

Maternal dietary fat alters amniotic fluid and fetal intestinal membrane essential n-6 and n-3 fatty acids in the rat

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Friesen, Russell, and Sheila M. Innis. Maternal dietary fat alters amniotic fluid and fetal intestinal membrane essential n-6 and n-3 fatty acids in the rat. *Am J Physiol Gastrointest Liver Physiol* 290: G505–G510, 2006. First published November 10, 2005; doi:10.1152/ajpgi.00257.2005.—We investigated whether maternal fat intake alters amniotic fluid and fetal intestine phospholipid n-6 and n-3 fatty acids. Female rats were fed a 20% by weight diet from fat with 20% linoleic acid (LA; 18:2n-6) and 8% α -linolenic acid (ALA; 18:3n-3) (control diet, $n = 8$) or 72% LA and 0.2% ALA (n-3 deficient diet, $n = 7$) from 2 wk before and then throughout gestation. Amniotic fluid and fetal intestine phospholipid fatty acids were analyzed at day 19 gestation using HPLC and gas-liquid chromatography. Amniotic fluid had significantly lower docosahexaenoic acid (DHA; 22:6n-3) and higher docosapentaenoic acid (DPA; 22:5n-6) levels in the n-3-deficient group than in the control group (DHA: 1.29 ± 0.10 and 6.29 ± 0.33 g/100 g fatty acid; DPA: 4.01 ± 0.35 and 0.73 ± 0.15 g/100 g fatty acid, respectively); these differences in DHA and DPA were present in amniotic fluid cholesterol esters and phosphatidylcholine (PC). Fetal intestines in the n-3-deficient group had significantly higher LA, arachidonic acid (20:4n-6), and DPA levels; lower eicosapentaenoic acid (EPA; 20:5n-3) and DHA levels in PC; and significantly higher DPA and lower EPA and DHA levels in phosphatidylethanolamine (PE) than in the control group; the n-6-to-n-3 fatty acid ratio was 4.9 ± 0.2 and 32.2 ± 2.1 in PC and 2.4 ± 0.03 and 17.1 ± 0.21 in PE in n-3-deficient and control group intestines, respectively. We demonstrate that maternal dietary fat influences amniotic fluid and fetal intestinal membrane structural lipid essential fatty acids. Maternal dietary fat can influence tissue composition by manipulation of amniotic fluid that is swallowed by the fetus or by transport across the placenta.

fetal intestinal development; docosahexaenoic acid

DIETARY FAT is a major modifiable environmental factor known to influence growth and development and susceptibility to disease. The n-6 fatty acid linoleic acid (LA; 18:2n-6) is the metabolic precursor for the synthesis of arachidonic acid (ARA; 20:4n-6), whereas α -linolenic acid (ALA; 18:3n-3) is the precursor for the synthesis of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (27). ARA and EPA are further metabolized to locally active autocooids collectively known as eicosanoids via the action of cyclooxygenases and lipoxygenases, whereas DHA can be further metabolized to docosanoids and other 22-carbon metabolites (6, 45). Whereas eicosanoids derived from ARA are proinflammatory, n-3 fatty acid-derived metabolites are anti-inflammatory or more weakly inflammatory. In addition, the n-3 fatty acids EPA and DHA have direct effects on inflammatory and

immune responses, carbohydrate and lipid metabolism, and cardiovascular and neural function through effects on gene expression and ion channel and G-coupled protein receptor activities (11, 12, 27, 33, 43).

Diets high in LA, as are characteristic of many Western nations (46), may result in increased tissue phospholipid ARA (6), and dietary intakes of n-3 fatty acids are low among many women (13, 30). Diets high in n-6/n-3 fatty acids are considered an important epigenetic factor contributing to the increased incidence of diseases involving immune, inflammatory, and oxidative response pathways, including coronary vascular disease, inflammatory bowel diseases, and aging-regulated cognitive and retinal disorders (6, 37, 44, 46). Premature infants are at risk for several diseases that involve oxidative and inflammatory tissue damage, such as bronchopulmonary dysplasia, necrotizing enterocolitis, and retinopathy of prematurity that may be modifiable by dietary polyunsaturated fatty acids (9, 24, 42). In postnatal animals, higher intakes of n-3 fatty acids result in increased membrane phospholipid n-3 fatty acids and a shift toward decreased n-6-derived eicosanoids and increased n-3-derived eicosanoids, with subsequent attenuation of inflammatory mediators and tissue responses (5, 6, 23). In addition, convincing evidence has been published that shows that maternal intakes of n-3 fatty acids influence the accretion of n-6 and n-3 fatty acids in the fetal liver and brain in animals and in red blood cell lipids in human neonates (15, 25, 27, 28). The effect of maternal dietary fat composition on amniotic fluid has not been described previously but could be important to fetal fatty acid accretion through fetal swallowing, thus influencing developing tissue organ lipids. In the present study, we established that maternal dietary fat has significance with respect to essential n-3 fatty acids and the n-6/n-3 fatty acid balance of amniotic fluid and fetal intestine membrane lipids. To our knowledge, this is the first report to demonstrate that the composition of dietary fatty acids influences amniotic fluid and fetal intestinal lipids.

MATERIALS AND METHODS

Animals and diets. Female Sprague-Dawley rats (175–200 g) were housed individually in a temperature-controlled animal facility with a 12:12-h light-dark cycle with food and water available ad libitum. Two weeks before being mated, rats were randomly assigned to one of two semisynthetic diets containing 20% fat by weight and identical in all macro- and micronutrients except for the composition of the fat (28). One diet contained 20% LA and 8% ALA with a n-6-to-n-3 (n-6/n-3) ratio of 2.5 (control diet, $n = 7$), and the other diet contained

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Table 1. Fatty acid composition of amniotic fluid total lipids

Fatty Acid	Control Diet	n-3-Deficient Diet
18:2n-6	7.11±0.52	10.3±0.45*
20:4n-6	17.9±0.3	20.9±1.41
22:4n-6	3.42±0.49	4.12±0.35
22:5n-6	0.73±0.15	4.03±0.22*
18:3n-3	0.17±0.01	0.13±0.0
20:5n-3	0.87±0.08	<0.01±0.00*
22:5n-3	0.45±0.07	<0.01±0.00*
22:6n-3	6.29±0.33	1.29±0.10*
18:1n-9	21.0±0.66	17.1±0.84
16:0	17.9±0.53	19.3±0.8
18:0	11.8±0.5	14.4±0.6

Values are means ± SE. (in g/100 g fatty acid). Pregnant rats were fed a diet adequate in n-3 fatty acids or deficient in n-3 fatty acids with high n-6 fatty acids. Amniotic fluid fatty acids were determined on gestation day 19. *Significant difference at $P < 0.01$.

72% LA and 0.2% ALA with a n-6/n-3 ratio of 370 (n-3-deficient diet, $n = 8$). Diets contained similar amounts of saturated fat, representing 7–10% fatty acids, but varied in the monounsaturated fatty acid oleic acid (18:1n-9) with 65% and 18% 18:1n-9 in control and n-3-deficient diets, respectively (28, 32). Studies on amniotic fluid and the fetal intestine were conducted on day 19 of gestation (normal gestation: 21 days). All procedures were approved and carried out in accordance with Animal Care Committee of University of British Columbia guidelines.

Tissue preparation and lipid analysis. On gestation day 19, rats were anesthetized with isoflurane, and the amniotic fluid was carefully withdrawn. Fetal intestines and livers were removed and rinsed with ice-cold PBS, and samples within a litter were pooled, frozen, and then stored at -70°C until analyzed. For lipid analysis, total lipids were extracted, and lipid classes were then separated by HPLC using a quaternary solvent system (29). After HPLC resolution, the column effluent was split to an evaporative light-scattering detector for quantification and to a fraction collector for recovery of separated lipid classes to allow further analysis of fatty acid components (29). Fatty acids in fetal intestinal and liver phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were converted to their respective methyl esters, separated, identified, and then quantified by gas-liquid chromatography (GLC) (26). Because of the small sample size, amniotic fluid cholesterol esters and PC within a diet group were pooled for further analysis of fatty acid components. Fatty acid components of amniotic fluid, reflecting the total fatty acid profile, were determined by direct methylation without prior resolution of individual lipid classes (31).

Statistical analysis. The effects of maternal dietary fat on amniotic and fetal intestinal and liver fatty acids was analyzed using Student's t -test, with the level of statistical significance set at $P < 0.05$, with each litter considered as $n = 1$. All the statistical procedures were performed using SPSS (version 12; Chicago, IL). Results are expressed as means ± SE.

RESULTS

Amniotic fluid fatty acids. Amniotic fluid from rats fed the diet deficient in n-3 fatty acids from only 2 wk before gestation showed marked fivefold lower total n-3 fatty acids and DHA levels compared with animals fed the control diet (Table 1). In addition, whereas EPA represented almost 1% of amniotic fluid fatty acids in the control group, EPA was <0.01% of fatty acids in amniotic fluid from the n-3-deficient group. There were no significant differences in saturated, monounsaturated, or ARA concentrations be-

tween the deficient and control groups. However, LA was significantly higher in amniotic fluid from animals fed the n-3-deficient diet, which was also higher in LA than the control diet. The amniotic fluid total n-6/n-3 fatty acid ratio was about sevenfold higher in the deficient group than in the control group (29.8 ± 0.92 and 4.12 ± 0.03 , respectively), explained by the lower amniotic fluid EPA, n-3 DPA, and DHA in the n-3-deficient group. Analysis of fatty acids in cholesterol esters and PC after HPLC separation of amniotic fluid lipids showed high amounts of ARA, representing 27–33% fatty acids in cholesterol ester and 20–24% fatty acids in PC in both groups; DHA, on the other hand, was 0.5% and 3.5% fatty acids in cholesterol esters and 1.8% and 8.2% fatty acids in PC, respectively, of the n-3-deficient group compared with the control group, respectively.

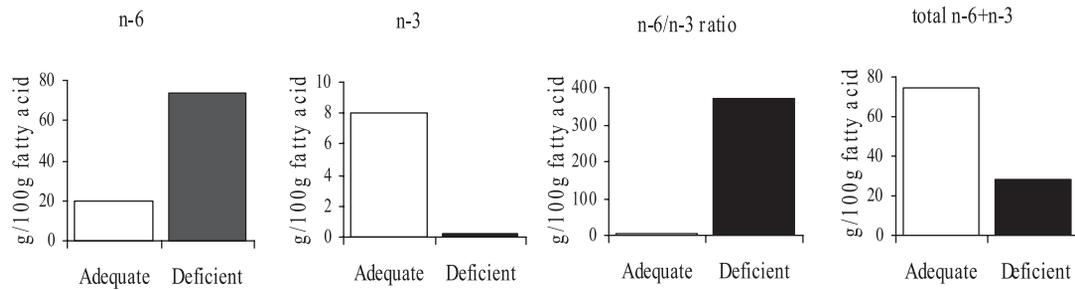
Fetal intestinal and liver phospholipid fatty acids. The difference in maternal dietary n-3 fatty acid intake was clearly reflected in fetal intestinal PE and PC. Fetal intestine PE in the n-3-deficient group had about sixfold lower DHA, significantly lower EPA and 22:5n-3, and higher 22:4n-6 and 22:5n-6 (DPA) than in the control group ($P < 0.05$; Table 2). Similarly, fetal intestine PC in the n-3-deficient group had significantly lower EPA and 22:5n-3 and DHA and higher LA, ARA, and DPA than in the control group. As in the amniotic fluid, the reduction in fetal intestine n-3 fatty acid caused by feeding rats a diet deficient in n-3 fatty acids during gestation led to a marked seven- to eightfold higher n-6/n-3 fatty acid ratio in intestinal PE and PC in the deficient group compared with the control group (Fig. 1). The replacement of DHA with n-6 DPA and, to a smaller extent, with 22:4n-6 in PE in the n-3-deficient group resulted in maintenance of the total n-6 plus n-3 fatty acid content similar to that in intestine PE of the control group. PC and PE had significantly higher LA, ARA, and n-6 DPA and lower EPA, n-3 DPA, and DHA in the liver of fetuses in the n-3 fatty acid-deficient group than in the control group (Table 3), similar to the effects of maternal dietary n-3 fatty acid deprivation found for the fetal intestine. Notable differences between fetal intestinal and liver phospholipid fatty acids include the higher ARA in the fetal liver

Table 2. Fetal intestine PC and PE fatty acids

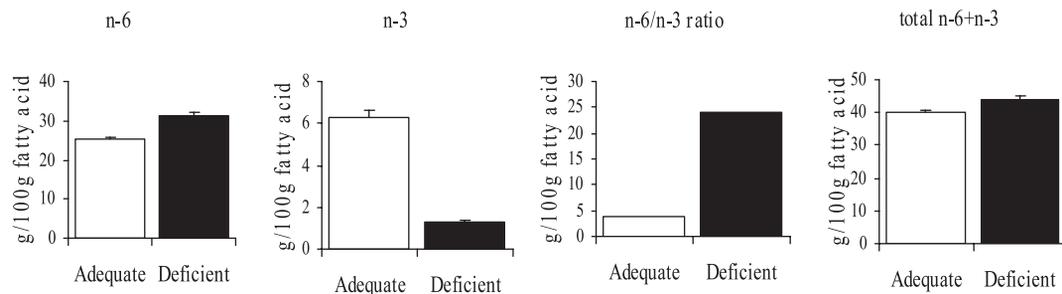
Fatty Acids	PC		PE	
	Control diet	n-3-Deficient diet	Control diet	n-3-Deficient diet
18:2n-6	4.67±0.09	7.23±0.40*	1.38±0.01	1.72±0.05
20:4n-6	8.81±0.16	10.8±0.1*	26.7±0.22	25.6±0.28
22:4n-6	0.83±0.03	1.30±0.09	9.12±0.02	10.6±0.23*
22:5n-6	0.25±0.03	1.66±0.03*	2.14±0.06	14.0±0.14*
18:3n-3	0.08±0.01	0.08±0.06	0.33±0.01	0.35±0.01
20:5n-3	0.32±0.02	0.09±0.03*	0.59±0.02	0.12±0.01*
22:5n-3	0.22±0.01	0.04±0.01*	1.67±0.09	0.09±0.01*
22:6n-3	2.67±0.10	0.54±0.03*	14.5±0.25	2.62±0.08*
18:1	28.9±0.31	24.0±0.34	12.5±0.39	11.1±0.13*
16:0	34.3±0.48	34.3±0.66	4.95±0.11	4.04±0.12
18:0	8.29±0.06	8.80±0.15	21.0±0.38	23.4±0.48

Values are means ± SE (in g/100 g fatty acid). Pregnant rats were fed a diet with adequate n-3 fatty acids or a diet deficient in n-3 fatty acids with high n-6 fatty acids throughout gestation. Fetal intestinal phosphatidylcholine (PC) and phosphatidylethanolamine (PE) fatty acids were analyzed on gestation day 19. *Significant difference at $P < 0.01$.

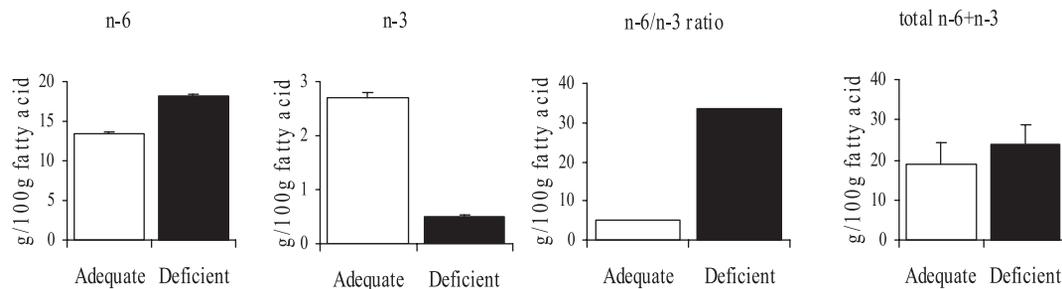
Maternal diet



Amniotic fluid



Fetal intestinal PC



Fetal intestinal PE

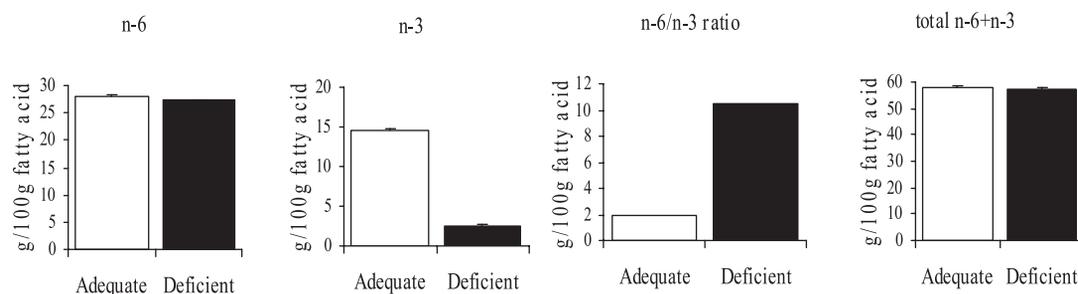


Fig. 1. Total n-6, n-3, n-6-to-n-3 (n-6/n-3) fatty acid ratio, and total n-6 plus n-3 fatty acids in the maternal diet and in amniotic fluid and fetal intestinal phosphatidylcholine (PC) and phosphatidylethanolamine (PE) at gestation *day 19* of rats fed a diet adequate or deficient in n-3 fatty acids. Values are means + SE; in some cases, SE values are too small to signify. * $P < 0.05$ compared with the adequate group.

than intestine PC and the higher 22:4n-6 in the fetal intestine than liver PE.

DISCUSSION

Our results provide the first evidence to show that maternal dietary n-6 and n-3 polyunsaturated fatty acids during gestation

influence the fatty acid composition of amniotic fluid lipids and structural membrane lipids in the fetal intestine. We used a well-controlled experimental design in which the composition of the maternal dietary fat was the only variable and demonstrated that amniotic fluid is abundant in n-6 fatty acid ARA (20:4n-6) and n-3 fatty acid DHA (22:6n-3) in rats fed a diet

Table 3. Fetal liver PC and PE fatty acids

Fatty Acids	PC		PE	
	Control diet	n-3-Deficient diet	Control diet	n-3-Deficient diet
18:2n-6	8.22±0.31	11.5±0.35*	3.40±0.18	4.92±0.13*
20:4n-6	12.3±0.33	16.1±0.34*	23.8±0.22	27.0±0.15*
22:4n-6	0.30±0.01	0.53±0.02*	1.33±0.03	2.38±0.07*
22:5n-6	0.33±0.03	2.91±0.07*	0.65±0.05	11.4±0.20*
18:3n-3	0.05±0.01	0.06±0.02	0.06±0.00	0.08±0.01
20:5n-3	0.76±0.06	0.03±0.01*	0.99±0.10	0.03±0.01*
22:5n-3	0.20±0.01	0.02±0.00*	0.65±0.04	0.07±0.01*
22:6n-3	6.40±0.21	1.24±0.06*	19.3±0.40	4.72±0.17*
18:1	22.5±0.3	18.0±0.20*	12.2±0.32	11.1±0.31
16:0	30.1±0.17	30.8±0.17	15.5±0.25	14.9±0.26
18:0	10.4±0.14	10.2±0.13	18.2±0.11	18.8±0.18

Values are means ± SE (in g/100 g fatty acid). Pregnant rats were fed a diet with adequate n-3 fatty acids or a diet deficient in n-3 fatty acids with high n-6 fatty acids throughout gestation. Fetal liver PC and PE fatty acids were analyzed on gestation day 19. *Significantly different at $P < 0.01$.

with about 1.6% energy as ALA in a LA-to-ALA ratio of 2.5:1. Feeding of a diet restricted in n-3 fatty acids and high in LA resulted in a fivefold decrease in DHA in amniotic fluid and in fetal intestine PC and PE. Human infant cortex PE is notable for enrichment and functional importance of DHA and contains about 12% DHA (17, 27, 36, 40). Dietary restriction of n-3 fatty acids in developing animals results in a reduction in DHA with a reciprocal increase in n-6 fatty acids, particularly n-6 DPA, in the brain such that the total n-6 plus n-3 fatty acids in intestine phospholipids remains constant (18, 26, 39). We provide the first demonstration that fetal intestine PE is also high in DHA, representing about 14% fatty acids, and that the total n-6 plus n-3 fatty acid content in PE is maintained similar to that in the brain through increased incorporation of n-6 fatty acids, particularly n-6 DPA, during n-3 deprivation.

Although phospholipids, including dipalmitoyl PC, the ratio of dipalmitoyl PC to sphingomyelin, and phosphatidylglycerol and cholesteryl palmitate, have been studied as indexes of fetal lung maturity (18, 34, 48), there is a paucity of information on the composition of amniotic fluid phospholipid n-6 and n-3 fatty acids. We are aware of no recent information using modern methods of fatty acid derivatization and capillary column GLC technology. The acidic phospholipids phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine are present in the epithelial lining of the postnatal lung but are found in very low concentrations in amniotic fluid and fetal lung lipids in the immature fetus (22). Consistent with this, phosphatidylglycerol, phosphatidylinositol, and phosphatidylserine were below the range of detection in rat amniotic fluid at gestation day 19, 2 days before term, in our studies; the major esterified lipids were cholesteryl esters and choline phospholipids in concentrations of about 30 and 25–30 mg/l, respectively. Analyses of lipid soluble phosphorous in human amniotic fluid at near-term gestation has found about 25 mg phospholipid/l (1).

The importance of amniotic fluid swallowing to the maintenance of amniotic fluid homeostasis and to fetal somatic and gastrointestinal development is well recognized (41). Recent advances, however, have shown the importance of direct transfer of water and solutes between fetal blood and amniotic fluid through the intramembranous pathway, including the fetal surface of the placenta, umbilical cord, and fetal skin, in

maintaining amniotic fluid and solute homeostasis (4). A contribution of amniotic fluid lipids to the accretion of essential n-6 and n-3 fatty acids in fetal tissues has not, to our knowledge, been raised previously, although experimental data have been published to show incorporation of amniotic fluid lipids into fetal tissues. Studies concerning amniotic fluid surfactant found 46% label from intra-amniotically administered dipalmitoyl PC in the fetal intestine, with a further 6.6% of the label in the liver (22). Similarly, intra-amniotic administration of DHA resulted in increased DHA in the fetal brain and liver (20). The physiological significance of amniotic fluid swallowing to fetal tissue DHA accretion cannot be addressed directly from our study. However, the term human fetus swallows 500–1,000 ml amniotic fluid/day, which contributes about 10% of total protein intake (3). The high concentration of DHA in the fetal intestine and amniotic fluid raises important new questions with respect to fetal lipid nutrition, tissue development, and diseases associated with an exaggerated inflammatory response or oxidative tissue damage. In this regard, studies by us and others have shown that diets high in n-3 fatty acids have a beneficial effect in suppressing inflammatory response in experimental models of colonic colitis (8, 32), whereas others have shown that polyunsaturated fat intakes in early life have lasting effects on intestinal nutrient transport function (47). In addition, in vitro studies have shown that DHA regulates the expression of ATPases in duodenal basal lateral membrane enterocytes (21), tight junction permeability in intestinal monolayer cells (49), and expression of peroxisome proliferator-activated receptor- α , NF- κ B, cyclooxygenase 2, retinoid X receptor, and VEGF in several cell lines (7, 14, 16, 38). DHA has also been shown to activate CTP:choline-phosphate cytidyltransferase, which is the rate-limiting enzyme in the synthesis of lung surfactant (35), and DHA increased dipalmitoyl PC in the fetal mouse and premature baboon lung (2, 10).

In conclusion, we have demonstrated that the composition and balance of n-6 and n-3 fatty acids in amniotic fluid, specifically the amounts of the long-chain n-3 fatty acids EPA and DHA, and the amounts and balance of n-6/n-3 fatty acids in amniotic fluid and fetal intestine membrane lipids are strongly influenced by maternal dietary lipids. We propose that amniotic fluid swallowing as well as placental fatty acid transfer contributes to n-6 and n-3 fatty acid accretion in developing fetal tissues and that both are subject to modification by an inadequate or unbalanced maternal diet n-6 and n-3 fatty acid intake. We and others have also shown that women following Westernized diets consume amounts of n-3 fatty acids that are below recommended intakes (13, 30). The physiological implications of DHA in amniotic fluid, the high amounts of DHA in fetal intestine aminophospholipids, and the effects of maternal polyunsaturated fatty acid intakes on fetal and subsequent infant tissue functions associated with n-6 and n-3 fatty acids require further investigation.

GRANTS

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