Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Mrudula et al. J Nutr Food Sci 2011, 1:1
DOI: 10.4172/2155-9600.1000102

Research Article Open Access

Effects of Prenatal and Postnatal Dietary Polyunsaturated Fatty Acids on Retinal Fatty Acid Composition and Gene Expression in Adult Rat

T. Mrudula, Ghafoorunissa, N. Saravanan and G. Bhanuprakash Reddy*

National Institute of Nutrition, Hyderabad, India

Abstract

Long chain polyunsaturated fatty acids (LC-PUFA) are the major determinants of the structure and function of retina, any impairment in their maternal and dietary supply might result in the defective retinal development, structure and function. The present study is aimed to investigate the effects of different pre- and postnatal diet varying only in their dietary n-6 and n-3 fatty acid content but not in the total energy on the molecular environment of retina. Female weanling Wistar-NIN rats received a diet enriched with either linoleic acid (LA) or alpha linolenic acid (ALA) or long chain n-3 PUFA (fish oil, FO) for 90 days, and mated with stock diet fed male rats. The first generation male pups of ALA and FO group were continued with the respective diet (ALA-ALA and FO-FO). Male pups delivered by LA fed mothers were divided into three groups; continued with either mother's diet (LA-LA) or assigned a diet enriched with either ALA (LA-ALA) or LCn-3PUFA (LA-FO). The effect of this dietary regimen on the expression of genes involved in fatty acid metabolism and phototransduction; total fatty acid composition, was analyzed in the retina after 105 days of post weaning feeding. The data show that prenatal and post weaning feeding of LCn-3PUFA enriched diet upregulated the expression of genes involved in phototransduction and fatty acid metabolism; decreased the level of arachidonic acid in retina. These results suggest that feeding LCn-3PUFA both at prenatal and postnatal could be beneficial for the molecular environment of retina.

Keywords: Maternal diet; n-6 and n-3 fatty acid; LCn-3PUFA; Rat retina; Fatty acid composition

Introduction

Long chain polyunsaturated fatty acids (LC-PUFA) are derived from shorter chain fatty acids through several desaturation and elongation reactions. The desaturase and elongase enzyme system is shared by linoleic acid 18:2n-6 (LA) and α-linolenic acid 18:3n-3 (ALA) for the formation of respective long chain products of n-6 and n-3 family. In tissues such as retina and brain, LC-PUFA represent up to 30% of total fatty acids suggesting their crucial role in the normal functioning of these specialized tissues [1-4]. Humans depend mainly on diet for the precursors of LC-PUFA such as LA and ALA. However the LC-PUFA could also be supplied directly through diet; meat products and sea foods are major source of the common LC n-6 and LC n-3 PUFA namely arachidonic acid (AA) and docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

The major LC-PUFA present in retina are DHA (22:6n-3) and AA (20:4n-6), found primarily in neural and vascular cell membrane phospholipids [5]. Highest body concentration of DHA per unit weight is found in photoreceptor outer segments of retina. Decreased availability of DHA due to metabolic abnormalities or decreased intake is associated with alterations in structure and function of visual system. Several evidences associate the higher content of DHA in retina and proper photoreceptor function [6,7]. Reports suggest that DHA-enriched cell membranes can favor rhodopsin conformational changes and function [8-10]. DHA and EPA, influence eicosanoid metabolism by regulating the metabolites of LC n-6 PUFA which are involved in the angiogenic and proinflammatory pathways [11]. Metabolites of LC-PUFA are known to function as ligands to transcription factors of many genes that are involved in cellular differentiation and growth and metabolism of lipid, protein, and carbohydrate. Thus the composition of n-6 and n-3 PUFA in the cell and subcellular membranes determine the metabolic fate of the cell by modulating growth, metabolism, expression of macromolecules and function [12-14].

Several evidences have shown that perturbation in the nutritional status of mother, particularly *in utero* conditions, may program the fetus for the development of diseases in the adulthood [15,16]. Recent studies suggest that nutritional imbalances during pregnancy cause perturbation of prenatal development which in turn permanently alters the structure, function and metabolism of various tissues and organs [17]. Studies also indicate that an increased intake of LC n-3 PUFA during pregnancy is directly proportional to the length of gestation and birth size [18] and beneficial in maintaining the overall metabolic status of the offspring after growth [19]. Whereas n-3 PUFA deficiency during pregnancy predisposes the offspring to metabolic abnormalities such as hypertension in adult life [20] indicating that maternal PUFA status during pregnancy is critical for the normal development of the fetus. Reports also suggest that maternal intake of saturated fatty acids (SFA) and high fat diet adversely affect the fetal health by predisposing the offspring to insulin resistance [21] and metabolic syndrome [22] respectively.

In recent years, the intake of SFA, n-6 PUFA and *trans*-fatty acids (TFA) has been increased whereas n-3 PUFA intake has decreased, which eventually resulted in increased ratio of n-6/n-3 PUFA in the diet. Studies on dietary fat intake among Indians showed that n-6 PUFA requirements are fully met owing to their high levels in cereal, millet and vegetable oils but n-3 PUFA intake is low and thus leading to higher n-6/n-3 ratio [23]. This change in dietary habits might be responsible for the increased prevalence of diet-related chronic diseases. In view of increasing interest in the role of LC-PUFA during

***Corresponding author:** G. Bhanuprakash Reddy, National Institute of Nutrition, Jamai-Osmania, Tarnaka, Hyderabad, India, Tel: 91-40-27197252; Fax: 91-40-27019074, E-mail: geereddy@yahoo.com

Accepted October 20, 2010; **Published** November 03, 2010

Citation: Mrudula T, Ghafoorunissa, Saravanan N, Reddy GB (2011) Effects of Prenatal and Postnatal Dietary Polyunsaturated Fatty Acids on Retinal Fatty Acid Composition and Gene Expression in Adult Rat. J Nutr Food Sci 1:102. doi:10.4172/2155-9600.1000102

fetal and early development, a study was conducted to understand the effects of diet varying only in the composition of the essential fatty acids during prenatal and postnatal period on metabolic programming of the offspring with respect to glucose metabolism, insulin sensitivity and tissue fatty acid profile. The dietary fats used in the study represented diets enriched with (a) 18:2n-6 (LA diet), (b) 18:3n-3 (ALA diet) and (c) LC n-3 PUFA (EPA and DHA diet). One of the findings of the study was that the maternal intake of ALA diet increased the levels of LC n-3 PUFA in the skeletal muscle of the offspring which is suggested to be beneficial in terms of increased insulin sensitivity [24]. Considering the importance of the study design and the role of LC-PUFA in the overall structure and function of the fetus and adult retina, we investigated the effect of prenatal and postnatal dietary n-6 and n-3 fatty acids on the retinal gene expression, retinal protein levels and retinal fatty acid profile in the adult rats as a part of the previous study [24].

Materials and Methods

Materials

Anti-glial fibrillary acidic protein (GFAP) antibody, anti-rabbit & anti-mouse IgG antibodies conjugated to peroxidase, TRI reagent and heptadecanoic acid were obtained from Sigma-Aldrich (St. Louis, MO). cDNA synthesis kit was procured from Invitrogen (Carlsbad, CA), IQTM SYBR Green supermix was from Bio-Rad (CA, USA), nitrocellulose membrane was of Millipore (Bedford, MA). Mouse monoclonal anti-rhodopsin antibody was from Chemicon (Temecula, CA). A chemiluminescence kit (Amersham Biosciences) was used for development and visualization of immunoreactive bands on Kodak X Omat AR Film. Authentic fatty acid standards were obtained from Nu-Chek (Elysian, MN).

Design of Animal experiment: The entire set of experiments of the present manuscript is a part of the previous study and the composition of the maternal and offspring diet, and fatty acid composition of diets were presented in detail in the previous report [24]. In brief, three week old male (n=12) and female (n=24) Wistar-NIN (WNIN) rats were obtained from National Centre for Laboratory Animal Sciences, Hyderabad, India and fed cereal-pulse based diet. While the male rats were fed on a stock colony diet, the female rats were randomly divided into three groups and fed the same stock diet enriched with either LA or ALA or LC n-3 PUFA (fish oil, FO) for 90 days (Table 1). Dietary carbohydrate provided 59% of energy while dietary protein and fat provided 20 and 21% of energy respectively.

Fatty acid diets*					
Mothers (n)	LA diet (12)			ALA diet (6)	LC-n3 PUFA diet (6)
Source and amount of fatty acids	Safflower seed oil and palm oil in the ratio of 4.5:5.5 that provided about 4 g LA and 0.14 g of ALA/ 100 g of diet with a n-6/n3 PUFA ratio of 28			Groundnut oil, palm oil and linseed oil in 3:3:4 ratio provided 3.02 g LA and 1.19 g ALA/ 100 g with a n-6/n3 PUFA ratio of 2.5.	Groundnut oil, palm oil and fish oil (4:5:1) provided 3.79 g LA, 0.15 g ALA and 0.2 g LC n-3 PUFA/ 100 g diet with n-6/n-3 ratio of 11
Duration	90 days				
Mating	Two females + one male**				
Pregnancy & lactation	LA-enriched diet			ALA diet	LC-n3 PUFA diet
Male pups (post weaning)	LA diet	ALA diet	LC-n3 PUFA diet	ALA diet	LC-n3 PUFA diet
(n)	(7)	(7)	(7)	(7)	(7)
Duration	105 days				

*Female rats received a diet that is similar to that of males wherein the fat source was replaced with respective oil blends.
**Male rats received a cereal-pulse based stock colony diet that contains 50% roasted chickpea, 22.5% wheat, 5% groundnut oil, 6% starch, 4% casein, 7% milk powder, 4% AIN-93 salt mixture, 0.5% AIN-93 vitamin mixture and 1% cellulose.

Table 1: Feeding protocol of different groups of mothers and offspring and the amount of fatty acids added to diet along with source of these fatty acids.

GENE		PRIMER SEQUENCE	AMPLICON SIZE
Hprt	F	CTT TGC TGA CCT GCT GGA TTA C	246 bp
	R	TTG GGG CTG TAC TGC TTA ACC	
Rhodopsin	F	CTT CCT GAT CTG CTG GCT TC	285 bp
	R	ACA GTG TCT GGC CAG GCT TA	
Gnat 1	F	TGA CGT GCA TCA TTT TCA TC	350 bp
	R	TTA AGC TCC AGG AAC TGC AC	
Pde6B	F	AGA TCC TGC CCA TGT TTG AC	350 bp
	R	GGC AGA GTC CAT ACC CAG TG	
Cone opsin	F	TGA GAT TTG ATG CTA AGC TGG	350 bp
	R	TGC CGG TTC ATA AAG ACA TAG	
Gnat 2	F	TCA AGA CAA CAG GCA TCA TC	350 bp
	R	AAG AGA ACG ATG GAC GTA GC	
Gfap	F	TTT CTC CAA CCT CCA GAT CC	350 bp
	R	AGC TTT AGG CCC TCA CAC TG	
Crx	F	GGA TGT GTA TGC ACG TGA GG	350 bp
	R	CCA CTG CTG TGG TAG GTG AA	
Elovl2	F	ACT ACG GCC TGT CTG TGT TC	313 bp
	R	TTA TTG AGC CTT CTT GTC CG	
Elovl4	F	ACA TAC AAT GAG CCG AAG CAG TC	401 bp
	R	CGG ATG TGA ACA GGA GGA GAG G	
Δ 5 Desaturase	F	AAG GGC CTT GTA GAA AAG TAT ATG	368 bp
	R	ACA AAA TGA TGT ACC AGG TGA TT	
Δ 6 Desaturase	F	CTA TAT CCC TTT CTA TGG CAT CTT	373 bp
	R	AGA CTT CTT CAG TGA ACT CAC AAT	

Table 2: Primer sequences and the amplicon size of the respective genes.

After 90 days of feeding, the female rats were mated with males (2 females + 1 male) and the pregnant rats were continued on their respective diets throughout the gestation. After delivery, the litter size of each mother was equalized to 7 male pups and the mothers were continued on the respective experimental diets. Upon weaning, the male pups of LA-fed mother were randomly split into three groups; one group was continued on the same diet (LA-LA), the other two groups were assigned to either ALA (LA-ALA) or LC n-3 PUFA (LA-FO) diets. Male pups born to mothers fed with ALA and FO were continued on the respective diet (ALA-ALA and FO-FO). All the procedures involved in animal experiments were conducted in accordance with guidelines and approval of institutional animal ethical committee. We adhered to the ARVO (Association for Research in Vision and Ophthalmology) Statement for the Use of Animals in Ophthalmic and Vision Research.

At the end of the experimental period (105 days), the adult rats were sacrificed by CO_2 asphyxiation. Eye balls were dissected out immediately and cut into two hemispheres to remove the lens and vitreous body. Care was taken to collect the retina intact from the concave regions of the eye ball adjacent to the optical nerve; retinas from two eyes were pooled and snap-frozen in liquid nitrogen and stored at -70^{0}C for further analysis. Retinas of three individual animals from each group were divided in to two portions and used for RNA extractions and western blotting. Retinas of the remaining four animals were pooled individually and used for the fatty acid analysis.

RNA extraction and quantitative real-time PCR: Total RNA was isolated from retinal tissue using TRI reagent and reverse transcription was performed with 5 µg total RNA in a 40 µl reaction volume using cDNA synthesis kit according to manufacturer's instructions. Quantitative real-time PCR was performed using 20 ng of cDNA of each sample in triplicates in a reaction mixture consisting of 10 µl of 2X IQTM SYBR Green supermix, 250 nM of each forward and reverse primers of target gene in a total reaction volume of 20 µl with a thermocycler (iQ Multicolour iCycler; Bio-Rad). The expression of genes involved in phototransduction like rhodopsin (Rho), MW-cone opsin (*O*psin), rod and cone transducins (Gnat1 & Gnat2), and genes involved in fatty acid metabolism like Δ5 & Δ6 desaturases, elongases (Elovl2, Elovl4) were analyzed. Hypoxanthine phosphoribosyltransferase (Hgprt) was also amplified from the samples of different dietary groups as house keeping control. The primer sequences and the amplicon sizes are given in Table 2. The target gene expression values were normalized to house keeping gene (Hgprt) according to comparative Ct-value method for relative quantification [25-27]. To confirm the specificity of amplified products, the PCR products were subjected to melting curve analysis and subsequently agarose gel electrophoresis.

Protein expression by immunodetection: Retina of individual animal was homogenized in lysis buffer containing 50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate and 0.1% triton-X-100. The homogenate was centrifuged at 8000 rpm for 10 minutes and supernatant was used as total retinal lysate. Equal amounts of protein from retinal lysate was separated on 10% SDS–PAGE and blotted onto nitrocellulose membrane. The membrane was immunolabeled with rabbit anti-GFAP antibody (1:100 dilution) or mouse monoclonal anti-rhodopsin antibody (1:100 dilution) and then with secondary anti-rabbit or anti-mouse IgG conjugated to peroxidase. A chemiluminescence kit was used for development and visualization of immunoreactive bands on the film. The immunoreactive bands were quantified using Quantity One software (Bio-Rad).

Extraction and analysis of retinal fatty acids: Total lipids were extracted from retinas by Folch's method, briefly two retinas of individual animal were homogenized in 2 ml of chloroform and lipids were extracted with chloroform/ methanol (2:1) and washed with water to remove protein contaminations from lipid preparations. Then the chloroform layers were pooled and evaporated to dryness in the presence of nitrogen. To the above, 5 ml of 2% sulphuric acid in methanol was added and incubated at 70^{0}C in a water bath for 4 h. After methylation, fatty acids methyl esters (FAME) were extracted using petroleum ether. FAME were analyzed by gas chromatography (GC; PerkinElmer, Norwalk, CN, USA) using Supelco SP 2330 fused silica capillary column (30 m x 0.2 µm film thickness, 0.25 mm internal diameter). Individual fatty acids were identified using authentic standards. Heptadecanoic acid (17:0) was used as internal standard [24].

Statistical analysis: Statistical analysis was done using the SPSS statistical program (SPSS, Inc, Chicago, IL) and the data were presented as mean along with their standard errors. Data were evaluated by One-way ANOVA and ANCOVA followed by least significant difference (LSD) post hoc tests. Log transformation of the data was done before One-way ANOVA and ANCOVA to stabilize variations. Similar results were observed in regression analysis also after controlling for other groups. Differences were considered significant at $p<0.05$.

Results

Gene expression studies

The relative fold expression of candidate genes in different

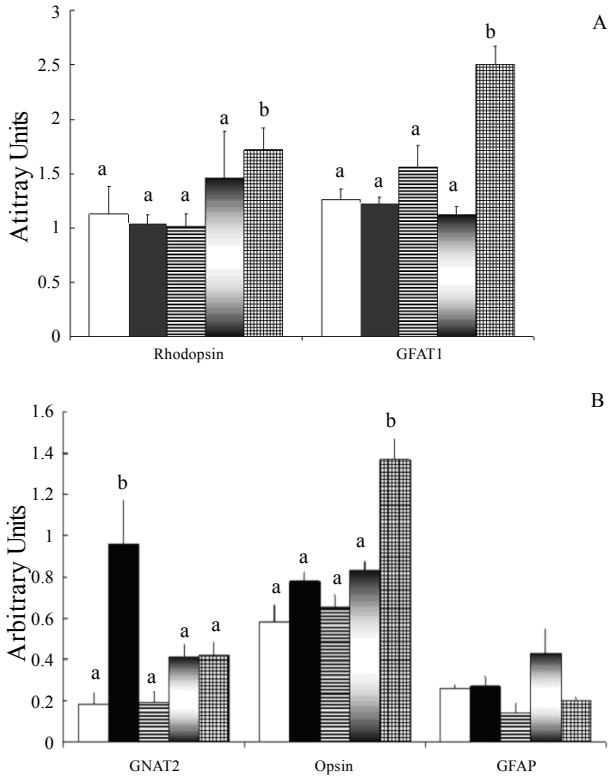

Figure 1: Relative expression of retinal genes in adult WNIN rats fed varying dietary fatty acids at pre and postnatal stages. The relative mRNA abundance was normalized to *Hgprt* and presented in arbitrary units. ***Panel A:*** Rhodopsin & rod transducin (*Gnat 1*); ***Panel B:*** Cone transducin (*Gnat 2*), cone opsin medium wavelength (*Opsin*) & glial fibrillary acidic protein (GFAP). The values are presented as mean ± SE of six independent observations. Different superscripts on the bars indicate values are statistically significantly different from other groups ($p< 0.05$). □sLA-LA, ■LA-ALA, ▀LA-FO, ■ALA-ALA and ▀FO-FO.

experimental groups was determined considering the expression of Hgprt as internal control which is unaltered due to different dietary treatment. Mean relative expression levels were calculated from three independent samples as fold change over Hgprt and presented as arbitrary units. The LA-LA wherein the mother and offspring met the n-6 PUFA requirement fully but not the n-3 PUFA [18] was taken as reference group for comparing the effects of other groups enriched with different dietary PUFA. In general, feeding LC n-3 PUFA enriched diet in the form of fish oil during pregnancy and post weaning period (FO-FO) significantly upregulated the expression of a number of genes in the retina. For example, the rod specific genes namely Rhodopsin and Gnat1; cone cell marker gene Cone Opsin were upregulated by 1.8 folds, 2.5 folds and 2.3 folds respectively in FO-FO group animals when compared to LA-LA animals (Figure 1A & 1B). Feeding LC n-3 PUFA throughout (pre and postnatal period, FO-FO) upregulated the genes involved in elongation of fatty acids >C22 (Elovl2, 5 folds) and very long chain fatty acids >C26 (Elovl4, 3.3 folds). While the increased expression of Elovl2 is statistically not significant, increase in Elovl4 is statistically significant. The Elovl4 gene is upregulated to the same extent even in the offspring of LA animals switched to post weaning

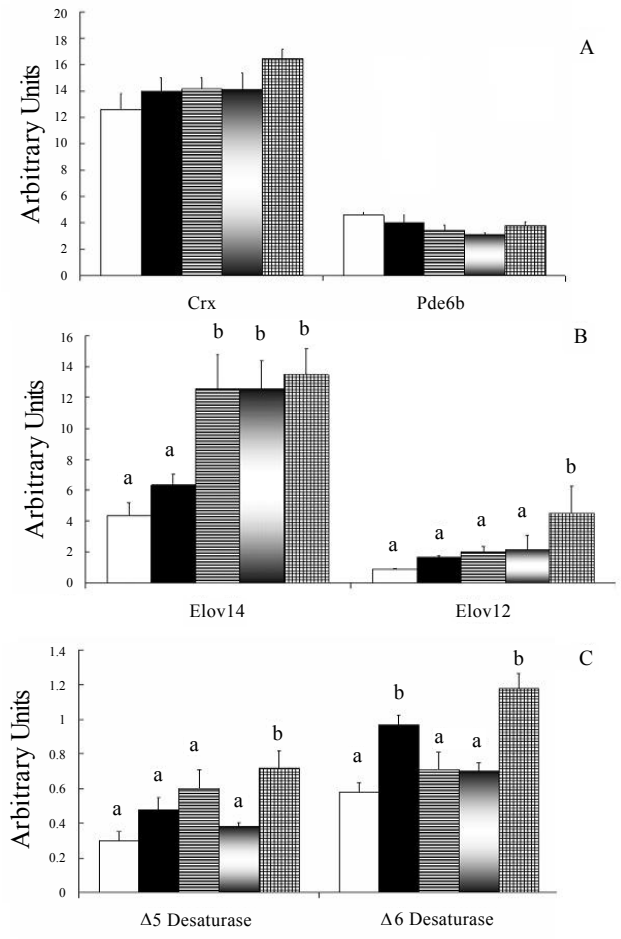

Figure 2: Relative expression of retinal genes in adult WNIN rats fed varying dietary fatty acids at pre and postnatal stages. The relative mRNA abundance was normalized to *Hgprt* and presented in arbitrary units. ***Panel A:*** Cone-rod homeobox gene (*Crx*) & phosphodiesterase 6B (*Pde6B*); ***Panel B:*** Elongases (*Elovl4 &*, *Elovl2*); ***Panel C:*** Δ5- & Δ6- desaturases. The values are presented as mean ± SE of six independent observations. Different superscripts on the bars indicate values are statistically significantly different from other groups (p< 0.05). □sLA-LA, ■LA-ALA, ■LA-FO, ■ALA-ALA and ■FO-FO.

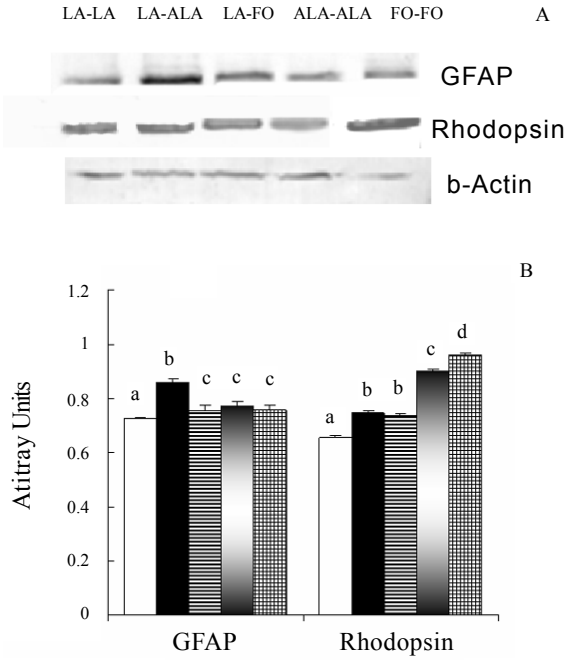

Figure 3: Immunodetection of glial fibrillary acidic protein (GFAP) & rhodopsin in the retina of WNIN rats fed with different dietary fatty acids. The abundance of individual protein were quantified, normalized with the levels of β-actin and presented in arbitrary units considering respective protein expression in PD-Ia group as one unit. ***Panel A:*** Representative immunoblot of rhodopsin, GFAP and β-actin; ***Panel B:*** The values are presented as mean ± SE of three independent observations. Different superscripts on the bars indicate values are statistically significantly different from other groups (p< 0.05). □sLA-LA, ■ LA-ALA, ■LA-FO, ■ALA-ALA and ■FO-FO.

ALA diet (LA-ALA) and animals fed throughout ALA diet (ALA-ALA) (Figure 2B). Feeding LC n-3 PUFA during prenatal and postnatal period also upregulated the gene expression of both Δ*5* (2.5 folds) and Δ*6* desaturases (2 folds) (Figure 2C). Expression of *Gnat2* and Δ*6* desaturase was also upregulated 5 and 2 folds respectively, upon shifting the pups from LA to ALA (Figure 1B and 2C). Whereas, expression of stress response protein, glial fibrillary acidic protein (Gfap) (Figure 1B) which is implicated in the gliosis of the amacrine cells of the retina, critical retinal transcription factor, cone-rod homeobox gene (Crx) (Figure 2A) and the expression of cGMP-dependent phosphodiesterase 6B (Pde6B) (Figure 2A) were not significantly altered by any of the different dietary treatments compared to LA-LA diet.

Protein expression

Immunodetection on retinal lysates of different groups was performed with selected retinal markers to validate the expression of retinal genes at protein level. The Western analysis showed no change in the expression of GFAP while the expression of rhodopsin is increased in a gradual manner upon shifting the postnatal diet from LA-LA to LA-ALA, LA-FO, ALA-ALA and FO-FO (Figure 3). The immunoblot analysis on the levels of rhodopsin validates its expression at transcript level observed in the present study (Figure 1A).

Retinal fatty acid composition

The data on retinal fatty acid composition showed no gross variations except for few notable changes. The percentage of saturated fatty acids is similar among all the groups. However, the level of AA (C20:4) is significantly (~17% of total AA) decreased in the FO-FO adult rats when compared to LA-LA animals (Table 3). This decrease

Fatty acids (nmole %)	LA-LA	LA-ALA	LA-FO	ALA-ALA	FO-FO
C 14:0	1.2 + 0.4	1.1 + 0.3	0.9 + 0.15	1.4 + 0.14	1.1 + 0.2
C 16:0	22.7 + 0.99	21.7 + 0.93	20.9 + 1.2	22.0 + 0.79	23.0 + 0.55
C 16:1	1.2 + 0.04	0.5 + 0.21	0.95 + 0.36	1.3 + 0.58	1.5 + 0.45
C 18:0	25.6 + 0.7	26.6 + 1.21	26.6 + 1.57	26.1 + 0.74	25.7 + 0.74
C 18:1	9.1 + 0.26	8.6 + 0.2	8.5 + 0.11	8.8 + 0.14	8.2 + 0.34*
C 18:2n-6	1.4 + 0.12	1.4 + 0.07	1.6 + 0.32	1.4 + 0.15	1.5 + 0.15
C 20:0	0.7 + 0.05	0.8 + 0.11	0.6 + 0.12	0.7 + 0.19	0.7 + 0.19
C 20:3n-6	1.2 + 0.53	1.2 + 0.79	0.8 + 0.49	0.9 + 0.65	1.3 + 0.96
C 22:0	0.6 + 0.07	0.9 + 0.3	0.7 + 0.09	0.7 + 0.2	0.7 + 0.13
C 20:4n-6	9.6 + 0.38	8.9 + 0.62	8.6 + 0.46	8.7 + 0.39	8.0 + 0.43*
C 24:0	0.97 + 0.25	1.0 + 0.33	0.67 + 0.2	0.89 + 0.28	0.78 + 0.18
C22:4n-6 & C 24:1	2.4 + 0.08	1.7 + 0.10	2.0 + 0.09	2.0 + 0.09	1.85 + 0.06*
C 22:5n-3	0.5 + 0.07	0.76 + 0.11	0.58 + 0.06	0.6 + 0.10	0.6 + 0.11
C 22:6n-3	22.0 + 0.6	24.0 + 0.8	25.6 + 1.5	24.3 + 0.6	24.4 + 0.3
Index					
$\sum$ (LA + AA)[a]	11.3	10.3	10.2	10.1	9.5
$\sum$ LC n-3 PUFA[b]	22.5	24.7	26.1	24.9	25.0
[a/b]Ratio	1.99	2.39	2.55	2.46	2.63

LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid; FO, fish oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. The values are presented as Mean ± SE of six independent observations. *Values are significantly different from other groups ($p < 0.05$). [a]Total LA and AA; [b]Total LC n-3 PUFA.

Table 3: Total phospholipids fatty acid composition in the retina of WNIN rat pups fed with a synthetic diet differing only in their fatty acid profile, during pregnancy and postnatal period.

in the AA levels in the FO-FO fed animals reflected in almost ~16% decrease in the total LA + AA levels in the retinal lipids. Further there was an increased ratio (1.32 fold) of total LC-n-3 PUFA (DPA + DHA) to LA + AA (biologically active n-6 PUFA) in the FO-FO fed animals (Table 3).

Discussion

Humans depend on diet as a major source for the LA and ALA, which are further elongated to LC-PUFA of respective fatty acid classes metabolically. Both LA and ALA and their LC-PUFA derivatives are required during normal fetal development to support the synthesis of structural lipids of brain and retinal tissue [28]. Diets in developed and developing countries are generally abundant of n-6 PUFA but poor in n-3 PUFA. Several evidences suggest that humans evolved on a diet with n-6/n-3 ratio of ~1 whereas now this ratio is about 15 in many parts of world including India [29-31]. The dietary ratio of n-6/n-3 is potentially important because the ratios of these fatty acids in the tissues are determined largely by their ratios in the diet.[32,33] The excessive amount of n-6 PUFA and a very high n-6/n-3 ratio is reported to promote the pathogenesis of many diseases including cardiovascular,[34] cancer, [35] inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFA (or a low n-6/n-3 ratio) is suggested to be beneficial [36-38]. Therefore, the present study was aimed to investigate the effect of maternal and post weaning (i.e. pre and postnatal) supply of diet enriched with (a) n-6 PUFA (LA) (b) n-3 PUFA (ALA) and (c) LC n-3 PUFA (EPA + DHA) with varying n-6/n-3 ratio on gene expression and fatty acid composition of adult rat retina.

Expression of many of the retinal genes tested that are critical for the retinal structure and functions are upregulated by pre and postnatal feeding of LC n-3 PUFA. The rod specific genes such as Rho, Gnat1, cone specific gene such as Opsin, the genes involved in the fatty acid metabolism such as $\Delta 5$ & $\Delta 6$ desaturases and elongases (Elovl2 & Elovl4) were significantly upregulated due to LC n-3 PUFA feeding throughout pregnancy, lactation and post weaning. The gene expression observed in the adults derived from mothers fed with ALA enriched diet does not show similar upregulation in the expression of retinal specific genes. Neither the animals shifted from LA to ALA (ALA rich diet) nor the animals shifted from LA to FO (LC n-3 PUFA rich diet) show the similar alterations in the gene expression, with the exception of Gnat2 & $\Delta 6$ desaturase (LA-ALA) and Elovl4 & $\Delta 5$ desaturase (LA-FO) respectively. These observations clearly suggest that maternal as well as post weaning feeding of LC n-3 PUFA is essential to have the beneficial effects in terms of increased retinal gene expression.

The rhodopsin protein expression, important for the normal structure and function of rod photoreceptor, is increased due to LC n-3 PUFA and the increase was also prominent in the other dietary groups when compared to LA-LA. Studies indicate that n-3 PUFA deficiency may cause decreased rhodopsin expression and thereby decrease the retinal sensitivity to light [39]. However, in the present study we demonstrate that by increasing the n-3 PUFA levels in the diet through ALA and LC n-3 PUFA, the rhodopsin expression could be increased. However, expression of a stress response protein, GFAP which is usually upregulated during pathological conditions such as in retinal atrophy, was not altered by different dietary fatty acid regimen.

The direct effects of dietary ALA on insulin sensitivity and increasing the levels of EPA and DHA in non-neural tissues has been shown earlier [40]. Our previous study with the same design also showed that maternal supplementation of n-3 PUFA through ALA and FO could improve the n-3 PUFA status in muscle phospholipids [24]. However, in the present study the maternal and post natal feeding of LA and ALA rich diet did not really influence either the levels of total SFA, total n-3 PUFA or DHA. Despite the higher amount of LA in the diet the amount of 18:2n-6 in the retina of LA-LA diet fed animals did not reflect the dietary levels. Moreover, ALA is the direct precursor for the LC n-3 PUFA in retina and the levels of which were also not altered in the retina in respective groups (LA-ALA and ALA-ALA) suggesting that the retina has a specific and robust mechanism to preserve and maintain the fatty acids profile even under the extreme conditions of deficiency [41].

Studies indicate that decreasing the levels of AA, which is the precursor for the inflammatory metabolites, could alleviate the harmful effects of neuro-inflammation and other age-related neural degeneration [42,43]. However, studies reporting a decrease in AA levels in retinal tissues are limited which can be attributed to unique fatty acid conservation mechanism of retina. Studies demonstrated that adequate n-3 PUFA in the diet could marginally (approximately 15%) decrease the levels of AA in F2 generation rats [44]. In the present study the intake of LC n-3 PUFA rich diet resulted in 17% decrease in the total AA levels in the retinal tissues of the animals of the F1 generation which is statistically significant when compared to the other groups. However, it is important to note that postnatal feeding of LC n-3 PUFA or ALA to the offspring born to LA fed mothers does not seem to be effective in decreasing the levels of AA. This observation in turn clearly suggests that both prenatal and postnatal feeding of LC n-3 PUFA might be needed to achieve a better n-6/n-3 ratio. Further it is also

interesting to note that LC n-3 PUFA fed animals showed significant decrease in the levels of AA in retina which in turn reflected a higher ratio of total LC n-3 PUFA (DPA + DHA) / biologically active n-6 PUFA (LA +AA). This ratio in FO-FO fed animals is 2.6 compared to LA-LA fed animals where the ratio is 2.0, further indicates that higher n-3/n-6 ratio would be beneficial to retinal structure and function. The present data is limited for the detection of fatty acid with the chain length up to C22. Therefore, it is not clear whether the up regulation of Elovl4 & Elovl2 reflected in the retinal fatty acids of chain length >C22, since these elongases act on fatty acids with chain length >C22.

In conclusion, the present study showed that feeding n-3 LC-PUFA is beneficial in terms of retinal gene expression and fatty acid composition only when it is supplied throughout pregnancy, lactation and post weaning period.

Acknowledgements

TM - Received a research fellowship from Andhra Pradesh Netherlands Biotechnology Programme (Currently Agri Biotech Foundation), India; GBR - Received grants from the Department of Biotechnology and Indian Council of Medical Research, Government of India. The authors thank Ms. Vani Acharya (National Institute of Nutrition) for her technical assistance in gas chromatography experiments.

References

1. Poulos A (1995) Very long chain fatty acids in higher animals-a review. Lipids 30: 1-14.
2. Neuringer M, Anderson GJ, Connor WE (1988) The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annu Rev Nutr 8: 517-541.
3. Catala A (2010) A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochem Biophys Res Commun 399: 318-323.
4. Liu A, Chang J, Lin Y, Shen Z and Bernstein PS (2010) Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. J Lipid Res 51: 3217–3229.
5. Fliesler SJ, Anderson RE (1983) Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 22: 79-131.
6. Futterman S, Downer JL, Hendrickson A (1971) Effect of essential fatty acid deficiency on the fatty acid composition, morphology, and electroretinographic response of the retina. Invest Ophthalmol 10: 151-156.
7. Wheeler TG, Benolken RM, Anderson RE (1975) Visual membranes: specificity of fatty acid precursors for the electrical response to illumination. Science 188: 1312-1314.
8. Wiedmann TS, Pates RD, Beach JM, Salmon A, Brown MF (1988) Lipid-protein interactions mediate the photochemical function of rhodopsin. Biochemistry 27: 6469-6474.
9. Aveldano MI (1988) Phospholipid species containing long and very long polyenoic fatty acids remain with rhodopsin after hexane extraction of photoreceptor membranes. Biochemistry 27: 1229-1239.
10. Mitchell DC, Straume M, Litman BJ (1992) Role of sn-1-saturated, sn-2-polyunsaturated phospholipids in control of membrane receptor conformational equilibrium: effects of cholesterol and acyl chain unsaturation on the metarhodopsin I in equilibrium with metarhodopsin II equilibrium. Biochemistry 31: 662-670.
11. Massiera F, Saint-Marc P, Seydoux J, Murata T, Kobayashi T, et al. (2003) Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? J Lipid Res 44: 271-279.
12. Innis SM (2004) Essential fatty acid metabolism during early development. In: Burrin DG, editor. Biology of metabolism in growing animals. BV. Amsterdam: Elsevier Science.
13. Innis SM (2003) Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. J Pediatr 143: S1-8.
14. Mukhopadhyay B, Liu J, Osei-Hyiaman D, Godlewski G, Mukhopadhyay P, et al. (2010) Transcriptional Regulation of Cannabinoid Receptor-1 expression in the liver by Retinoic Acid acting via Retinoic acid Receptor-γ. J Biol Chem 285: 19002-19011.
15. Barker DJ (2002) Fetal programming of coronary heart disease. Trends Endocrinol Metab 13: 364-368.
16. Simmons R (2005) Developmental origins of adult metabolic disease: concepts and controversies. Trends Endocrinol Metab 16: 390-394.
17. Holness MJ, Langdown ML, Sugden MC (2000) Early-life programming of susceptibility to dysregulation of glucose metabolism and the development of Type 2 diabetes mellitus. Biochem J 349 Pt 3: 657-665.
18. Olsen SF, Sørensen JD, Secher NJ, Hedegaard M, Henriksen TB, et al. (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. Lancet 339: 1003-1007.
19. Joshi S, Rao S, Golwilkar A, Patwardhan M, Bhonde R (2003) Fish oil supplementation of rats during pregnancy reduces adult disease risks in their offspring. J Nutr 133: 3170-3174.
20. Armitage JA, Pearce AD, Sinclair AJ, Vingrys AJ, Weisinger RS, et al. (2003) Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. Lipids 38: 459-464.
21. Siemelink M, Verhoef A, Dormans JA, Span PN, Piersma AH (2002) Dietary fatty acid composition during pregnancy and lactation in the rat programs growth and glucose metabolism in the offspring. Diabetologia 45: 1397-1403.
22. Buckley AJ , Keserü B, Briodyc J, Thompson M, Ozanne SE, et al. (2005) Altered body composition and metabolism in the male offspring of high fat-fed rats. Metabolism 54: 500-507.
23. Ghafoorunissa (1996) Fats in Indian diets and their nutritional and health implications. Lipids 31 Suppl: S287-291.
24. Ibrahim A, Ghafoorunissa, Basak S, Ehtesham NZ (2009) Impact of maternal dietary fatty acid composition on glucose and lipid metabolism in male rat offspring aged 105 d. Br J Nutr 102: 233-41.
25. Mandal MN, Vasireddy V, Reddy GB, Wang X, Moroi SE, et al. (2006b) CTRP5 is a membrane-associated and secretory protein in the RPE and ciliary body and the S163R mutation of CTRP5 impairs its secretion. Invest Ophthalmol Vis Sci 47: 5505-13.
26. Vasireddy V, Jablonski MM, Mandal MN, Raz-Prag D, Wang XF, et al. (2006a) Elovl4 5-bp-deletion knock-in mice develop progressive photoreceptor degeneration. Invest Ophthalmol Vis Sci 47: 4558-4568.
27. Reddy GB, Vasireddy V, Mandal MN, Mrudula T, Wang ZF, et al. (2009) A Novel Rat Model with Obesity-Associated Retinal Degeneration. Invest Ophthalmol Vis Sci 50: 3456-3463.
28. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF (2001) The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 40: 1-94.
29. Crawford MA (1968) Fatty-acid ratios in free-living and domestic animals. Possible implications for atheroma. Lancet 1: 1329-1333.
30. Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 54: 438-463.
31. Dwarkanath P, Muthayya S, Thomas T, Vaz M, Parikh P, et al. (2009) Polyunsaturated fatty acid consumption and concentration among South Indian women during pregnancy. Asia Pac J Clin Nutr 18:389-394.
32. Cleland LG, Gibson RA, Hawkes JS, James MJ (1990) Comparison of cell membrane phospholipid fatty acids in five rat strains fed four test diets. Lipids 25: 559-564.
33. Lands WE, Libelt B, Morris A, Kramer NC, Prewitt TE, et al. (1992) Maintenance of lower proportions of (n - 6) eicosanoid precursors in phospholipids of human plasma in response to added dietary (n - 3) fatty acids. Biochim Biophys Acta 1180: 147-162.
34. Simopoulos AP (1994) Is insulin resistance influenced by dietary linoleic acid and trans fatty acids? Free Radic Biol Med 17: 367-372.
35. Ge Y, Chen Z, Kang ZB, Cluette-Brown J, Laposata M, et al. (2002) Effects of adenoviral gene transfer of C. elegans n-3 fatty acid desaturase on the lipid profile and growth of human breast cancer cells. Anticancer Res 22: 537-543.

36. Bartram HP, Gostner A, Scheppach W, Reddy BS, Rao CV, et al. (1993) Effects of fish oil on rectal cell proliferation, mucosal fatty acids, and prostaglandin E2 release in healthy subjects. Gastroenterology 105: 1317-1322.

37. Bartram HP, Gostner A, Reddy BS, Rao CV, Scheppach W, et al. (1995) Missing anti-proliferative effect of fish oil on rectal epithelium in healthy volunteers consuming a high-fat diet: potential role of the n-3:n-6 fatty acid ratio. Eur J Cancer Prev 4: 231-237.

38. James MJ, Cleland LG (1997) Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Semin Arthritis Rheum 27: 85-97.

39. Bush RA, Malnoë A, Remé CE, Williams TP (1994) Dietary deficiency of N-3 fatty acids alters rhodopsin content and function in the rat retina. Invest Ophthalmol Vis Sci 35: 91-100.

40. Blank C, Neumann MA, Makrides M, Gibson RA (2002) Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. J Lipid Res 43: 1537-1543.

41. Wiegand RD, Koutz CA, Stinson AM, Anderson RE (1991) Conservation of docosahexaenoic acid in rod outer segments of rat retina during n-3 and n-6 fatty acid deficiency. J Neurochem 57: 1690-1699.

42. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, et al. (2007) Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med 13: 868-873.

43. Sanchez-Mejia RO, Newman JW, Toh S, Yu GQ, Zhou Y, et al. (2008) Phospholipase A2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. Nat Neurosci 11: 1311-1318.

44. Moriguchi T, Loewke J, Garrison M, Catalan JN, Salem N Jr (2001) Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum. J Lipid Res 42: 419-427.