The role of n-3 polyunsaturated fatty acids in brain: Modulation of rat brain gene expression by dietary n-3 fatty acids

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Rats were fed either a high linolenic acid (perilla oil) or high eicosapentaenoic + docosahexaenoic acid (fish oil) diet (8%), and the fatty acid and molecular species composition of ethanolamine phosphoglycerides was determined. Gene expression pattern resulting from the feeding of n-3 fatty acids also was studied. Perilla oil feeding, in contrast to fish oil feeding, was not reflected in total fatty acid composition of ethanolamine phosphoglycerides. Levels of the alkenylacyl subclass of ethanolamine phosphoglycerides increased in response to feeding. Similarly, levels of diacyl phosphatidylethanolamine molecular species containing docosahexaenoic acid (18:0/22:6) were higher in perilla-fed or fish oil-fed rat brains whereas those in ethanolamine plasmalogens remained unchanged. Because plasmalogen levels in the brains of rats fed a n-3 fatty acid-enriched diet increased, it is plausible, however, that docosahexaenoic acid taken up from the food or formed from linolenic acid was deposited in this phospholipid subclass. Using cDNA microarrays, 55 genes were found to be overexpressed and 47 were suppressed relative to controls by both dietary regimens. The altered genes included those controlling synaptic plasticity, cytosceleton and membrane association, signal transduction, ion channel formation, energy metabolism, and regulatory proteins. This effect seems to be independent of the chain length of fatty acids, but the n-3 structure appears to be important. Because n-3 polyunsaturated fatty acids have been shown to play an important role in maintaining normal mental functions and docosahexaenoic acid-containing ethanolamine phosphoglyceride (18:0/22:6) molecular species accumulated in response to n-3 fatty acid feeding, a casual relationship between the two events can be surmised.

Lipids in central nervous tissue of vertebrates are characterized by high content of docosahexaenoic acid (22:6n-3, DHA), which is particularly enriched in aminophospholipids, ethanolamine, and serine phosphoglycerides (1). This and other n-3 polyunsaturated fatty acids occupy the sn-2 position of phospholipids, and it has been suggested that the brain tissue has "an absolute molecular species requirement" for its function (2). Its importance in maintaining structural and functional integrity of membranes is highlighted by the fact that DHA cannot easily be depleted from the brain (3, 4); usually two generations are necessary to reduce its level (5, 6). One of the major functions of DHA can be related to its involvement in cognitive processes. Once its level had been reduced, the animal models underperform in learning tests and other mental functions (7–9), which can be reversed by supplying DHA or other n-3 polyunsaturated fatty acids, such as linolenic acid (18:3n-3, LNA) to the animals (8). It also has been demonstrated that infants receiving long-chain polyunsaturated fatty acid during pregnancy or nursing showed higher scores in the Brunet–Lézine psychomotor development test than those deficient in these fatty acids (10). Indeed, cerebral cortex DHA level is higher in breast-fed infants than in formula-fed ones (11). Most of the brain DHA is supplied by the liver during pregnancy (12, 13) and by breast feeding after delivery (11). The exact mode of action of DHA-containing phospholipids on cognitive functions is not known, but there might be a relationship between their effect on blood–brain barrier (14), membrane fluidity (15), activity of some enzymes (8, 16), neural signaling (17), ionic channels (18), or control of nerve growth factor (19). Evidently cognitive processes are very complex and cannot be traced back to a simple mechanistic accumulation of DHA in neural membranes. It can be hypothesized that several genes also will be activated by dietary n-3 fatty acids and that some gene products separately or combined with membrane effects of these fatty acids exert their beneficial effect on mental functions such as learning, memory, etc. The fact that LNA and DHA activate several genes in other tissues, like liver or adipose tissue, has already been demonstrated (20, 21), but no data are available on brain. To address this question, essential fatty acid-sufficient rats were fed from conception with oils rich either in LNA, the precursor of DHA, or in eicosapentaenoic acid (EPA)+DHA only for one generation, and the expression of genes in brains was tested when the rats reached adulthood. In this article an attempt is made to correlate the alterations in molecular composition of ethanolamine phosphoglycerides caused by dietary n-3 polyunsaturated fatty acids with their effect on gene expression and cognitive performance.

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Experimental Procedures

Animals and Diets. Male Wistar rats were kept from conception until adulthood on rat chow supplemented either with perilla oil or fish oil (8%). Fatty acid composition of the diets is given in Table 1. Brains were removed, snap-frozen in liquid nitrogen, and kept until processing at −70°C.

Analysis of Brain Phospholipids. Lipids were extracted from hemispheres according to Folch *et al.* (22). Phospholipids were obtained by column chromatography on Silicagel using chloroform to elute neutral lipids and methanol to elute polar lipids (phospholipids). Fatty acid compositions of the separated phospholipids by TLC were determined by gas chromatography using a FFAP column (30 m, 0.25 mm i.d., Supelco). Molecular species composition of ethanolmine phosphoglycerides after segregation into diacyl, alkylacyl, and alkenylacyl subclasses was determined according to the method of Takamura and Kito (23) on a Supelcosyl LC 18 column (25 cm, 1 mm i.d., Supelco). Identification of peaks was done as described in ref. 24. Quantitation of phosphatidylethanolamine subclasses was done by adding known amounts of derivatized 12:0/12:0 diglyceride to the spots.

Construction of Microarrays. Rat brain, liver, and ganglion cDNA libraries were constructed in TriplEx cloning vector according to

Abbreviations: DHA, docosahexaenoic acid (22:6n-3); LNA, linolenic acid (18:3n-3); EPA, eicosapentaenoic acid.

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Table 1. Fatty acid composition of diets

Fatty acids, wt%	Control	Perilla	Fish
16:0	13.6	8.9	7.5
16:1n-7	0.8	TR	1.3
18:0	3.1	2.5	2.2
18:1n-9	17.0	19.5	16.2
18:1n-7	—	1.2	1.5
18:2n-6	51.0	28.0	23.0
18:3n-3	6.2	39.4	2.5
20:1n-9	—	—	1.3
20:4n-6	0.1	—	—
20:5n-3	0.7	—	12.0
22:5n-3	0.2	—	2.6
22:6n-3	1.2	—	26.9
Lipid content, wt%	8	8	8

Only the most common fatty acids are listed. TR, traces.

the manual of the SMART cDNA construction kit (CLONTECH). Inserts were PCR-amplified with TriplEx vector-specific primers, purified with a MultiScreen-PCR plate (Millipore), dried, resuspended in 50% DMSO/water, and distributed in 384-well plates. Aminoalkylsilanized slides (Silane-Prep Slides, Sigma) were used as the solid support for construction of the array. A total of 3,200 array-ready cDNA clones were spotted in duplicate by using a MicroGrid Total Array System spotter (BioRobotics, Cambridge, U.K.) with 16 pins with a 4 × 4 format. After printing the slides were treated as described (25).

Brain Samples, RNA Preparation, and Amplification. Three rat groups each containing eight animals were examined. They were either (*i*) kept on normal rat chow, (*ii*) fed with fish oil, or (*iii*) fed with perilla oil from conception until reaching adulthood. Their brains were dissected while frozen and immediately subjected to RNA preparation. For gene expression analysis, total RNA was purified from each group (25–25 mg tissue from eight rats) with a NucleoSpin RNA purification kit (Macherey & Nagel) according to the manufacturer's instructions. RNA preparations from each group were pooled, their quantity was assessed by gel electrophoresis, and their OD_{260}/OD_{280} ratios were determined. Total RNA was amplified by using an antisense RNA amplification protocol by *in vitro* transcription by a modified version of Ebervine (http://www.microarrays.org/pdfs/ModifiedEberwine.pdf; ref. 26). Briefly, 20 μg total RNA was reverse-transcribed in the presence of 2 μg HPLC-purified, anchored oligo(dT) primer having T7 promoter sequence at its 5′ end. After first-strand synthesis, double-stranded DNA was prepared with *Escherichia coli* DNA polymerase, *E. coli* ligase, and RNase H (Life Technologies, Vienna, Austria) at 16°C for 5 h and purified with a PCR purification kit (Macherey & Nagel). After sample concentration in vacuum, antisense RNA synthesis was performed with a RiboMax *in vitro* transcription kit (Promega) according to the manufacturer's manual. The RNA was purified, concentrated, and measured spectroscopically.

Generation of Microarray Probes, Hybridization, and Reverse Transcription–PCR. Three micrograms of pooled, amplified, antisense RNA was labeled during reverse transcription with Cy3-labeled or Cy5-labeled dCTP. After hydrolysis of the RNA, labeled cDNA was purified, vacuum-concentrated, and reconstituted in 15 μl of hybridization buffer (50% formamide/5× SSC/0.1% SDS/100 μg/ml salmon sperm DNA) containing 200 ng/μl poly(dT). Probes generated from the control and oil-fed samples were mixed and applied onto the array after denaturation by heating for 1 min at 90°C. The slide was covered by a 24 mm × 32 mm coverslip, and DPX Mountant (Fluka) was poured to its sides to prevent evaporation. Slides were incubated at 42°C for 20 h in a humid hybridization chamber. After hybridization the mountant was removed, and the arrays were washed by submersion and agitation for 10 min in 1× SSC with 0.1% SDS, 10 min in 0.1% SSC with 0.1% SDS, and 10 min in 0.1× SSC at room temperature, then rinsed briefly in deionized water and dried. From each RNA pool four probes were generated: two Cy5-labeled and two Cy3-labeled ones to perform replicate "color-flip" experiments suggested by other studies (27, 28).

Relative quantitative reverse transcription–PCR was performed on a Light Cycler Instrument (Roche Diagnostics) with gene-specific primers and standard protocol to validate the gene expression changes observed with microarrays. Relative expression ratios were normalized to glyceraldehyde-phosphate dehydrogenase. Six of the genes exhibiting alterations in gene expression (see Table 5) were analyzed, and both techniques showed similar expression ratios (data not shown).

Scanning and Data Analysis. Each array was screened under a green laser (532 nm) and a red laser (660 nm) by using a ScanArray Lite (GSI Lumonics, Billerica, MA) scanning confocal fluorescent scanner with 10-μm resolution. The resulting images were quantified by using the software program SCANALYZE2 (ref. 29; www.microarrays.org/software.html). Each spot was defined by manual positioning of grid circles over the image. The average pixel intensity and the local background of each spot were determined. A measure (MRAT, denotes the median of the set of background-corrected single pixel values of channel 2 fluorescence for all pixels within the spot) was determined (30). This average expression ratio for all genes on the array was normalized to 1.0. For background corrections those data were calculated as negatives where the average intensity of the spot was smaller than two times of the average background of the same area. Those results also were excluded where the replicate spots from a different site of the same array or results from the replicate experiments were significantly different. The expression ratios reported are the average and standard deviations from separate experiments. Data analysis and visualization of scatter images were performed with Microsoft EXCEL software.

Results

We expected that dietary n-3 fatty acids would bring about some, so far unrecognized, changes in molecular composition of membrane phospholipids and previously unrecognized gene expression patterns in the brains of the experimental animals. Total phospholipids were investigated in this study under the assumption that the fatty acid and molecular species composition of individual membranes is close to that of total phospholipids.

This article deals only with fatty acid and molecular species composition of phosphatidylethanolamines. Table 2 shows the fatty acid compositions of rat brains in relation to dietary lipids. As seen, fatty acid composition of ethanolamine phosphoglycerides is almost identical in the three dietary regimens with the exception of DHA, which is slightly higher in brains receiving fish oil.

Table 3 shows the distribution of ethanolamine subclasses in relation to dietary fatty acids. Table 3 reveals that there is an increase in the levels of alkenylacyl subclass in order control < perilla oil < fish oil. This trend is also evident when comparing the amounts of total ether lipids (56.3%, 58.3%, 60.6%, respectively). Lin *et al.* (31) also found higher levels of ethanolamine plasmalogens in brain cortex of fish oil-fed monkeys versus soy oil-fed monkeys (40.6% vs. 29.5%). Levels of DHA containing diacyl phosphatidylethanolamine molecular species were almost identical in rat brain and monkey brain cortex (30.1% vs. 31.5%), but in our case lower values for $18{:}0_a/22{:}6$ species (where a is alcohol) were obtained (20% in rat brains vs. 37% in monkey brain cortex) (Table 4). There is an accumulation of diacyl 18:0/22:6 species in response to n-3 fatty acid feeding, and this trend agrees with the data of Lin *et al.* (31).

To test the differences in relative gene expression levels we used a DNA microarray technique (32). To balance individual

Table 2. Fatty acid composition of ethanolamine phosphoglycerides of rat brains

Fatty acid	Control ($n = 8$)	Perilla ($n = 10$)	Fish oil ($n = 10$)
DMA16:0	5.11 ± 0.51	4.71 ± 0.86	4.00 ± 0.93
16:0	8.31 ± 1.53	8.28 ± 2.6	6.34 ± 2.20
16:1n-7	0.68 ± 0.21		1.08 ± 0.36
DMA18:0	9.08 ± 2.00	9.21 ± 2.16	6.46 ± 2.19
DMA18:1n-9	3.29 ± 0.36	3.00 ± 1.58	2.29 ± 0.50
DMA18:1n-7	2.14 ± 0.40	1.83 ± 0.46	1.79 ± 1.18
18:0	17.05 ± 2.00	17.13 ± 1.45	19.00 ± 2.90
18:1n-9	12.18 ± 1.49	12.23 ± 1.19	12.75 ± 2.56
18:1n-7	1.60 ± 0.27	1.57 ± 0.49	1.44 ± 0.30
18:2n-6	0.80 ± 0.02	0.88 ± 0.34	0.71 ± 0.32
18:3n-3	1.90 ± 0.17	ND	0.42 ± 0.22
20:4n-6	9.64 ± 1.57	10.10 ± 1.48	9.07 ± 3.46
20:5n-3	ND	ND	0.1
22:4n-6	4.22 ± 1.29	4.32 ± 0.86	2.68 ± 0.39
22:5n-3	0.52	TR	1.05 ± 0.36
22:6n-3	19.73 ± 2.00	18.39 ± 2.92	22.16 ± 2.86
Total saturates	25.36	25.41	25.34
Total n-6	14.66	15.30	12.46
Total n-3	22.15	18.39	22.60

DMA, dimethyl acetal. ND, not determined.

difference in gene expression patterns we pooled eight rats for each experiment. Each experiment was performed four times by using fluorescent dyes for labeling to reduce the number of false-positive or false-negative ratios deriving from possible uneven incorporation of different dyes during labeling or from other experimental variables introduced by hybridization, washing conditions, or array features. The expression profiles of rat brains on control diets served as control.

The expression levels of 102 cDNAs, representing 3.4% of the total DNA elements on the array, were significantly altered in brains of animals fed either perilla oil (rich in LNA) or fish oil (rich in EPA and DHA) (Table 5). The changes ranged from −5-fold to +7-fold. It was interesting to observe that the two kinds of oils exerted almost the same effect on the gene expression profile. Only one gene, coding for a membrane protein with unknown function, exhibited specific up-regulation in response to fish oil feeding. Again, only one gene (homolog to mouse cDNA clone with unknown function) was specifically down-regulated in perilla oil-fed rat brain. Altogether expression of 55 genes increased whereas the expression of 47 genes was decreased by fish or perilla oil feeding.

Discussion

The observation that n-3 fatty acids, LNA and EPA+DHA, affect expression levels of a number of genes in brain opens the way toward understanding the role of these fatty acids in the function of central nervous tissue. Until now, one of the major functions of long-chain polyunsaturated fatty acids, particularly DHA, and polar lipids esterified with these fatty acids, was believed to be to maintain the proper biophysical property and structural integrity of neural membranes. This paper assigns an additional function to these fatty acids/phospholipids, namely to control gene expression in nervous tissue. From the number of genes involved one can conclude that this control might be very complex and concerted even if we don't know the exact role of certain genes. Altogether 55 genes were detected overexpressed and 47 were suppressed in rats fed n-3 polyunsaturated fatty acids (Table 5). It can be speculated that in essential fatty acid deficiency the balance between genes overexpressed and suppressed is upset. In this respect there was only a minor difference between LNA, which does not accumulate or is even absent in brain phospholipids (Table 2), and DHA, which is the major polyunsaturated fatty acid in brain structural lipids and accumulates after a long-term feeding period. Indeed, LNA was found equally active compared with dietary DHA in DHA accretion in rats (33, 34) and primates (35) and in affecting mental functions (33). In agreement with this, the magnitude of the response of most of the genes up-regulated was almost identical with 18:3n-3 and EPA+DHA (Table 4). Clarke and Jump (36) proposed that delta-6 desaturation of LNA is necessary to evoke the effect of gene expression in liver. This might not be true for brain. Table 2 shows that the desaturation end product of LNA, DHA, does not accumulate in the phosphatidylethanolamines of perilla oil-fed rats. However, because our fish oil was also abundant in EPA, it is not clear whether this fatty acid effectively contributed to this effect. It is interesting that EPA, which was absent in control brains, appeared in low amounts (0.1%) in phosphatidylethanolamines of fish oil-fed rat brains (Table 2). Because the difference between LNA and EPA or DHA are two or four carbonic atoms and two or three double bonds, respectively, we believe that the n-3 structure, and not the chain length, is important in controlling the mode of gene expression.

From the results of Table 3 it is evident that feeding with both n-3 polyunsaturated fatty acids brought about an increase in proportion of ethanolamine plasmalogens. In addition, both fatty acids brought also some alteration of 18:0/22:6 diacyl phosphatidylethanolamine subclasses (Table 4), indicating that (*i*) 18:3n-3 must have been converted to DHA either in liver (12, 13) or in astrocytes as suggested by Spector and Moore (37), and (*ii*) n-3 polyunsaturated fatty acids affect brain lipid metabolism. Although the level of 18:0/22:6 containing ethanolamine plasmalogen species was identical in all three food regimens, a roughly 20% increase in the level of this subclass in fish oil-fed rat brains indicates that a portion of DHA, in addition to diacyl subclass, was deposited in ethanolamine plasmalogens. Based on these observations, alterations in biophysical parameters of brain membranes can be expected. The question is whether these fatty acids affect the rat brain genome in free form or through their effect on composition and biophysical properties of neural membranes. Altered lipid composition of nuclear membranes may affect permeability properties, allowing them to pass through different compounds (messengers), which may affect

Table 3. Subclass composition (%) of ethanolamine phosphoglycerides in relation to dietary fatty acids

Subclass	CTR ($n = 5$)	PER ($n = 5$)	FO ($n = 5$)
Diacyl	43.7	41.7	39.3
Alkylacyl	13.7	9.4	8.6
Alkenylacyl	42.6	48.9	52.1

CTR: control; PER: perilla oil; FO: fish oil.

Table 4. DHA containing phosphatidylethanolamine molecular species in rat brain in relation to dietary fatty acids (% of total)

Diet	CTR ($n = 5$)	PER ($n = 5$)	FO ($n = 5$)
PE subclass: Diacyl			
18:1/22:6	1.93 ± 0.34	1.72 ± 0.49	2.10 ± 0.61
16:0/22:6	6.55 ± 2.18	6.12 ± 1.32	8.17 ± 1.73
18:0/22:6	30.12 ± 2.42	32.37 ± 1.09	34.15 ± 0.38
PE subclass: Alkylacyl			
$18:1_a/22:6$	3.63 ± 0.52	3.89 ± 1.00	5.67 ± 0.42
$16:0_a/22:6$	9.35 ± 2.18	7.39 ± 1.50	8.25 ± 0.93
$18:0_a/22:6$	12.60 ± 0.82	13.17 ± 0.85	10.56 ± 0.90
PE subclass: Alkenylacyl			
$18:1_a/22:6$	3.09 ± 0.16	3.01 ± 0.45	5.00 ± 0.75
$16:0_a/22:6$	8.92 ± 0.31	9.50 ± 1.00	10.97 ± 2.12
$18:0_a/22:6$	19.00 ± 2.42	19.22 ± 0.57	20.84 ± 1.21

a: alcohol; CTR: control; PER: perilla; FO: fish oil; PE, phosphatidylethanolamine.

Table 5. Effects of fish and perilla oil on rat brain gene expression and genes with altered gene expression

Fish oil Mean	SD	Fold-Δ	Perilla oil Mean	SD	Fold-Δ	Gene product	Acc. no.
Lipid metabolism							
5.65	1.45		4.13	0.79		Serine palmitoyl transferase	AF111168
5.35	0.85		5.23	1.29		Farnesyl pyrophosphate syn. (testis)	NM_031840
−3.34	0.11		−3.89	0.1		Dihydrolipoamide transacylase E2	AA410009
−1.74	nd		−2.09	0.02		Phospholipase D	D8862
Membrane proteins							
6.39	2.4		6.03	0.52		Sec24 prot. (Sec24A isoform)	AJ131244
1.52	0.25		1.92	0.05		B cell receptor ass. prot. 37	U75392
−1.9	0.02		−1.98	0.1		Syntaxin binding prot. Munc18-2	U20283
−2.94	0.13		−4.76	0.19		Transthyretin	NM_012681
−3.04	0.06		1.17	nd		Membrane prot. (human ch. 3p25)	L09260
Endocytosis, synaptic vesicle recycling, formation							
2.05	0.03		2.02	0.45		D-cadherin precursor	AF135156
4.5	0.35		5.23	0.23		Clathrin-ass. adaptor chain mu 1A	AF139405
3.15	0.89		3.12	0.36		Adaptor-rel. prot. AP-3 mu2 subunit	L07074
1.72	0.17		2.16	0.22		Profilin IIa (Pfn2)	AF228737
Synaptic proteins							
1.97	0.21		2.03	0.23		Synuclein, alpha	NM_019169
2.37	0.27		2.34	0.21		Synuclein, gamma	NM_031688
Cytoskeleton							
2.58	nd		2.97	nd		Actin-rel. prot. 2	XM_002674
1.78	0.11		1.89	0.47		Alpha-tubulin (Tuba1)	NM_022298
1.25	0.38		2.02	0.32		Microtubule-ass. prot. 1A/1B chain 3	NM_022867
−1.5	0.12		−2.09	0.27		Axoneural dynein heavy chain 8	U61747
−1.62	0.09		−1.65	0.15		Microtubule-ass. prot. 4	AW142042
−2.57	0.11		−4.95	0.03		Unconventional myosin 3 (Myr5)	NM_012981
−2.13	0.04		−2.86	0.03		Capping prot. (actin filament)	AW541453
Signal transduction							
2.66	1.12		2.86	0.89		RAB6B small GTPase	W17800
1.68	0.13		1.98	0.43		Calmodulin (RCM3)	NM_017326
1.93	0.24		2.3	0.27		Calmodulin 1 (Calm 1)	NM_031969
1.79	0.14		1.8	0.13		Calmodulin	AF178845
1.62	0.21		1.79	0.2		LIM-domain prot. LMP-1	AF095585
2.99	0.07		3.63	0.28		SH3-containing prot. SH3P4	AF009603
2.13	0.36		2.11	0.33		Pleiotrophin (Hbnf, HB/GAM)	NM_017066
−2.21	0.03		nd	nd		Bromodomain prot. CELTIX1	AF213969
−1.65	0.2		−1.3	0.22		Ajuba (Jub) (*M. musculus*)	AW536166
−2.53	0.11		−2.86	nd		L1cam locus (*M. musculus*)	AA409744
−1.43	0.15		−1.76	0.07		Ras oncogene neuroblastoma, Nras	W15662
Others							
1.91	0.51		2.04	0.34		Translin	NM_021762
6.57	2.17		7.7	2.14		Ubiquitin-prot. ligase Nedd4-2	AF277232
2.99	0.8		2.36	1.1		Parathyroid hormon reg. sequence	AA290355
4.42	2.1		6.28	1.58		Elongation factor 1-alpha	X63561
4.37	2.04		5.44	0.97		Beta-globin	X16417
4.14	1.27		5.6	nd		Ribosomal prot. L7a	X15013
1.91	0.17		1.64	nd		Dipeptidyl peptidase III	D89340
−1.59	nd		−1.96	0.7		RNA-binding prot. AB0169092	AA038452
−5.24	0.02		−2.62	0.24		U1 small nuclear ribonucleoprot. hom.	AW189878
−2.26	0.14		−2.16	0.08		Ubiquitin carrier prot. E2-C	AW536176
−2.19	0.17		−2.2	0.09		BAC 10818 cont. the Ercc-4 gene	AA407398
−1.67	0.17		−2.06	0.11		Ring finger prot. 14 (Rnf14)	NM_020012
−1.67	0.14		−1.95	0.16		RNA1 homolog (Fug1)	AW536168
−1.36	0.05		−1.63	0.14		Similarities to BAT2	AW545609
−1.61	0.2		−1.59	0.19		NIPSNAP2 prot.	AA925098
−1.57	0.21		−1.61	0.11		14-3-3 prot. gamma-subtype	AW542425

nd, not determined; EST, expressed sequence tag; BAC, bacterial artificial chromosome.

gene expression or suppression. Clarke and Jump (36) proposed that n-3 polyunsaturated fatty acids or some metabolites are transferred to the nucleus where they may modify certain nuclear proteins and thus regulate DNA/protein interactions.

n-3 polyunsaturated fatty acids also were shown to modify gene expression in liver (20) or adipose tissue (21), but their targets were different. In liver mitochondria both LNA and DHA increased the levels of some β-oxidation enzymes such as carnitine palmitoyl-

Table 5. Continued

Fish oil Mean SD Fold-Δ		Perilla oil Mean SD Fold-Δ		Gene product	Acc. no.
Energy metabolism					
3.53	0.37	3.22	1.28	ATP synthase subunit d (Atp5jd)	NM_019383
2.63	0.38	2.49	0.47	ATP synthase, H+ transport, F0	NM_013795
4.47	1.78	4.3	0.74	Cytochrome b	AF295545
5.24	2.11	4.12	0.31	Cytochrome c oxidase subunit I	M27315
6.13	0.58	5.43	1.59	Cytochrome c oxidase subunit II	AF378830
1.91	0.74	2.41	0.28	Cytochrome c oxidase subunit VIc	X06146
3.77	1.23	4.56	0.83	NADH dehydrog. hom./cyt. c. I	X13220
3.53	0.67	3.88	0.92	Liver cytochrome c oxidase VIII	L48209
4.9	1.75	5.55	0.02	NAD+-isocitrate dehydr., gamma	X74125
1.97	0.2	1.92	0.38	Ubiquinol-cytochrome c red. (6.4kDa)	XM_009253
Receptors, ion channels, neurotransmission					
1.92	0.12	2	0.25	Vasopressin V1b receptor	AF314527
2.22	0.45	1.97	0.32	Somatostatin	NM_012659
−1.99	0.12	−1.43	0.1	Integrin alpha 5	AW544851
−2.65	0.06	−4.03	0.05	Putative E1–E2 ATPase	RSU78977
Regulatory proteins kinases, phosphorylases					
1.92	0.06	2.01	0.38	Prot. phosphatase 2A	NM_022209
1.8	0.28	1.86	0.16	Prot. phosphatase 2, alpha isoform	NM_017039
4.75	nd	7.14	0.64	Transcription factor-like p. MRGX	AF100620
1.41	0.12	1.51	0.23	Tax1 binding prot. TXBP151	W35945
−1.51	0.21	−1.6	0.15	STK-1 (serine/threonine kinase)	AA410169
−2.06	0.1	−2.42	0.08	Nonreceptor tyrosine kinase 2	W07947
−1.46	0.09	−1.56	0.09	Transcriptional repressor deltaEF1	D76433S
−1.73	0.16	−2	0.03	Goliath prot. hom. Zn-finger prot.	AA288977
−2.26	0.02	−2.22	0.11	Kruppel-type zinc finger (C2H2)	AW540949
−3.1	0.15	−2.23	0.03	Methyl CpG binding prot. 2	NM_022673
Unknown					
3.26	0.88	4.35	0.26	Clone RP11-68L6	AC008062
2.56	0.66	1.95	0.05	MO25 prot. (LOC51719)	NM_016289
2.34	0.07	2.6	0.02	Similar to CGI-89 prot.	XM_029457
7.14	2.85	6.6	1.75	Cerebellum cDNA	AK005360
2.55	nd	3.98	0.92	RIKEN library, clone: 1300006O23	AK004920
4.34	1.86	5.3	0.66	Clone: 2010305H07	AK008527
1.5	0.05	2.35	0.19	RIKEN cDNA 6330579B17 gene	NM_026494
4.63	1.45	4.58	0.23	No hits found	
2.02	0.09	2.44	0.71	No hits found	
5.97	1.6	5.73	1.38	No hits found	
5.92	1.94	6.05	0.05	No hits found	
5.18	2.22	6.61	1.24	No hits found	
−1.82	0.21	−2.03	0.23	EST (A. Thaliana)	AA409629
−2.11	0.18	−2.29	0.24	Genomic 142000013386055	AA409609
−1.96	0.17	−1.86	0.22	EST	AA288548
−2.66	0.03	nd	nd	BAC RP5-1185J21	AW544929
−2.04	0.12	−2.59	0.04	BAC clone RP11-319F3	AC006039
−2.92	0.07	−2.3	0.14	Clone PLACE1004814	AW536179
−2.32	0.06	−2.07	0.13	CGI-121 prot.	AA409886
−2.25	0.19	−2.54	0.05	Clone RP11-497124	AW544503
−4.98	0.08	−4.63	0.02	Cosmid clone U39B3	AW545277
−1.74	0.08	−2.35	0.09	HSPC016 mRNA	AF077202
−1.61	0.09	−1.71	0.21	Clone 705O1	AP000845
−2.15	0.13	−3	0.1	Clone RP3-419C19	AW544861
−1.76	0.05	−1.64	0.05	EST clone RP4-622L5	AW544872
−2.73	0.08	1.14	nd	Clone rp21-580p20	AA409442
−2.16	0.2	−2.44	0.29	Clone ct7-326b16	AA409624
−2.08	0.36	−1.62	0.08	Clone 45N8	AW544672
−1.56	0.11	−1.74	0.14	Unknown EST	AA396314

nd, not determined; EST, expressed sequence tag; BAC, bacterial artificial chromosome.

transferase I, II, mitochondrial trifunctional enzyme subunit, and 2,4-dienoyl-CoA reductase (20). In retroperitoneal, but not s.c., adipose tissue DHA inhibited fatty acid synthase, hormone sensitive lipase, phosphoenolpyruvate carboxykinase, or leptin mRNA levels. In our case only a few genes connected to lipid metabolism were influenced; among them, phospholipase D, which participates in phospholipid metabolism, was suppressed. In rat brain the ATP-generating machinery, ATP synthase subunit d, ATP synthase H^+, cytochrome *c* oxidase subunits, or NAD^+ isocytrate dehydrogenase, gamma subunit, among others, were most heavily affected (Table 5). It is striking that in different tissues different genes respond to the same fatty acids although each cell is supplied

with identical genome. One possible explanation is that the regulatory sequence of genes reacting with the unknown complex of n-3 polyunsaturated fatty acids or with their putative DNA-binding proteins varies from tissue to tissue.

As seen in Table 5 the ATP-generating machinery of brain responded to n-3 fatty acids most intensively. Brain is known to exhibit a high metabolic rate, and it has been suggested that half of the ATP formed is used to maintain Na^+/K^+ ATPase activity, which controls ionic flow resulting from nerve transmission (8). The main function of the brain is to accept and process information received from the inner or outer environment. The present results show that several genes participating in signal transduction processes were overexpressed, almost to the same extent, by both LNA and EPA+DHA. It is worth mentioning that genes encoding synuclein α and γ also were overexpressed. Synuclein α plays a role in neural plasticity and is related to learning in the brains of songbirds (38). Its role in synaptogenesis has also been suggested (38), and in this context it is interesting to note that learning causes synaptogenesis in the cerebellar cortex of adult rats (39). It has been shown that synucleins are associated with synaptosomes (40). It must, however, be considered that a fragment of α-synucleins is a part of senile plaques of patients with Alzheimer's disease (AD) (41). However, it also must be considered in this connection that there is no evidence to show that n-3 fatty acids would induce this disease. In contrast, a given mixture of n-6 and n-3 fatty acids improve the life quality of AD patients (42). In fact, the prevalence of AD negatively correlates with fish consumption (43). Moreover, n-3 polyunsaturated fatty acids improve cognitive functions in very old people (44). In the current study we found that the same mixture of the above fatty acids results in an increase of 18:0/22:6 diacyl phosphatidylethanolamine levels in rat brains, but no data are available as yet to indicate whether brains of very old individuals give the same response.

Increased levels of proteins involved in signal transduction (calmodulins, etc.) may enhance communication between neurons during this process. Ca^{2+}/calmodulin signaling recently has been suggested to have a special role in the stimulant-induced plasticity of the central nervous system (45). On the other hand, the gene encoding transthyretin, which regulates neuroproliferation and differentiation (46) in some brain regions, was suppressed. This gene was up-regulated in mice treated with Ginkgo biloba extract (47). Gingko biloba is known to counteract some neurological disorders such as loss of short-term memory, lack of attention, or even Alzheimer's disease. This contradiction can be overcome if we consider that we investigated brain hemispheres of rats, whereas in the above study the subject was the hippocampus of mice. Evidently much more can be learned about the role of genes not discussed here and also about genes suppressed by LNA and EPA+DHA. For instance, to the best of our knowledge, no data are available on the possible role in mental functions of regulatory proteins, protein kinases, and phosphorylases stimulated or inhibited by n-3 polyunsaturated fatty acids.

Transcript of several genes differently expressed in the brain tissue of 18:3n-3-fed rats and 20:5n-3 + 22:6n-3-fed rats is involved in the regulation of the cytosceleton. Alteration in the cellular architecture along with alterations in molecular composition of membranes might influence a wide range of brain functions: stabilization of axons and dendrites, cell shape, polarity, neural plasticity, vesicle formation and transport. It is tempting to speculate that genes overexpressed by n-3 polyunsaturated fatty acids and alterations in molecular composition of neural membranes collectively contribute to the well-demonstrated effect of these fatty acids to improvement of mental performance.

Further investigations must decide the velocity of the above responses, keeping in mind that in our experiments the rats were fed for several months with n-3 fatty acids, but in some tissues the gene expression or suppression takes place in a matter of hours. It would be interesting also to learn which gene(s) respond first to n-3 fatty acid stimulation/inhibition as well as what is the lowest dietary dose of these fatty acids. In this experiment the rats, when adult, digested 65 mg/day 18:3n-3 and 62 mg/day 20:5n-3 + 22:6n-3.

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