seen on the cognitive performance of animals in different dietary groups, we examined the brain fatty acid profile (Table VI). In males from group IV, a significant increase ($P = 0.008$) was seen in the γ-linolenic acid (GLA) content of brain. There was no significant difference in the other fatty acids in males. No significant difference was seen in any of the fatty acids in females in any treatment group.

Serum Glucose

Glucose levels were significantly ($P = 0.01$) higher in group II males than in control males (5.62 ± 0.73 versus 4.69 ± 0.28 mM/L). Group III rats also showed higher ($P = 0.010$) glucose levels (5.69 ± 0.46 mM/L) than did control rats. In contrast, glucose values in group IV were similar (4.22 ± 0.68 mM/L) to those in the control group. Thus, fish oil supplementation showed

TABLE IV.

BODY WEIGHTS (GRAMS) OF MALE AND FEMALE RATS IN DIFFERENT GROUPS*

Weeks	Group I	Group II	Group III	Group IV
Males (*n*)	18	16	13	20
3 wk	47.9 ± 3.9	42.9 ± 3.8†	48.6 ± 2.0	46.6 ± 3.5
7 wk	153.1 ± 15.9	136.5 ± 43.2	157.5 ± 17.9	141.1 ± 29.3
11 wk	280.7 ± 21.8	263.7 ± 65.5	286.3 ± 27.8	263.3 ± 55.8
15 wk	356.9 ± 23.4	315.5 ± 80.2†	357.8 ± 27.9	320.4 ± 56.9†
19 wk	407.8 ± 30.2	354.4 ± 80.7†	397.7 ± 60.6	364.4 ± 55.5†
23 wk	448.1 ± 43.5	396.5 ± 67.1†	437.9 ± 48.6	388.8 ± 56.8†
25 wk	459.7 ± 54.1	401.6 ± 81.8†	450.9 ± 41.9	396.7 ± 63.1†
Females (*n*)	23	15	23	16
3 wk	46.9 ± 5.1	44.7 ± 3.2	45.8 ± 4.4	46.7 ± 3.7
7 wk	113.3 ± 22.4	137.2 ± 6.4†	119.2 ± 13.1	129.8 ± 22.9†
11 wk	187.5 ± 16.9	198.5 ± 14.7	188.0 ± 14.7	185.1 ± 44.3
15 wk	228.5 ± 18.6	231.0 ± 15.2	222.1 ± 19.9	217.3 ± 30.7
19 wk	250.2 ± 22.1	249.5 ± 21.5	240.7 ± 29.2	231.7 ± 31.7†
23 wk	264.4 ± 25.8	268.9 ± 23.4	253.7 ± 31.0	245.5 ± 33.4
25 wk	268.2 ± 27.2	274.9 ± 21.3	259.9 ± 30.9	248.2 ± 38

* Mean ± standard deviation. Dams received the following diets during pregnancy: 18% protein plus 2 mg of folic acid/kg of diet (group I, control); 12% protein without folic acid supplementation (group II); 12% protein plus 8 mg of folic acid/kg of diet (group III); 12% protein plus 70 g of cod liver oil/kg of diet.
Offspring received the control diet during the growth period.
† $P < 0.05$, control versus treatment groups.

a beneficial effect on maintaining glucose levels into adulthood. In contrast, there was no significant change in glucose concentrations in females in any treatment group.

DISCUSSION

Folic acid supplementation during pregnancy is one of the most common nutritional interventions in many developing countries.[7,18] Among other micronutrients, there is growing interest in investigating the role of essential fatty acids, especially ω-3 fatty acids, in promoting fetal growth.[19] Recent reports[5] have found that low birth weight increases risks for adult diseases, and maternal undernutrition is one of the major determinants in developing countries. Therefore, it is imperative to examine the role of these micronutrients in reducing such risks at later ages, apart from their known role in fetal growth. In reported studies, supplementation of folic acid[20] or ω-3 fatty acids[21,22] is given at adequate protein levels. However, because maternal diets in developing countries are often inadequate in protein, it would be relevant to investigate how dietary manipulations ameliorate the negative effects of maternal malnutrition. We therefore examined the effect of maternal supplementation of folic acid and fish oil at marginal levels of protein during pregnancy on birth size, cognitive performance, and serum glucose in 6-mo offspring in a rat model,[23,24] a commonly used model for examining the long-term consequences of undernutrition in utero. Our study showed that folic acid supplementation is beneficial in terms of weight gain during pregnancy and postnatal growth, and that fish oil supplementation is more beneficial in maintaining glucose at age 6 mo.

Protein restriction during pregnancy does not affect food intake[25] but does affect weight gain. Marin et al.[26] found that mean body weights of pregnant dams fed 15%, 10%, and 5% protein were 79%, 72%, and 58%, of control dams, respectively. We observed that folic acid supplementation at 12% protein level during pregnancy resulted in a weight gain similar to that of control rats, and that its deficiency resulted in lower weight gain (*P*

TABLE V.

ABSOLUTE AND RELATIVE BRAIN WEIGHTS OF MALE AND FEMALE RATS AT AGE 6 MO AGE IN DIFFERENT GROUPS*

	Group I	Group II	Group III	Group IV
Males (*n*)	6	6	5	6
Absolute weights (g)	1.96 ± 0.07	1.82 ± 0.07†	1.89 ± 0.08	1.84 ± 0.10†
Relative weights (%)	0.43 ± 0.04	0.49 ± 0.10	0.43 ± 0.06	0.53 ± 0.06†
Females (*n*)	6	6	6	6
Absolute weights (g)	1.81 ± 0.06	1.73 ± 0.05	1.75 ± 0.08	1.77 ± 0.08
Relative weights (%)	0.67 ± 0.05	0.63 ± 0.06	0.71 ± 0.06	0.75 ± 0.12

* Mean ± standard deviation.
†$P < 0.05$, control versus treatment groups.
Group I, casein plus 18% protein (control); group II, 12% protein without folic acid supplementation; group III, 12% protein plus 8 mg of folic acid; group IV, 12% protein plus ω-3 fatty acid (cod liver oil)

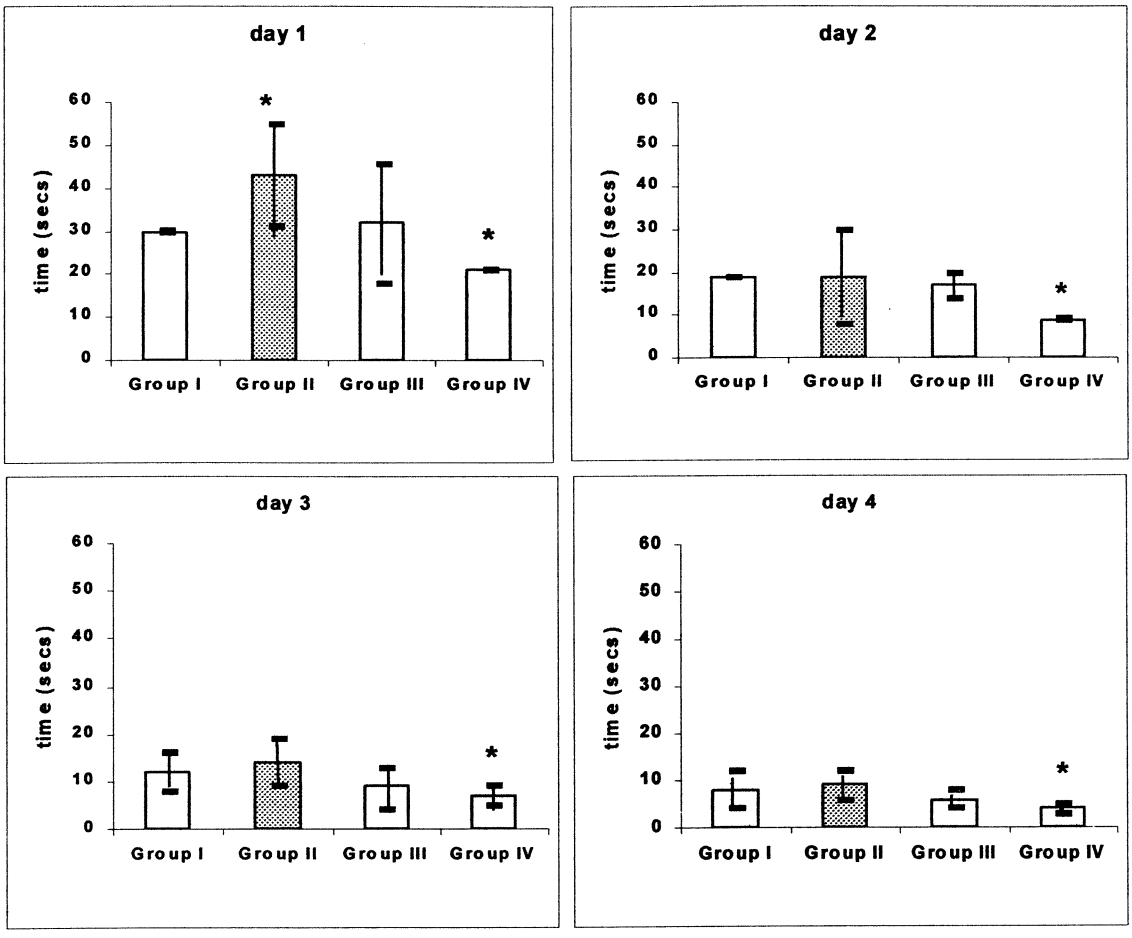

FIG. 2. Cognitive performance of males on the water maze (*$P < 0.05$, control versus treatment groups). All values are mean ± standard deviation. Group I, control diet of casein plus 18% protein; group II, diet of 12% protein and deficient in folic acid; group III, diet of 12% protein plus 8 mg of folic acid; group IV, diet of 12% protein plus ω-3 fatty acid (cod liver oil).

< 0.006) when compared with the control group (18% protein), indicating the beneficial effect of folic acid supplementation at marginal protein levels. Lower weight gain, although not significant, in group IV (12% protein with cod liver oil supplementation) did not affect the length of gestation or litter size but did result in lower ($P < 0.04$) litter weight. These observations are similar to those reported for fish oil supplementation.[22,27]

The group II diet (12% protein without folic acid) showed a profound effect on growth because, even with dietary repletion, mean litter weights before weaning were lower despite the dams being shifted to the control diet from the day of delivery. This effect was also seen after weaning but only in males. In contrast, offspring from group III (12% protein and folic acid supplementation) had mean body weights comparable to control animals throughout postnatal period. These observations are in accordance with studies reporting beneficial effects of maternal folic acid supplementation on birth outcome, with different levels,[28] except at an excess level of folic acid, i.e., 40 mg/kg.[20] Animals in the fish oil group had mean body weights lower than those of the control animals, but the differences were significant only for males. Cod liver oil is a rich source of EPA (20:5 ω-3) and DHA (22:6 ω-3), and its supplementation during pregnancy is expected to compromise the level of arachidonic acid (20:4 ω-6) due to the inhibitory action of ω-3 fatty acids on δ-6-desaturation in the fetus,[27] which affects growth.[29] However, in the present study, our finding of no significant differences in brain arachidonic acid across groups cannot be explained and needs further exploration.

Relative brain weights of rats in groups II (12% protein without folic acid) and III (12% protein plus folic acid) were comparable to those of control rats (18% protein). However, males in group IV (12% protein plus cod liver oil) showed significantly higher relative brain weights ($P = 0.036$) and better cognitive performance ($P = 0.039$) than did those in the control group. Improved cognitive performance at age 6 wk in offspring of dams supplemented with fish oil during gestation has been reported.[21] Our results suggested that EPA and DHA in fish oil likely contribute to improvement in cognitive performance. No significant difference in cognitive performance was seen in female offspring. In fact, most of the results were significant in males rather than females. This observation may be related to the greater nutritional sensitivity in the male due to faster tissue growth.[23]

Although folic acid alters LC PUFA metabolism, the effect of folic acid supplementation on the brain fatty acid profile is rarely examined. Fish oil in the form of cod liver oil dramatically alters the LC PUFA composition of brain in male Wistar rats.[30,31] We observed higher levels of GLA in males from the group supplemented with fish oil, which requires additional study. Large amounts of ω-3 fatty acids consumed during gestation may have an inhibitory effect on δ-5- and δ-6-desaturases.[32] This may partly explain the increase in brain GLA levels. Further, such an increase in GLA content not being observed in the folate-supplemented and folate-deficient groups supports this explanation.

Serum glucose values at age 6 mo were significantly higher in group II animals (12% protein without folic acid). Group III (12%

TABLE VI.

BRAIN FATTY ACID PROFILE OF MALE AND FEMALE RATS FROM DIFFERENT GROUPS*

	Group I	Group II	Group III	Group IV
Males (*n*)	3	5	5	5
Docosahexaenoic acid (22:6 ω-3)	7.5 ± 3.43	9.79 ± 0.79	10.9 ± 0.37	8.33 ± 2.56
Eicosapentaenoic acid (20:5 ω-3)	1.94 ± 0.48	1.95 ± 0.25	2.24 ± 0.89	1.76 ± 0.63
Arachidonic acid (20:4 ω-6)	11.58 ± 1.24	11.21 ± 1.83	13.0 ± 2.57	10.77 ± 0.75
Linoleic acid (18:2 ω-6)	0.8 ± 0.42	0.76 ± 0.72	0.74 ± 0.73	0.71 ± 0.56
γ-Linoleic acid (18:3 ω-6)	1.05 ± 0.27	1.19 ± 0.89	1.02 ± 0.34	2.75 ± 0.80†
α-Linolenic acid (18:3 ω-3)	0.45 ± 0.2	0.34 ± 0.18	0.28 ± 0.05	0.28 ± 0.06
Oleic acid (18:1 ω-9)	33.56 ± 5.22	28.86 ± 3.52	26.55 ± 6.63	32.63 ± 2.94
Stearic acid (18:0)	18.48 ± 1.3	19.27 ± 3.01	19.11 ± 1.81	18.83 ± 0.70
Palmitic acid (16:0)	19.70 ± 1.14	18.11 ± 1.67	19.05 ± 3.56	19.69 ± 1.95
Myristic acid (14:0)	1.41 ± 0.38	0.91 ± 0.52	1.02 ± 0.17	0.84 ± 0.34
Females (*n*)	6	6	6	6
Docosahexaenoic acid (22:6 ω-3)	11.29 ± 1.18	10.69 ± 0.64	11.0 ± 2.47	10.72 ± 1.42
Eicosapentaenoic acid (20:5 ω-3)	1.46 ± 0.41	1.54 ± 0.68	1.27 ± 0.63	1.78 ± 0.31
Arachidonic acid (20:4 ω-6)	8.86 ± 4.27	10.93 ± 0.83	12.45 ± 2.78	11.53 ± 2.17
Linoleic acid (18:2 ω-6)	0.43 ± 0.34	0.33 ± 0.07	0.32 ± 0.07	0.45 ± 0.24
γ-Linoleic acid (18:3 ω-6)	1.52 ± 0.96	1.21 ± 0.28	1.37 ± 0.56	0.93 ± 0.17
α-Linolenic acid (18:3 ω-3)	0.32 ± 0.09	0.32 ± 0.08	0.29 ± 0.02	0.28 ± 0.08
Oleic acid (18:1 ω-9)	29.67 ± 5.83	28.97 ± 2.44	28.50 ± 2.19	29.98 ± 2.18
Stearic acid (18:0)	19.41 ± 1.46	20.93 ± 1.39	19.63 ± 2.48	20.36 ± 1.19
Palmitic acid (16:0)	18.76 ± 1.87	16.98 ± 1.91	19.73 ± 2.84	18.20 ± 1.57
Myristic acid (14:0)	0.74 ± 0.46	0.77 ± 0.26	0.95 ± 0.43	0.48 ± 0.18

*Mean ± standard deviation (g/100 g of fatty acid).

† $P < 0.05$, control versus treatment groups.

Group I, casein plus 18% protein (control); group II, 12% protein without folic acid supplementation; group III, 12% protein plus 8 mg of folic acid; group IV, 12% protein plus ω-3 fatty acid (cod liver oil).

protein plus folic acid) animals also showed increases in glucose concentrations, unlike group IV animals (12% protein plus cod liver oil) that had values similar to those of the control animals (18% protein). In a previous study, insulin levels were significantly high in group II animals (deficient in folic acid) but low in group III animals and were comparable to group IV (control) animals.[33] Prior administration of GLA and other LC PUFAs have been shown to prevent alloxan-induced damage to β-cells and attenuate the development of diabetes.[34,35] Therefore, our results suggest that GLA may have a role in maintaining serum glucose levels, but further studies are needed to confirm this proposition.

Long-term effects of undernutrition during gestation differ with respect to brain and metabolic parameters in offspring. Brain sparing is a feature of intrauterine growth retardation in humans[36] and experimental animals,[37] but the reverse is true with regard to metabolic parameters. Observations from Dutch famine studies have indicated that adaptations that enable the fetus to continue to grow may have adverse consequences in terms of increased risks for coronary heart diseases.[38] Insulin plays a central role in the regulation of fetal growth, and it is believed that one fetal adaptation to undernutrition is to alter insulin and glucose metabolism.[39] Rats that are weaned prematurely have an increased serum cholesterol concentration in later life, which becomes apparent only after 7 mo.[40] Thus, evidence from human and animal studies suggest that fetal adaptation to undernutrition during gestation may have several adverse consequences in adult offspring.

In a prospective study[41] on rural Indian mothers, consumption of green leafy vegetables, a food source of α-linolenic acid, was strongly associated with birth size. Because this relationship was seen predominantly in undernourished women, the investigators concluded that, when maternal diets are inadequate in macronutrients, micronutrients may play an important compensatory role in fetal development. The present findings identified ω-3 fatty acid as one of the important micronutrients involved not only in fetal development but also in metabolic programming determining the risks associated with increased glucose levels in adulthood. These findings have wider implications in the context of "fetal origin of adult diseases" hypothesis that associates the risks of adult diseases with a suboptimal in utero nutritional environment. It emphasizes the need for supplementing micronutrients such as LC PUFA to existing nutrition interventions for pregnant women to prevent adult diseases such as hypertension, diabetes, coronary heart disease, and hyperlipidemia. This could be better achieved through food-based interventions than through distribution of tablets containing one or two micronutrients. However, several other micronutrients may be involved in improving fetal growth, and evaluating their long-term effects with regard to adult health is an important area for further research.

SUMMARY

Maternal folic acid deficiency showed higher serum glucose levels in offspring at age 6 mo. Dams supplemented with folic acid at marginal protein levels during gestation showed cognitive performance similar to the control dams but produced increased serum glucose levels in the offspring. In contrast, fish oil supplementation produced better cognitive performance and maintained serum glucose levels into adulthood.

ACKNOWLEDGMENTS

The authors thank Dr. V. S. Rao, Director, Agharkar Research Institute, Pune and Dr. S. Shivram, Director, National Chemical Laboratory, Pune for providing necessary facilities to carry out this

research work. Thanks are also due to Dr. S. P. Mahadik, Medical College of Georgia, Augusta, USA for giving valuable advice regarding cognitive tests and brain fatty acid estimations.

REFERENCES

1. 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
2. Das UN. Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent hypertension in adult life? Hypertension 2001;38:E6
3. Das UN. Is metabolic syndrome an inflammatory condition? Exp Biol Med 2002;27:989
4. Das UN. Can perinatal supplementation of long-chain polyunsaturated fatty acids prevent diabetes mellitus? Eur J Clin Nutr 2003;57:218
5. Barker DJP. Foetal programming and public health. In: O'Brien PMS, Wheelar T, Barker DJP, eds. *Foetal programming: influences on development and disease in later life*. London: Royal College of Obstetricians and Gynaecologists, 1999:3
6. Wagner C. Symposium on the subcellular compartmentation of folate metabolism. J Nutr 1996;126:1228S
7. Iyengar L, Rajalakshmi K. Effect of folic acid supplement on birth weight of infants. Am J Obstet Gynecol 1975;122:332
8. Gheye S, Lakshmi AB, Krishna TP, Krishnaswamy K. Fibrinogen and homocysteine level in coronary artery disease. Indian Heart J 1999;51:499
9. Olson SF, Sorensen JD, Secher NJ, et al. Randomised controlled trial of effect of fish oil supplementation on pregnancy duration. Lancet 1992;339:1003
10. Sattar N, Berry C, Greer IA. Essential fatty acids in relation to pregnancy complications and foetal development. Br J Obstet Gynecol 1998;105:1248
11. Fernstrom JD. Can nutrient supplements modify brain function? Am J Clin Nutr 2000;71:1669S
12. Raghuramulu N, Nair KM, Kalyansundaram S. *A manual of laboratory techniques*. Hyderabad: National Institute of Nutrition, 1983, 277
13. 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
14. Gopalan C, Rama Sastri BV, Balasubramanian SC. *Nutritive value of Indian foods*. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research, 1996, 94
15. Stewart CA, Morris RG. The water maze. In: Saghal A, ed. *Behavioral science: a practical approach, Vol 1*. New York: New York University Press, 1993:107
16. Manku MS, Horrobin DF, Huang S, Morse N. Fatty acids in plasma and red cell membranes. Lipids 1983;18:906
17. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative glucose acceptor. Ann Clin Biochem 1969;6:24
18. O'Scholl T, Johnson WG. Folic acid: influence on the outcome of pregnancy. Am J Clin Nutr 2000;71:1295S
19. Elevoll EO, James DG. Potential benefits of fish for maternal, fetal and neonatal nutrition: a review of the literature. Food Nutr Agric 2000;27:28
20. Achon M, Alonso-Aperte E, Reyes L, Ubeda N, Moreiras V. High dose folic acid supplementation in rats: effects on gestation and methionine cycle. Br J Nutr 2000;83:177
21. Yonekubo A, Honda S, Okano M, Takahashi K, Yamamoto Y. Effects of dietary fish oil during the fetal and postnatal periods on the learning ability of postnatal rats. Biosci Biotech Biochem 1994;58:799
22. Saste MD, Carver JD, Stockard JE, Benford VJ, Chen LT, Phelps CP. Maternal diet fatty acid composition affects neurodevelopment in rat pups. J Nutr 1998; 128:740
23. Lucas A, Baker BA, Desai M, Hales CN. Nutrition in pregnant or lactating rats programs lipid metabolism in the offspring. Br J Nutr 1996;76:605
24. Latorracea MQ, Carneiro EM, Boschero AC, Mello MA. Protein deficiency during pregnancy and lactation impairs glucose induced insulin secretion but increases the sensitivity to insulin in weaned rats. Br J Nutr 1998;80:291
25. 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
26. Marin MC, Tomas MED, Serres C, Mercuri O. Protein energy malnutrition during gestation and lactation in rats affects growth rate, brain development and essential fatty acid metabolism. J Nutr 1995;125:1017
27. Amusquivar E, Ruperez FJ, Barbas C, Herrera E. Low arachidonic acid rather than α tocopherol is responsible for the delayed postnatal development in offspring of rats fed fish oil instead of olive oil during pregnancy and lactation. J Nutr 2000;130:2855
28. Lin GWJ. Effect of dietary folic acid levels and gestational ethanol consumption on tissue folate contents and rat fetal development. Nutr Res 1991;11:223
29. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tollry EA. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci USA 1993;90:1073
30. Bourre JM, Bonneil M, Dumont O, et al. Effect of increasing amounts of dietary fish oil on brain and liver fatty composition. Biochim Biophys Acta 1990;1043: 149
31. Wainwright PE, Xing HC, Ward GR, et al. Water maze performance is unaffected in artificially reared rats fed diets supplemented with arachidonic acid and docosahexaenoic acid. J Nutr 1999;129:1079
32. Herrera E. Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development—a review. Placenta 2002;23:S9
33. Joshi SR, 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
34. Mohan IK, Das UN. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition 2001;17:126
35. Suresh Y, Das UN. Protective action of arachidonic acid against alloxan induced cytotoxicity and diabetes mellitus. Prostaglandins Leukot Essent Fatty Acids 2001;64:37
36. Lumbers ER, Yan Yu ZU, Gibson KJ. The selfish brain and the Barker hypothesis. Clin Exp Pharmacol Physiol 2001;28:942
37. Dressino V, Orden B, Oyhenart EE. Sexual responses to intrauterine stress: body and brain growth. Clin Exp Obstet Gynecol 2002;29:100
38. Roseboom TJ, Van Der Meulen JP, Ravelli ACJ, et al. Effects of prenatal exposure to the Dutch famine on adult disease in later life: an overview. Mol Cell Endocrinol 2001;185:93
39. Erickson JG, Forsen T, Tuomilehto J, et al. Effects of size at birth and childhood growth on the insulin resistance syndrome in elderly individuals. Diabetologia 2002;45:342
40. Barker DJP, Martyn CN, Osmond C, Fall CHD. Growth in utero and serum cholesterol concentrations in adult life. Br Med J 1993;307:1524
41. Rao S, Yajnik CS, Kanade AN, Fall CHD, et al. 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