Differential Cerebral Cortex Transcriptomes of Baboon Neonates Consuming Moderate and High Docosahexaenoic Acid Formulas

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INTRODUCTION

The vertebrate central nervous system (CNS) is rich in the long chain polyunsaturated fatty acids (LCPUFA) docosahexaenoic acid (DHA) and arachidonic acid (ARA), and this composition is highly conserved across species[1]. Within the CNS, DHA and ARA are found at highest concentration in gray matter[2], and DHA is particularly concentrated in retinal photoreceptor membranes where it has long been known to play a key role in visual excitation[3]. In humans, DHA and ARA accumulate perinatally[4] and many studies of DHA/ARA supplemented formula show improvements in visual acuity[5] and cognitive function[6].

Despite the high demand for LCPUFA during perinatal CNS development, the best current evidence indicates that ARA and DHA can be synthesized only very inefficiently from dietary precursors and must be obtained from the diet[7]. DHA and ARA are present in all human milks studied to date[8], however their concentration is variable. For DHA it is closely linked to the mother’s intake of preformed DHA, which is in turn reflective of the mother’s intake of fatty fish or fish/marine oil supplements[9,10,11,12]. Dietary factors associated with ARA are less well understood[13]. High levels of precursor fatty acids LA and ALA in formulas yield negligible or at most moderate increases in plasma ARA and DHA concentrations[14,15]. However, in randomized controlled studies where preterm and term infants are fed preformed DHA and ARA supplemented formula, improvements in LCPUFA status as well as cognitive development and visual functions are observed[16,17,18,19,20].

While the importance of LCPUFA in infant nutrition has been established, the underlying mechanisms are only beginning to be understood. Brain accretion of LCPUFA is most intense during the brain growth spurt in the third trimester of pregnancy and during early childhood[21,22,23,24]. Selective incorporation and functional properties of LCPUFA, especially DHA, in retinal and neural membranes suggests a specific role in the modulation of protein-lipid interactions, membrane bound receptor function, membrane permeability, cell signaling, regulation of gene transcription, membrane excitation[3,25] and many studies of DHA/ARA supplemented formula show improvements in visual acuity[5] and cognitive function[6].

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expression and neuronal growth [25,26,27,28,29,30]. Additionally, LCPUFA mediate metacrine regulation and changes in gene expression by interacting with nutrient sensitive transcription factors [18,31]. Accordingly, poor nutrition during prenatal life and early infancy may have a lasting influence on neural function, as well as adult risk for chronic diseases [32,33,34]. Studies suggest that infant diets low in LCPUFA can lead to health complications such as insulin resistance, obesity, or blood pressure changes later in life [35,36].

DHA and ARA were introduced in 2002 to infant formulas in the United States, but initial concentrations varied over more than a factor of two (range of DHA 8-19 mg/kcal; ARA 21-34 mg/kcal) [37] and there are no dose response studies in humans or non-human primates available as a guide to optimal levels. A previous study in our laboratory on 4-week-old baboon neonates with preformed DHA and ARA (0.33%,w/w DHA and 0.67% ARA) in formulas showed DHA concentrations in various regions of the brain similar to breastfed controls, with the important exception of the cerebral cortex; ARA concentrations were not much altered by inclusion of dietary preformed ARA [2]. These results inspired our present study on 12 week old baboon neonates with the higher level of 1.00% DHA, along with 0.67% ARA. We report elsewhere [38] that DHA in the precentral gyrus of cerebral cortex increased beyond that achieved for 0.33% DHA, while regions such as the basal ganglia that reached DHA concentrations similar to breastfed animals at 0.33% DHA did not show further increases with 1.00% DHA. These data demonstrate that formula DHA in the high normal range of breastmilk DHA supports enhanced cortex DHA, but do not reveal how this compositional change may influence metabolic function.

To gather mechanistic information on the role of DHA and ARA in the primate cerebral cortex, we investigated global gene expression for cerebral cortex of animals in this study, consuming two different levels of formula DHA both within the range found in human breastmilk [8]. We report here changes in expression of thousands of genes in 12-week-old baboons in response to two different levels of LCPUFA: 0.33% DHA and 0.67% ARA; 1.00% DHA and 0.67% ARA. We have reported in detail on consequences for tissue fatty acid composition [38] and other factors elsewhere (Hsieh et al., 2007, submitted).

RESULTS AND DISCUSSION
Significance analysis (P<0.05) identified changes in expression levels of 1108 probe sets (ps) for comparisons of L3/C and/or L/C, representing 2.05% of the total >54,000 ps on the oligoarray. Most ps showed <2-fold change. For the L/C comparisons, 554 ps were upregulated, and 574 ps were downregulated, while for the L3/C comparisons, 666 ps were upregulated and 442 ps were downregulated, showing that more genes were upregulated in the cerebral cortex in response to increasing formula ARA and DHA. Functional characterization by gene ontology of these differentially regulated genes assigns them to diverse biological processes including lipid and other metabolism, ion channel and transport, development, visual perception, G-protein and signal transduction, regulation of transcription, cell cycle, cell proliferation, apoptosis etc. Known functions were assigned to 702 probe sets with 2-fold change. For the L/C comparisons, 666 ps were upregulated and 442 ps were downregulated, while for the L3/C comparisons, 534 ps were upregulated, and 574 ps were downregulated. Because the L and L3 groups have the same amount of ARA but different amounts of DHA, our treatments do not strictly represent a DHA dose response. The L/C comparison corresponds to inclusion of DHA and ARA at current levels near the worldwide breastmilk means, while the L3 group corresponds to DHA near the worldwide high [8].

Nine genes were tested by quantitative real time PCR to confirm the array results, as shown in Table S4. All were qualitatively consistent with the gene array results.

We highlight results in several categories of gene ontology as follows.

Lipid (fatty acid and cholesterol) Metabolism
Table 1 presents results from genes related to lipid metabolism that are regulated by dietary LCPUFA.

| Metabolism       | Gene Symbol | Unigene ID | L  | L3 |
|------------------|-------------|------------|----|----|
| Lipid            | ATP8B1      | Hs.569910  | 1.28| 1.36|
|                  | PDE3A       | Hs.386791  | 1.08| 1.30|
|                  | ELOVL5      | Hs.520189  | 1.02| 1.11|
|                  | ACSL3       | Hs.471461  | 1.13| 1.08|
|                  | HNF4A       | Hs.116462  | 1.06| 1.16|
|                  | CLPS        | Hs.1340    | 1.02| 1.16|
|                  | ALDH5B2     | Hs.87539   | 1.05| 1.16|
|                  | PLCE1       | Hs.20022   | 1.10| 1.19|
| Fatty acid oxidation | ACAD5B     | Hs.81934   | 1.10| 1.38|
|                  | ACAD10      | Hs.331141  | 1.08| 1.10|
|                  | GLYAT       | Hs.274336  | 1.01| 1.30|
|                  | ADH5        | Hs.78989   | 1.03| 1.22|
|                  | CPT2        | Hs.145384  | 1.10| 1.22|
| Energy           | LEP         | Hs.194236  | 1.01| 1.17|
| Ceramide         | NSMAF       | Hs.372000  | 1.04| 1.31|
| Steroid          | LASS5       | Hs.270525  | 1.06| 1.11|
| Glycosphingolipid| SPTLC2      | Hs.435661  | 1.27| 1.40|
| Steroid          | OSBP2       | Hs.517546  | 1.17| 1.35|
| Phospholipid     | UGT2B15     | Hs.150207  | 1.04| 1.21|
| Prostaglandin and Leukotriene | PLA2G6     | Hs.170479  | 1.09| 1.20|
|                  | TEBP        | Hs.50425   | 1.02| 1.52|
|                  | ANXA3       | Hs.480042  | 1.26| 1.04|
|                  | LTC45       | Hs.456     | 1.33| 1.24|
| Cholesterol      | DHCR24      | Hs.498727  | 1.18| 1.17|
|                  | PRKAG2      | Hs.131133  | 1.07| 1.09|
|                  | PRKAA1      | Hs.43322   | 1.09| 1.02|
|                  | SOAT1       | Hs.496383  | 1.09| 1.12|
|                  | FDFT1       | Hs.546253  | 1.01| 1.13|

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ative disorders involving iron accumulation [39], as well as the underlying factor in infantile neuroaxonal dystrophy, a neurodegenerative disorder caused by accumulation of iron in the globus pallidus and resulting in death by age 10[40]. In a previous study of four week old breastfed baboons, the globus pallidus was found to have 15.8±0.5% DHA (w/w of total fatty acids) and was the richest in DHA of 26 CNS regions examined[2]. The globus pallidus is also rich in ARA, with 10.3% (w/w) in four week old baboons. PLA2 are a superfamily of enzymes that liberate fatty acids from the sn-2 position of phospholipids; in the globus pallidus DHA and ARA are the most abundant acyl groups at this site.

Remarkably, among the elongation and desaturation enzymes associated with LCPUFA synthesis, only a single elongation enzyme was differentially expressed. The human ELOVL3 transcript was downregulated slightly in the L/C group and upregulated in the L3/C group. This enzyme, also called HELO1, catalyzes the two carbon elongation of polyunsaturated 18 and 20 carbon fatty acids [41,42].

We also found that DGKE was upregulated in the L3/C comparison. Genes involved in ceramide metabolism (NAMAF, LASS5), glycosphingolipid metabolism (SPTLC2) and steroid metabolism (OSBP2, UGT2B15) showed increased expression in L3/C group, whereas NAMAF and OSBP2 were downregulated in L3/C group.

The best studied role of ARA is as a precursor for eicosanoids including prostaglandins, leukotrienes, and thromboxanes. One of the genes derived from membrane-bound ARA, which catalyze the first step in the biosynthesis of cysteinyll leukotrienes, Leukotriene C4 synthase (LTC4S), is downregulated in both DHA-ARA groups. LTC4S is a potent proinflammatory and anaphylactic mediator [43]. An elevated level of mRNA for PGES3 (prostaglandin E synthase 3) was observed in both the groups. PGES3 is also known as TEBP (telomerase-binding protein p23) or inactive progesterone receptor, 23-KD (p23). p23, a ubiquitous highly conserved protein which functions as a co- chaperone for the heat shock protein, HSP90, participates in the folding of a number of cell regulatory proteins [44,45]. p23 has been demonstrated to bind to human telomerase reverse transcriptase (hTERT) and contribute to telomerase activity [46]. Decreased levels of Annexin A5 (AXL5) also known as Lipocorin III was observed with increasing DHA.

Genes involved in fatty acid oxidation (ACADS, ACAD10 and GLYAT) were upregulated, and carnitine palmitoyltransferase II (CPT2) downregulated, in the L3/C group. ACADs (acyl-CoA dehydrogenases) are a family of mitochondrial matrix flavoproteins that catalyze the dehydrogenation of acyl-CoA derivatives and are involved in the β-oxidation and branched chain amino-acid metabolism [47,48]. Both the ACADs family members ACADSB and ACAD10 were upregulated in L3/C group, consistent with greater energy production in the high DHA group. Mitochondrial-specific GLYAT (glycine-N-acetyltransferase) also known as acyl CoA:glycine N-acetyltransferase (ACGAT), conjugates glycine with acyl-CoA and participates in detoxification of various drugs and xenobiotics [49,50]. Mawal et al [50] suggested that delayed development of GLYAT might impair detoxification process in children.

Genes involved in cholesterol biosynthesis, DHCR24, PRKAG2, PRKAA1, SOAT1, and FDF7T1 showed significant associations with LCPUFA levels. Increasing DHA upregulated DHCR24 and PRKAG2, downregulated PRKAA1, SOAT1 and FDF7T1. DHCR24 (24-dehydrocholesterol reductase) also known as selective AD indicator 1 (SELAD1) catalyzes the reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis [51]. SELAD1 may activate estrogen receptor in the brain and protect from beta-amyloid-mediated toxicity [32]. Decreased expression of SELAD1 is observed in brain regions of patients with Alzheimer’s disease [53]. PRKAG2 (protein kinase, AMP-activated, gamma 2) is a member of AMP-activated protein kinase (AMPK) family. AMPKs perform multifunctional roles in calcium signaling, weight loss, regulation of energy metabolism in heart [34,35,36].

SOAT1 (sterol O-acyl transferase) or Acyl-coenzyme A: cholesterol acyl transferase (ACAT) is an intracellular protein which catalyzes the formation of choles terol esters in endoplasmic reticulum and is involved in lipid droplets that are characteristic of foam cells of atherosclerotic plaques [57,58,59].

Increased expression was detected for ATP8B1, PDE3A in both groups, comparatively more in L3/C, while transcripts involving HNFP4 (Hepatic nuclear factor-4z), CLPS and ALDH8B2 showed decreased expression with increasing DHA. Intrahepatic cholestasis, or impairment of bile flow, is an important manifestation of inherited and acquired liver disease resulting in hepatic accumulation of the toxic bile acids and progressive liver damage. Bile acids enhance efficient digestion and absorption of dietary fats and fat-soluble vitamins, and are the main route for excretion of sterols. Expression of ATP8B1 is high in the small intestine, and mutations in ATP8B1 gene have been linked to intrahepatic cholestasis [60,61]. ATP8B1 expression was confirmed by real time PCR (Table S1). PDE3A (phosphodiesterase 3A, cGMP-inhibited) is a 120 kDa protein found in myocardium and platelets [62]. Ding et al[63] showed significantly decreased expression of PDE3A in the left ventricles of failing human hearts. PDE3A expression is required for the regulation of penile erection in humans [64].

Leptin (LEP), which has a role in energy metabolism, was upregulated in L3/C group. Leptin is a secreted adipocyte hormone that plays a pivotal role in the regulation of food intake and energy homeostasis [65,66]. Leptin suppresses feeding and decreases adiposity in part by inhibiting hypothalamic Neuropeptide Y synthesis and secretion [67,68].

Ion Channel and Transport

Expression levels of transcripts involved in ion channel and transporter activity were altered by dietary LCPUFA (Table S5). Uncoupling protein 2, LOC313173 (hypothetical protein) and ATP11C, which have ion channel activity, are upregulated in both the groups but moreso in L3/C. Other transcripts with ion channel activity, including VDAC3, FTH1, KCN3, KCNH7 and TRPM1 were upregulated in L3/C group and downregulated in L/C. GLRA2, TRPV2 and HFE are upregulated in L/C and repressed in L3/C. P2RX2, GRIA1 and CACNA1A are repressed in both the groups.

One of our significant observations is the increased expression of uncoupling protein 2 (UCP2), a mitochondrial, proton carrier. Our data shows, for the first time, increased expression of UCP2 in neonatal cerebral cortex associated with dietary LCPUFA; increased expression is observed in both the groups but more in L3/C. QRT-PCR confirmed the array results (Table S4). Nutritional regulation and induction of mitochondrial uncoupling proteins resulting from dietary n3-PUFA in skeletal muscle and white adipose tissue have been observed [69,70]. Increased UCP2 expression is beneficial in diseases associated with neurodegeneration, cardiovascular and type 2 diabetes[71]. Dietary fats in milk increased the expression and function of UCP2 in neonatal brain and protected neurons from excitotoxicity [72].

VDAC3 (voltage-dependent anion channel 3) belongs to a group of pore forming proteins found in the outer mitochondrial membrane and in brain synaptic membranes [73,74]. Massa et al [75] observed a significant reduction of VDAC3 mRNA levels in the skeletal muscle and brains of dystrophin-deficient mdx mice during postnatal development. Mice lacking VDAC3 exhibit infertility [76]. All the transcripts (VDAC3, KCN3 and KCNH7)
having voltage-gated anion channel porin activity were upregulated with increasing DHA. FTH1 (Ferritin heavy chain 1) is required for iron homeostasis and it has been previously shown to be expressed in human brain [77].

Genes encoding small molecule transporters were differentially expressed, including carriers of glucose (SLC2A1, SLC5A4), chloride (SLC12A6), sodium (SLC13A5), monoamine (SLC18A2) and others (SLC26A4, SLC17A6). These transporters might help in exchange of nutrients and metabolites. Members of the cytosome P and B family of proteins were also differentially expressed. Transcripts encoding VDP, RSAF1, G1Q6 and OXAH1 were significantly repressed by increasing DHA.

G-Proteins and Signaling

Numerous genes encoding G-protein activity were differentially regulated (Table S3), and the majority were induced by high DHA. GNA15, GNA14, PTHR2, RCP9 and FZD3 showed increased expression in both DHA groups. EDG7, SHSTC2, GNHR, ADRA1A, BLR1, GPR101, GPR29 and OR8G2 were downregulated in L/C and upregulated in L3/C. NPY1R is downregulated in both the groups.

DHA regulates G-protein signaling in the brain and retina [78]. G-proteins are membrane-associated proteins which promote exchange of GDP for GTP in GDP and regulate signal transduction and membrane traffic [79]. GNA13 deficiency impairs angiogenesis in mice [80] while GNA14 activates the NF-κB signaling cascade [81]. Parathyroid hormone receptor 2 (PTHR2) is activated by parathyroid hormone and is relatively abundant in the CNS [82,83]. RCP9, also known as calcitonin gene-related peptide receptor component protein, may have a role during hematopoiesis [84]. Tibis and Goffinet [85] showed expression of FZD3 during postnatal CNS development in mice. FZD3 array results were confirmed by SYBR green real time PCR assay (Table S4).

Neuropeptide Y is a 36-amino acid peptide with strong orexigenic effects in vivo [86]. Two major subtypes of NPY (Y1 and Y2) have been defined by pharmacologic criteria. NPY1R was suggested to be unique for the control of feeding [87]. Pedrazzini et al [88] observed a moderate but significant decrease in food intake in mice lacking the NPY1R gene.

EDG7 (endothelial differentiation, lysophosphatidic acid G-protein-coupled receptor, 7) mediates calcium mobilization [89]. Mutation in the SHSTC2 gene causes childhood-onset of a neurodegenerative disorder affecting motor and sensory neurons [90].

Several signaling proteins (NFI, WSBI, SOCS4, RT1, CD6B1, OR2AP9 and RERG) were upregulated in both groups. Genes that are upregulated in L3/C and downregulated in L/C were also observed, specifically PDE4D, KRA5, IIGA2, PLCXD3, WNT8A, ARHGAP4, RAPGEF6, OR2F1/OR2F2, CCMI and SFRP2, while a few genes (WNT10A, ADCG2, OGT, DDAH1 and BCL9) were upregulated in L/C and downregulated in L3/C. IQGAP3, GGR, APLN, CNTF1, GRP, LPHN3, CKN1, Y1A3 and MCF2 were downregulated in both the groups (Table S5).

NFI is a tumor-suppressor gene; mutations in this gene cause neurocutaneous defects [91]. NFI gene expression and function are needed for normal fracture healing [92]. NFI expression levels were confirmed by QRT-PCR (Table S4). WSBI is a SOCS-box-containing WD-40 protein expressed during embryonic development in chicken [93]. RAS and RAS related gene families of small GTPases (RT1, KRA5, RERG and RAPGEF6) were upregulated by increasing DHA.

Diets deficient in n-3 PUFA induce substitution of n-6 DPA (22:5n-6) in neural membranes, and impairment of functions mediated by G protein mediated signaling, such as visual perception, learning and memory, and olfactory discrimination. Abundant evidence indicates that this results in reduced rhodopsin activation, and signaling in rod outer segments compared to DHA-replete animals [78,94,95,96,97].

Development

Table 2 shows differential expression of 24 genes related to development. The products of 11 transcripts play a role in nervous system development. The expression of TIMM8A, NRG1, SEM3D and NUMB genes were upregulated in both L/C and L3/C groups. HES1 and SIM1 were downregulated in both the groups. GDF11, SMA3/SMA5, SESGL3 were downregulated in L/C and upregulated in L3/C. The mRNA levels of growth factors FGFI and FGFI4 displayed increased abundance in L/C and decreased abundance in L3/C.

TIMM8A also known as Deafness/Dystonia Peptide 1 (DDP1) is a well conserved protein organized in mitochondrial intermembrane space. Loss-of-Function mutations in the TIMM8A gene cause Mohr-Tranebjaerg syndrome (a progressive neurodegenerative disorder with deafness, blindness, dystonia and mental deficiency) and Jensen syndrome (opticocochlear nerve atrophy with dementia) [98,99,100]. TaqMan assay confirmed the array results (Table S4). NRG1 is essential for the development and function of the CNS facilitating the neuronal migration and axon guidance [101,102]. NUMB negatively regulates notch signaling and plays a role in retinal neurogenesis, influencing the proliferation and differentiation of retinal progenitors and maturation of postmitotic neurons [103].

Table 2. Development gene fold-changes in expression profiles.

| Development | Gene Symbol | Unigene ID | L | L3 |
|-------------|-------------|------------|---|----|
| Nervous system | TIMM8A | Hs.447877 | 1.04 | 1.57 |
|  | NRG1 | Hs.453951 | 1.02 | 1.21 |
|  | SEM3D | Hs.201340 | 1.10 | 1.14 |
|  | NUMB | Hs.585653 | 1.01 | 1.10 |
|  | HES1 | Hs.250666 | -1.30 | -1.63 |
|  | SIM1 | Hs.520293 | -1.16 | -1.16 |
|  | GDF11 | Hs.591023 | -1.18 | 1.09 |
|  | SMA3/SMA5 | Hs.482414/484969/588240 | -1.08 | 1.06 |
| Muscle | SH3GL3 | Hs.270055/458285 | 1.16 | 1.04 |
|  | FGFI | Hs.37055 | 1.08 | -1.20 |
|  | FGFI4 | Hs.591206 | 1.01 | -1.10 |
| Skeletal | C6orf97 | Hs.130239 | -1.03 | 1.34 |
|  | CALD1 | Hs.490203 | 1.09 | 1.14 |
| Heart | GAP4 | Hs.527698 | 1.03 | -1.22 |
|  | S100A7 | Hs.4243987 | 1.02 | 1.22 |
| Epidermis | S100A7 | Hs.112408 | -1.06 | 1.27 |
|  | FGFI7 | Hs.5911206 | 1.14 | 1.02 |
|  | SCEI | Hs.115166 | -1.01 | -1.13 |
| Ectoderm/ | SMURF1 | Hs.189329 | 1.15 | 1.32 |
| Mesoderm | TCF21 | Hs.78061 | -1.12 | -1.18 |
| Gametogenesis | OTEX | Hs.196956 | 1.09 | 1.24 |
|  | TCP11 | Hs.453371 | -1.02 | 1.08 |
|  | CDV1 | Hs.528382 | -1.01 | -1.10 |
|  | SPAG6 | Hs.527698 | -1.03 | -1.22 |
neurons [103]. HESI (Hairy/Enhancer of Split, Drosophila, Homolog of, 1) a basic helix-loop-helix protein is downregulated. Decreased expression of HESI is observed as neurogenesis proceeds and in case of persistent expression differentiation of neuronal cells are blocked in the CNS [104].

Visual Perception

Nine transcripts having a role in visual perception were differentially expressed (Table 3). Genes coding for LUM, EML2, TIMP3 and TTCT8 were upregulated in both the supplement groups. TIPG1 was upregulated in L3/C and downregulated in L/C. RGS16 and TULP2 were upregulated in L/C and downregulated in L3/C. RAX and IMPDH1 were downregulated in both the supplement groups.

Lumican (LUM), is an extracellular matrix glycoprotein and a member of the small-leucine-rich-proteoglycan (SLRP) family [105]. It is widely distributed in the corneal stroma and connective tissues [106]. Lumican helps in the establishment of corneal stromal matrix organization during neonatal development in mice. Those lacking lumican exhibit several corneal related defects [107]. It is important for corneal transparency in mice [108]. TaqMan assay showed 5-fold more upregulation of LUM more than the microarray data (Table S4). Mutations in LUM are membrane associated molecular motors which play essential role in cytoskeleton and cell adhesion (Table S5). EVER1, PER1, Cep192, SSEA2, LPA12, TMEM29, TM6SF1 were upregulated in both the groups. ORMDL3, SEZ6L, HIDIN, TA-LRRP, PEDLI1 were upregulated in L3/C and downregulated in L/C. MFAP5L was upregulated in L/C and downregulated in L3/C. Transcripts of GP2 and SFNGR2 were downregulated in both the groups.

Numbers of transcripts were upregulated by increased DHA in the formulas. LCPUFA can affect biological membrane functions by influencing membrane composition and permeability, interaction with membrane proteins, membrane-bound receptor function, photoreceptor signal transduction and transport [111, 112, 113]. Mutations in EVER1 or transmembrane channel-like 6 (TM6C) gene cause epidermolysis bullosa verruciformis, a type of skin disorder [117]. HIDIN is a novel gene and markedly-down regulated by mutations causes congenital hydrocephalus in mice [118]. The exact function of GP2 is unknown, but it has been associated with the secretory granules in the pancreas [119].

Programmed Cell Death/Apoptosis

Transcripts with apoptotic activity were differentially expressed (Table S5). Seven out of nine transcripts in our study were upregulated with increasing DHA, including CARD6, TIA1, BNP1, TAF1, GULP1, CASP9 and FJ13491. Programmed cell death (PCD) plays an important role during the development of immune and nervous systems [120]. Jacobson et al [121] proposed PCD as an important event in eliminating unwanted cells during development. Mice with targeted deletion of CASP3 die perinatally due to vast excesses of cells deposition in their CNS as a result of decreased apoptotic activity [120]. CARD6 (caspase recruitment domain protein 6) is upregulated in both the groups. It is a microtubule-interacting protein that activates NF-kB and takes part in the signaling events leading to apoptosis [122]. TIA1 is upregulated in L3/C and downregulated in L/C. TIA1 is a member of RNA-binding protein family with pro-apoptotic activity, and it silences the translation of cyclooxygenase-2 (COX2). Narayanan et al, [123] suggested that DHA indirectly increases the expression of genes which downregulate COX2 expression. The COX2 enzyme catalyzes the rate-limiting step for prostaglandin production, which influence many processes including inflammation [124]. Downregulation of TIA1 in L/C could be due to the influence of ARA, the major COX2 substrate, rather than that of DHA which is a competitive inhibitor. GULP1 assists in removal of the apoptotic cells by phagocytosis [123]. CASP9 activates caspase activation cascade and is an important component of mitochondrial apoptotic pathway [126].

Cytoskeleton and Cell adhesion

Dietary LCPUFA regulated expression of several transcripts involved in cytoskeleton and cell adhesion (Table S5). The expression of 27 ps involved in cytoskeleton was altered. MYO1A and MYO5A were upregulated with increasing amounts of DHA whereas MYO1E showed decreased expression. Myosin-1 isoforms are membrane associated molecular motors which play essential roles in membrane dynamics, cytoskeletal structure and signal transduction [127]. COLA6 and COLA3 showed increased expression whereas COLA2 and COLA2 showed decreased expression with increasing DHA. Type IV collagen is the major component of the basement membrane. Mild forms of Alport nephropathy is associated with deletion in COLA4 gene [128] and eye abnormalities are common in people afflicted with Alport syndrome [129]. WASL, also known as neural WASP (WASP), was upregulated in both the groups. Actin cytoskeleton regulation is vital for brain development and function. WASL is an actin-

| Gene Product | Unigene ID | L | L3 |
|--------------|------------|---|----|
| LUM (Lumican) | Hs.406475 | 1.03 | 1.30 |
| Interphotoreceptor matrix proteoglycan 1 (IMPG1) | Hs.590893 | 1.03 | 1.18 |
| Echinoderm microtubule associated protein like 2 (EML2) | Hs.24178 | 1.07 | 1.15 |
| TIMP metalloproteinase inhibitor 3 (TIMP3) | Hs.297324 | 1.28 | 1.05 |
| Tetraoctapeptide repeat domain 8 (TTCT8) | Hs.303055 | 1.10 | 1.01 |
| IMP (inosine monophosphatase dehydrogenase 1 (IMPDH1)) | Hs.534808 | 1.20 | 1.12 |
| Tubby like protein 2 (TULP2) | Hs.104636 | 1.07 | 1.15 |
| Retina and anterior neural fold homeobox (RAX) | Hs.278957 | 1.10 | 1.24 |
| Regulator of G-protein signalling 16 (RGS16) | Hs.413297 | 1.01 | 1.26 |

Table 3. Visual perception gene fold-changes in expression profiles.
regulating protein and mediates filopodium formation [130,131,132]. HIPP1 (huntingtin interacting protein 1) and HOOK2 (hook homolog 2) were downregulated in both the groups.

The expression levels of 15 transcripts involved in cell adhesion changed as a result of dietary LCPUFA (Table S5). BTBD9, CD44, ARM4, GOS1, LOC389722 and PCDHBI3 showed increased expression in both the groups. Glycoprotein CD44 is a cell-surface adhesion molecule that is involved in cell-cell and cell-matrix interactions [133] while PCDHB3 is a member of protocadherin beta family of transmembrane glycoproteins [134]. NLGN3 and CIR61 were downregulated in both groups.

Peptidases
Several transcripts having peptidase activity were differentially expressed (Table S5). SERPINB6 is significantly upregulated in L3/C and downregulated in L/C. Of note, the ADAM families of proteins ADAM17, ADAM33, and ADAMT516 were upregulated and ADAMT515 was downregulated in both the supplement groups. ADAM proteins are membrane-anchored glycoproteins named for two of the motifs they carry: an adhesive domain (disintegrin) and a degradative domain (metalloprotease) [135]. These proteins are involved in several biological processes including cell-cell interactions, heart development, neurogenesis and muscle development [136,137,138,139]. ADAM17 is required for proteolytic processing of other proteins and have been reported to participate in cleaving of the amyloid precursor protein [140,141]. Loss of ADAM17 is reported in abnormalities associated with heart, skin, lung and intestines [142,143,144]. Real time PCR confirmed array results of ADAM17 (Table S4). ADAM33 has been recently implicated as an asthma and bronchial hyperresponsive-ness gene [145]. It is required for smooth muscle development in the lungs helps in airway wall “modeling”, and proper functioning of lungs throughout life [146,147].

CTSB (Cathepsin B) also known as amyloid precursor protein secretase (APP5) was upregulated. It is involved in the proteolytic processing of amyloid precursor protein [148]. Felbor et al [149] reported deficiency of CTSB results in brain atrophy and loss of nerve cells in mice. CTSB (Cathepsin C) was downregulated in the L/C group and upregulated in the L3/C group. Loss of function mutations in CTSB gene are associated with tooth and skin abnormalities [150].

NAALAD2 was upregulated while PAPLN, RNF130, TPMRSS2, PGC, CPI2, FUREN were downregulated. CPI2 interacts with WNT proteins and may regulate embryonic development, however; its expression in adult tissues is less abundant [151]. TPS2 and SPP2 were showed increased expression in L/C and decreased expression in L3/C. PAPP1, GZMA, SERPINA1, IQCT1 transcripts were downregulated in L/C and upregulated in L3/C. Several hypothetical transcripts (FLJ10504, FLJ30679, FLJ90651, FLJ25179, DKFz606L1818) were differentially expressed.

Cell Cycle, Cell Growth and Cell Proliferation
Fifteen transcripts having a role in cell cycle regulation, growth and proliferation were differentially expressed (Table S3). Four of the transcripts SEXN3, RAD1, GAS1 and PARD6B involved in cell cycle regulation were upregulated in both the groups. Cell growth factors, INHBC and GOG were induced in both the groups. FGRFRIOP is a positive regulator of cell proliferation and showed increased expression. KIAALDI, CDC20 and CSDK2C were downregulated.

Growth arrest specific gene 1 (GAS1) expression is positively required for postnatal cerebellum development. Mice lacking GAS1 had significantly reduced cerebellar size compared to wild type mice [152]. Liu et al [152] proposed that GAS1 perform dual roles in cell cycle arrest and in proliferation in a cell autonomous manner. PARDB6 has a role in axonogenesis [153].

INHBC is a member of transforming growth factor-beta superfamily (TGF-beta) and is involved cell growth and differentiation [154,155]. Osteoglycin (OGN) is also known as Minecan and Osteoinductive factor (OIF). Minecan is a member of small-leucine rich proteoglycan gene family and is a major component of cornea and other connective tissues [156,157]. It has a role in bone formation , cornea development and regulation of collagen fibrillogenesis in corneal stroma [157,158,159]. CDC20 regulates anaphase-promoting complex [160].

Response to Stress
MSRA, SOD2, GSTA3 and GSR genes were differentially expressed (Table S5). MSRA was upregulated in both the supplement groups. SOD2 is downregulated in L/C and upregulated in L3/C. GSR is upregulated in the L/C and downregulated in the L3/C. GSTA3 is downregulated in both the groups.

Oxidative damage to proteins by reactive oxygen species is associated with oxidative stress, aging, and age-related diseases [161,162,163]. MSRA is expressed in the retina, neurons and the nervous system [162]. Knock-outs of the MSRA gene in mice result in shortened life spans both under normoxia and hyperoxia conditions [164]. MSRA also participates in the regulation of proteins [165]. MSRA plays an important role in neurodegenerative diseases like Alzheimer’s and Parkinson’s by reducing the effects of reactive oxygen species [163]. Overexpression of MSRA protects human fibroblasts against H2O2-mediated oxidative stress [166]. SOD2 belongs to the iron/manganese superoxide dismutase family. It encodes a mitochondrial protein and helps in the elimination of reactive oxygen species generated within mitochondria [167]. In our study increased amount of DHA reduced the expression of glutathione-related proteins GSR and GSTA3.

Kinases and Phosphatases
Phosphorylation and dephosphorylation of proteins control a multitude of cellular processes. Several proteins having kinase activity were altered (Table S5). Of note, transcripts involving STK3, STK6, HINT3, TK1, DRF1, GUCY2C and NEK1 were significantly upregulated with increasing DHA. A number of MAP kinases were downregulated in L3/C group, including MAPK4, MAPK12, MAPK52 and MAPK63. Other transcripts which showed significantly decreased expression were CKM, LMTK2, NEK11, TNK1, BRD4 and MEG3796.

Transcripts having dephosphorylation activity, including ACPL2, KIAA1240, PPP2R3A, PPP1R12B, PTPRG, PPP3CA and ACP were upregulated in L3/C group (Table S5). MTMR2, PPP1R7, PTPR2 and HDHD3 were significantly downregulated with increasing DHA.

Transcription Factors
Several transcription factors are differentially expressed by dietary LCPUFA (Table S5). Zinc finger proteins, Homeo box proteins and RNA Pol II transcription factors were among them. Several of the Zinc finger proteins were upregulated in L3/C, which include ZNF611, ZNF384, ZNF481, ZNF273, ZNF457, MLLX, BTB11, PRDM7, JAZ1, ZNF582, MLLT10, ZNF567, ZNF44, ZNF296, ZF3, NAB1, ZNF198, ZNF457 and ZNF207, while PCH2, ZBTB9, ZNF297, WHSCIL1, SALLA, ZNF585, ZFP, ZNF414, ZNF419 and ZNF479 were repressed in L3/C group. Zinc finger proteins exhibit varied biological functions in eukaryotes including activation of transcription, protein folding, regulation of apoptosis, lipid
Ingenuity Network Analysis

We explored relationships among sets of genes using Ingenuity Systems network analysis. Out of 1108 differentially expressed probe sets in our data, 387 probe sets (34.93%) were found in the Ingenuity Pathway Analysis (IPA) knowledge database, and are labeled “focus” genes. Based on these focus genes, IPA generated 41 biological networks (Table S6). Among these 41 networks, 24 had scores of >8 and the top 2 networks with 35 genes had scores of 49. We focus here on the most significant network.

The top network identified by IPA is associated with nervous system development and function, cellular growth and proliferation (Figure 1). Epidermal growth factor receptor (EGFR) is the most outstanding interaction partner found within the network. EGFR interacts with TIMP3, NRG1, ADAM17, EDG7 and FGFR; all are upregulated, and involved in neural or visual perception development. EGFR signaling is implicated in early events of epidermal, neural and eye development. Loss of EGFR signaling results in reduced brain size and loss of larval eye and optic lobe in drosophila [171]. EGFR expression is required for postnatal forebrain and astrocytes development in mice [172]. Functional pathway analysis conducted on this network using the IPA tool set identified three genes, ADAM17, NUMB and HES1, involved in the Notch signaling pathway which regulates nervous system and eye development [173,174]. ADAM17 and NUMB were upregulated while HES1 was repressed in both the groups. This analysis suggests that LCPUFA influence many processes with influences that converge on EGFR.

LCPUFA are known to directly interact with nutrient sensitive transcription factors such as peroxisome proliferator-activated receptors (PPARs), liver receptors, hepatic nuclear factor-4, sterol regulatory binding proteins, retinoid receptors and NF-KB. Upon ingestion, LCPUFA can elicit a transcriptional response within minutes[31,175,176,177]. Microarray studies on LCPUFA-supplementing animals have identified several tissue-specific pathways regulated by LCPUFA, particularly involving the liver, adipose, and brain tissue transcriptome [26,178,179]. Using murine 11K Affymetrix oligoarrays, Berger et al [178] [180] showed increased hepatic expression of lipolytic and decreased expression of lipogenic genes. However, in the hippocampus brain region, increased expression of HTR4 and decreased expression of TTR and SIATBE, genes involved in the regulation of cognition and learning, as well as POMC, a gene associated with appetite control, was identified. The first paper published on the brain gene transcriptome with respect to LCPUFA supplementation by Kitajka et al. in 2002 [181] demonstrated that feeding fish oil (DHA 26.9%) to rats increased expression of genes involved in lipid metabolism (SPPL2C, FPS), energy metabolism (ATP synthase subunit d, ATP synthase H, cytochromes, IDH3G), cytoskeleton (Actin related protein 2, TUBA1), signal transduction (Calmodulins, SH3P4, RAB6B small GTPase), receptors, ion channels and neurotransmission (Vasopressin V1b receptor, Somatostatin), synaptic plasticity (Synucleins) and regulatory proteins (proteins phosphatases). In the same study, fish oil supplementation also significantly reduced the expression of phospholipase D and Transhyretin. In related work, Kitajka et al [26], using rat cDNA microarrays with 3,200 spots, found results similar to those previously reported. Barcelo-Coblijn et al. [182] were the first to report moderation of age-induced changes in gene expression in rat brain as a result of diets rich in fish oil (DHA 11.2%). In this study, 2 month old rats showed increased expression of SNCA and TTR, however, 2-year old rats exhibited no significant changes. In addition, Puskas et al. [183] demonstrated that administration of omega-3 fatty acids from fish oil (5% EPA and 27% DHA; total fat content: 8%) for 4 weeks in 2 year

Binding etc [168]. Homeobox transcription factors, TGF2, PHTF1, OTP and HHEX were induced whereas PHOX2A, IRX1 and MITF were repressed in L3/C. RNA Pol II transcription factors (BRCAl, TFCP2, CHD2, THRAP3, SMCAD2 and NFE2L2) showed increased expression in L3/C. However, transcripts for UTF1, POU2F2, ELL, POLR2C, THRAP5, FGFR and GLSI1 showed decreased expression in L3/C. SOX7 and SOX12, high mobility group (HMG) box proteins, were also differentially expressed. NF1 array expression results were confirmed by real time PCR (Table S4).

Receptor Activity

Transcripts performing receptor activities were differentially expressed (Table S5). While increasing levels of DHA were associated with decreased expression of CD40, ITGB7, IL20RA, CD14, DOK3, MIR1, B2R3P4, RARA, CD3D, IL1R1, MCP, HOMER3 transcripts, increased expression was detected for FCGR2B, IL3RA, MRC2, SCUBE3, CR2, NCR2, CFL2, SLAMF1, EGF and KIR3DL2. Interestingly, retinoic acid receptor ÿ (RARß) activity was decreased in both the groups, EGF expression levels were confirmed by QRT-PCR (Table S4).

Ubiquitin Cycle

Twenty-five probe sets having a role in the ubiquitination process were differentially expressed (Table S5). Interestingly, five members of F-box protein family (FBXL7, FBXL4, FBXL17, FBXW4 and FBXW9) showed increased expression in L3/C group. F-Box proteins participate in varied cellular processes such as signal transduction, development, regulation of transcription and transition of cell cycle. They contain protein-protein interaction domains and participate in phosphorylation-dependent ubiquitination [169,170]. Proteins associated with anaphase-promoting complex (CDC23 and ANAPC1) were downregulated in L3/C group.

Others

Transcripts involved in 1) calcium ion binding (MGC33630, UMODL1, FLJ23818, S100Z, MGC2458, ITSN2 and PRG3), 2) zinc ion binding (FGD5, ZFIVE29, PDLIM4, ZCCHC6, ZNF518 and INSM2), 3) ATP binding (AML141 and C6orf102), 4) GTP binding (DOCK3, DOCK6, DOCK10, MKN1 and GTP), 5) nucleic acid binding (IFH1, C10orf10, DDX30, TNRGC6, RSN, ZCCHC5, DJ467N11.1, MGC24399 and LOC124245), 6) DNA binding (KIAA1305, HIP1-BP74, HDAP1, C17orf31, HST1H2BD and HST1H1E), 7) protein binding (ABTB1, MGC50721, RANBP5, STXB4, BTB5 and KLHL4), and 8) protein folding (HSPB5, DAX1B2, FKBP11 and TBCC) were all differentially expressed. Also, several transcripts which play a role in RNA processing events were differentially expressed. SFRS2IP, LOC81691, EXOSC2, SFPQ, SNRPV and SFRS5 showed increased expression, whereas, NOL5A, RBM19, NCBP2 and PHEF4 showed decreased expression with increasing DHA. Transcripts related to immune response are also differentially expressed. HLA-DPB1, MX2 and IGHG1 were upregulated and PLUNC was downregulated with increasing DHA.

Finally, 406 transcripts with no known gene ontology functions were differentially expressed (Table S5). Several of these transcripts were among the most differentially expressed, among these, H3G, LOC283460, FLJ13611, PARP6, C6orf111, C10orf67, TTTY8, C11orf1 and PHAX were upregulated, whereas transcripts for CHRDL2, TSGA13, RP4-622L3, MGC5391, RF126P1, E1M1942 and NOB1P were repressed considerably.
old rats induced expression of transthyretin and mitochondrial creatine kinase and decreased expression of HSP86, ApoC-I and Makorin RING zinc-finger protein 2, genes in hippocampus brain region. Finally, Flachs et al [179] showed increased expression of genes for mitochondrial proteins in adipose tissue.

In comparison with previous brain transcriptome analyses, the present study employing the use of high-density Affymetrix oligoarrays (>54,000 ps) revealed genes differentially regulated by LCPUFA at ranges mimicking breastmilk. With the exception of SPTLC2, which we also found to be upregulated in the L/C and L3/C comparisons, none of the remaining, previously identified genes, were differentially expressed in our dataset. Many factors are likely to contribute to the observed differences in differentially expressed genes between our study and previous work. One likely source is the difference in dietary DHA/ARA, which is within the range of human and baboon breastmilk; previous studies used much higher amounts of DHA, from 11.2% to 27% [182,183]. Also, interactions between levels of ARA and DHA supplied in our

Figure 1. Ingenuity network analysis showing gene interactions generated from L3/C comparisons. Network is graphically represented as nodes (genes) and edges (the biological relationship between genes).

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study add some complexity to the interpretation since the three treatments do not represent a strict dose response to DHA. However, our DHA and ARA come from sources that are routinely consumed by human infants in commercial infant formulas, and thus are directly relevant to that group. Despite lower levels of DHA/ARA, genes in our data set show subtle changes in expression. Moreover, the magnitude of these results is not surprising given the nutritional focus of the study, in which subtle, widespread shifts in transcription may have profound biological effects. Our data indicate that LCPUFA supplementation within the ranges of breastmilk will induce global changes in gene expression across numerous biological processes.

Conclusions
The impact of DHA and ARA on infant baboons was both significant and widespread. We identified several novel differentially-expressed transcripts in 12-week old baboon cerebral cortices modulated by dietary LCPUFA. The majority of probe sets showed subtle changes in gene transcription. In the cerebral cortex, we observed increased expression of mitochondrial proton carrier, UCP2 (uncoupling protein 2) in both groups, but more in L3/C. PLA2G6, implicated in childhood neurodegeneration, was differentially expressed. TSH, a沉默的 of the COX2 gene transcription is upregulated in L3/C. Increased expression was observed for TIMM8A, NRG1, SEMA3D and NMEB, genes involved in neural development. LEM, EMZ2, TIMP3 and TGFβ genes with roles in visual perception were upregulated. Hepatic nuclear factor-4α (HNF4A) showed decreased expression with increasing DHA. RARA was repressed in both the groups. A network involving 35 genes attributed to neural development and function was identified using Ingenuity pathway analysis, emphasizing EGFR as the most outstanding interaction partner in the network. In this network EGFR interacts with genes involved in neural or visual perception, TIMP3, NRG1, ADAM17, EDG7 and FGF7. Although subtle, the upregulation of Numb and downregulation of HES1 in the Notch signaling pathway, not previously shown to interact with fatty acids, supports the involvement of LCPUFA, particularly DHA, in neural development. Interestingly, no known desaturases and only one elongase, LCPUFA biosynthetic enzymes, were differentially expressed in cerebral cortex. In a study of liver gene expression in preparation, fatty acid desaturases SCD and FADS1 were significantly downregulated in liver, where we identified a multifunctional protein TOB1 which is significantly upregulated.

These data represent the first comprehensive transcriptome analysis in primates and have identified widespread changes in cerebral cortex genes that are modulated by increases in DHA, induced by dietary means. Importantly, the range of DHA used here is within limits of human and primate breastmilk, the natural food for humans, and indicate that CNS gene expression responds to LCPUFA concentrations.

MATERIALS AND METHODS
Details of experimental design, animal characteristics, and tissue sampling are available elsewhere [30] and will be outlined briefly here.

Animals and Diets
The animal phase took place at the Southwest Foundation for Biomedical Research (SFBR), San Antonio, TX, and was approved for animal care and research protocols from SFBR and Cornell University Institutional Animal Care and Use Committee (IACUC). Twelve baboon neonates born spontaneously around 182 days gestation were randomized into 3 groups (n=4 per group). They were fed for 12 weeks on one of three formulas: C: Control (no DHA-ARA); L: 1×LCPUFA (0.33%DHA-0.67%ARA); L3: 3×LCPUFA (1.00%DHA-0.67%ARA). Formulas in color-coded cans were kindly provided by Mead-Johnson Nutritional (Evansville, IN) in ready-to-feed form, 2 colors per treatment, so that investigators were masked to the treatments.

Sampling and Array Hybridization
Twelve week old baboon neonates were anesthetized and euthanized at 84.4±1.1 days. Tissue collected from the precentral gyrus of the cerebral cortex was placed in RNA Later according to vendor instructions and was used for the microarray analysis and validation of microarray results.

Microarray studies utilizing baboon samples with human oligonucleotide arrays have been successfully carried out previously[194,185]. Cerebral cortex global messenger RNA in the three groups was analyzed using Affymetrix GeneChip™ HG-U133 Plus 2.0 arrays <http://www.affymetrix.com/products/arrays/ specific/hgu133plus2.affx>. The HG-U133 Plus 2.0 has >54,000 probe sets representing 47,000 transcripts and variants, including 30,000 well-characterized human genes. One hybridization was performed for each animal (12 chips total). RNA preparations and array hybridizations were processed at Genome Explorations, Memphis, TN <http://www.genome-explorations.com/>. The completed raw data sets were downloaded from the Genome Explorations secure ftp servers.

Microarray Data Analysis
Raw data (.CEL files) were uploaded into Iobion’s Gene Traffic Multi 3.2 (Iobion Informatics, La Jolla, CA, USA) and analyzed by using the robust multi-array analysis (RMA) method. In general, RMA performs three operations specific to Affymetrix GeneChip arrays: global background normalization, normalization across all of the selected hybridizations, and log2 transformation of perfect match oligonucleotide probe values [186]. Statistical analysis using the significance analysis tool set in Gene Traffic was utilized to perform Multiclass ANOVA on all probe level normalized data. Pairwise comparisons were made between C vs L, and C vs L3 and all probe set comparisons reaching P <0.05 were included in the analysis. Gene lists of differentially expressed probe sets were generated from this output for functional analysis.

Bioinformatics analysis
Expression data was annotated using NIH DAVID <http://apps1.niaid.nih.gov/david/> [187] and NetAffx <http://www. affymetrix.com/analysis/index.affx/>. Genes were grouped into functional categories and pathways based on the Gene Ontology Consortium <http://www.geneontology.org/>, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway Database <http://www.genome.jp/kegg/pathway.html> and <BioCarta <http://www.biocarta.com/>>. Data presented in this manuscript is accessible through GEO Series accession number GSE6519 (GEO, http://www.ncbi.nlm.nih.gov/geo/).

RNA Isolation and RT PCR
RT PCR was conducted on nine genes to confirm the results of the array analysis. Total RNA from 30 mg samples of baboon cerebral cortex brain tissue homogenates was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA). Each RNA preparation was treated with DNase I according to the manufacturer’s instructions.
The yield of total RNA was assessed by 260 nm UV absorption. The quality of RNA was analyzed by 260/280 nm ratios of the samples and by agarose gel electrophoresis to verify RNA integrity.

One microgram total RNA from each group (C, L, L3) was reverse-transcribed into first strand cDNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The iScript reverse transcriptase is a modified MMLV-derived reverse transcriptase and the iScript reaction mix contains both oligo(dT) and random primers. The generated first strand cDNA is stored at −20°C until used.

Quantitative real-time PCR using SYBR green and TaqMan assay methods was used to verify the differential expression of selected genes that were upregulated in L3/C comparison. All the primers were gene-specific and generated from human sequences (<www.ensembl.org>). PCR primers were designed with PrimerQuest software (IDT, Coralville, IA) and ordered from Integrated DNA Technologies (IDT, Coralville, IA). Initially primers were used.

Network Analysis

We used a web-delivered bioinformatics tool set, Ingenuity pathway analysis (IPA 3.0) (<http://www.ingenuity.com>), to identify functional networks influenced by our dietary treatments. IPA is a knowledge database generated from the peer-reviewed scientific publications that enables discovery, visualization and exploration of functional biological networks in gene expression data and delineates the functions most significant to those networks. The 1108 differentially expressed probe sets identified by microarray data, as discussed below, were used for network analyses. Affymetrix probe set ID’s were uploaded into IPA and queried against all other genes stored in the IPA knowledge database to generate a set of networks having up to 35 genes. Each Affymetrix probe set ID was mapped to its corresponding gene identifier in the IPA knowledge database. Probe sets representing genes having direct interactions with genes in the IPA knowledge database are called “focus” genes, which were then used as a starting point for generating functional networks. Each generated network is assigned a score according to the number of differentially regulated focus genes in our dataset. These scores are derived from negative logarithm of the P indicative of the likelihood that focus genes found together in a network due to random chance. Scores of 4 or higher have 99.9% confidence level of significance as defined in detail elsewhere [188].

SUPPORTING INFORMATION

**Table S1A** Genes with known function upregulated by L3 (1.00%DHA-0.67%ARA) in Brain

Found at: doi:10.1371/journal.pone.0000370.s001 (0.16 MB XLS)

**Table S1B** Genes with known function downregulated by L3 (1.00%DHA-0.67%ARA) in Brain

Found at: doi:10.1371/journal.pone.0000370.s002 (0.18 MB XLS)

**Table S1C** Genes without known function upregulated by L3 (1.00%DHA-0.67%ARA) in Brain

Found at: doi:10.1371/journal.pone.0000370.s003 (0.08 MB XLS)

**Table S1D** Genes without known function downregulated by L3 (1.00%DHA-0.67%ARA) in Brain

Found at: doi:10.1371/journal.pone.0000370.s004 (0.07 MB XLS)

**Table S2** Probe sets showing ≥1.4 fold changes in gene expression

Found at: doi:10.1371/journal.pone.0000370.s005 (0.02 MB XLS)

**Table S3** Primers and Probe Sequences

Found at: doi:10.1371/journal.pone.0000370.s006 (0.02 MB XLS)

**Table S4** Comparison of microarray versus QRT-PCR gene expression values (Fold-changes)

Found at: doi:10.1371/journal.pone.0000370.s007 (0.01 MB XLS)

**Table S5** Classification According to Gene Ontology Functions for Brain

Found at: doi:10.1371/journal.pone.0000370.s008 (0.28 MB XLS)

**Table S6** Ingenuity functional network analysis

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Author Contributions

Conceived and designed the experiments: JB KK JA AH. Performed the experiments: PN KK AH. Analyzed the data: JB BP KK. Wrote the paper: JB KK. Other: Contributed to experimental design: PN. Edited the manuscript: BP AH PN JA.
REFERENCES

1. Crawford MA, Casperd NM, Sinclair AJ (1976) The long chain metabolites of linoleic acid linolenic acids in liver and brain in herbivores and carnivores. Comp Biochem Physiol B 34: 395–401.
2. Diaz CV, HN, Verbruggen T, Sarkadi-Nagy EA, Wijerandran V, Nathanielsz PW, et al. (2005) The influence of long chain polyunsaturated supplementation on docosahexaenoic acid and arachidonic acid in baboon neonate central nervous system. BMC Med 3: 11.
3. Brensike RM, Anderson RE, Wheeler TG (1973) Membrane fatty acids associated with the electrical response in visual excitation. Science 182: 1253–1254.
4. Martínez M (1992) Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr 120: S129–S130.
5. Morale SE, Hoffman DR, CASTANeda YS, Wheaton DH, Burns RA, et al. (2005) Duration of long-chain polyunsaturated fatty acids availability in the diet and visual acuity. Early Hum Dev 81: 197–203.
6. Marszalek JR, Lodish HE (2005) Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: Breast milk and fish are good for you. Annual Review of Cell and Developmental Biology 21: 633–657.
7. Brunna JT (2002) Efficiency of conversion of alpha-linoleic acid to long chain n-3 fatty acids in man. Curr Opin Clin Nutr Metab Care 5: 127–132.
8. Brunna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, et al. (2006) Docosahexaenoic and arachidonic acid concentrations in human breastmilk worldwide. Pediatrics submitted.
9. Innis SM, Kuhnlein HV (1988) Long-Chain N-3 Fatty-Acids in Breast-Milk of Inuit Women Consuming Traditional Foods. Early Human Development 18: 105–119.
10. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE (1983) Breast milk composition: content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 41: 787–800.
11. Wang L, Shimizu Y, Kaneko S, Hanaka S, Abe T, et al. (2000) Comparison of the fatty acid composition of total lipids and phospholipids in breast milk from Japanese women. Pediatr Res 47: 14–20.
12. Makrides M, Neumann MA, Gibson RA (1996) Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr 50: 352–357.
13. Lauritzen L, Jorgensen MH, Hansen HS, Michaelsen KF (2002) Fluctuations in human milk long-chain PUFAs levels in relation to dietary fish intake. Lipids 37: 237–244.
14. Jensen CL, Prager TC, Fraley JK, Chen HM, Anderson RE, et al. (1997) Effect of dietary linoleic acid and arachidonic acid on growth and visual function of term infants. Journal of Pediatrics 131: 200–209.
15. Makrides M, Neumann MA, Jeffrey B, Lien EL, Gibson RA (2000). A randomized trial of different ratios of linoleic to alpha-linolenic acid in the diet of term infants: effects on visual function and growth. American Journal of Clinical Nutrition 71: 120–129.
16. Gibson RA, Chen W, Makrides M (2001) Randomized trials with polyunsaturated fatty acid interventions in preterm and term infants: functional and clinical outcomes. Lipids 36: 873–883.
17. Willatts P, Forsyth JS, DiModugno MK, Varma S, Colvin M (1998) Developmental profile of LCPUFA and Brain Transcriptome. PLoS ONE | www.plosone.org 11 April 2007 | Issue 4 | e370
18. Birch EE, O’Connor J, Pan DA, Krikets AD, Strohlein LH (1998) The fatty acid composition of skeletal muscle membrane phospholipid: its relationship with the type of feeding and plasma glucose levels in young children. Metabolism 47: 106–112.
19. Sarkadi-Nagy E, Wijerandran V, Dian GY, Chao AC, Hsieh AT, et al. (2004) Formula feeding potentiates docosahexaenoic and arachidonic acid bio-synthesis in term and preterm baboon neonates. J Lipid Res 45: 1–8.10.
20. Haub AT, Anthony JC, Diemens-Schade DA, Rumsby MC, Lawrence F, et al. (2007) The influence of moderate and high dietary docosahexaenoic acid on baboon neonatal neural fatty acids. Ped Res In Press.
21. Morgan NV, Westaway SK, Morton JE, Gregory A, Gissen P, et al. (2006) PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. Nat Genet 38: 732–734.
22. Khaete SB, Hauser OH, Hof R, Shelef I, Narkis G, et al. (2006) PLA2G6 Mutation Underlies Infanteile Neuroaxonal Dystrophy. Am J Hum Genet 79: 942–948.
23. Leonard AE, Bobik EG, Dorado J, Kroeger PE, Chuang LT, et al. (2000) Cloning of a human cDNA encoding a novel enzyme involved in the elongation of long-chain polyunsaturated fatty acids. Biochem J 350 Pt 3: 765–770.
24. Leonard AE, Kelder B, Bobik EG, Chuang LT, Lewis CJ, et al. (2002) Identification and expression of mammalian long-chain PUFAs elongation enzymes. Lipids 37: 733–740.
25. Welch DJ, Credly DP, Hauser SD, Mathis KJ, Krivi GG, et al. (1994) Molecular cloning and expression of human leukotriene-C4 synthase. Proc Natl Acad Sci U S A 91: 9743–9749.
26. Buchner J (1999) Hsp90/Ca-a holding for folding. Trends Biochem Sci 24: 191–195.
27. Weaver AJ, Sullivan WP, Fels SJ, Owen BA, Todd DO (2000) Crystal structure and activity of human p23, a heat shock protein 90 co-chaperone. J Biol Chem 275: 23045–23052.
28. Holt SE, Anser DL, Baur J, Tesmer VM, Dym M, et al. (1999) Functional requirement of p23 and Hsp90 in telomerase complexes. Genes Dev 15: 817–826.
29. Rozen R, Vockley J, Zhou L, Milos R, Willard J, et al. (1994) Isolation and expression of a cDNA encoding the precursor for a novel member (ACADSB) of the acyl-CoA dehydrogenase gene family. Genomics 24: 289–297.
30. Ye X, Ji C, Zhou C, Zeng L, Gu S, et al. (2004) Cloning and characterization of a human cDNA ACAD10 mapped to chromosome 12q24.1. Mol Biol Rep 31: 174–181.
31. Birch EE, CASTANeda YS, Wheaton DH, Birch DG (2000) A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Developmental Medicine and Child Neurology 42: 574–181.
32. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH (1993) Growth in utero and serum cholesterol concentrations in adult life. BMJ 307: 1524–1527.
33. de Rooij SR, Painter RC, Roseboom TJ, Phillips DJ, Oomund C, et al. (2006) Glucose tolerance at age 58 and the decline of glucose tolerance in comparison with age 50 in people prenatally exposed to the Dutch famine. Diabetologia 49: 637–643.
34. Birch DJ (2003) Coronary heart disease: a disorder of growth. Horm Res 59 Suppl 1: 35–41.
35. Weisgerg HS, Armitage JA, Sinclair AJ, Vingrys AJ, Burns PL, et al. (2001) Perinatal omega-3 fatty acid deficiency affects blood pressure later in life. Nat Med 7: 258–259.
36. Baur LA, O’Connor J, Pan DA, Krikets AD, Strohlein LH (1998) The fatty acid composition of skeletal muscle membrane phospholipid: its relationship with the type of feeding and plasma glucose levels in young children. Metabolism 47: 106–112.
effects and stimulates the expression of selective Alzheimer’s disease indicator-1, a recently discovered antiangiogenic gene in human neuroblastoma long-term cell cultures. J Clin Endocrinol Metab 90: 1775–1782.

54. Evans AM (2006) AMP-activated protein kinase and the regulation of Ca2+ transport in O2-sensing cells. J Physiol 280: 3467–3467.

55. Kuthe A, Magert H, Uckert S, Forssmann WG, Stief CG, et al. (2000) Gene cloning of the mouse obese gene and its human homologue. Nature 372: 543–546.

56. Dooley CM, James J, McGlade CJ, McIlwaine J, Ahmed S, et al. (2003) Involvement of Numb in vertebrate retinal development: Evidence for multiple roles of Numb in neural differentiation in the central-nervous-system. Embo Journal 13: 1799–1805.

57. Usdin TB, Wang T, Niles K, Mezey E, Palkovits M (2000) New members of the parathyroid hormone/parathyroid hormone receptor family: the parathyroid hormone 2 receptor and tuberoinfundibular peptide of 39 residues. Front Neuroendoendocrinol 21: 349–353.

58. Gehlert DR (1998) Multiple receptors for the pancreatic polypeptide (PP-fold) family: physiological implications. Proc Soc Exp Biol Med 218: 7–22.

59. Pedrazzini T, Seydoux J, Kusmpter P, Aubert JF, Grouzmann E, et al. (1998) Cardioregulatory response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat Med 4: 722–726.

60. Shafir I, Feng W, Shoshan-Barmata V (1998) Voltage-dependent anion channel (VDAC) in normal and dystrophic skeletal muscle. J Muscle Res Cell Motil 21: 433–442.

61. Bialon E, Bedden A, Lint J, McBride ER, Forte M (1994) Human elongated untranslated region: new findings and insights. Analyst 123: 41–50.
134. Wu Q, Magnussen T (1995)Localization of Mouse Lumican (Keratan Sulfate Proteoglycan) to Distal Chromosome 1o. Mammalian Genom 6: 367–368.

135. Wollberg TG, Straight PD, Gruen RL, Huwiler AJ, Primaolok P, et al. (1995)Adam, a Widely Distributed and Developmentally-Regulated Gene Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain. Developmental Biology 169: 378–383.

136. Wolfsberg TG, Primaolok P, Mules DG, White JM (1993)Adam, A Novel Family of Membrane-Proteins Containing a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

137. Xu W, Hatini J, Zou XX, Hong M, Zheng C, et al. (2005)Identification of components of the ADAM family encoded by the autosomal recessive disorder CDS2. Human Molecular Genetics 14: 2057–2059.

138. Yang J, Flaherty BL, Cornish MK, Knuist S, Kucharski T, Nabeshima Y, et al. (1995)A metallolproteinase-disintegrin participating in myoblast fusion. Nature 377: 652–656.

139. Baxbaum JD, Liu KN, Luo VX, Slack JL, Stocking KL, et al. (1998)Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor. Journal of Biological Chemistry 273: 27765–27767.

140. Zandir K, Postina R, Schroeder A, Mueller U, Fahrenholz F (2005)Shedding of the amyloid precursor protein APPL2 by disintegrin-metallolproteinases. FEBS J 272: 3801–3820.

141. Jackson LF, Qiu TH, Sunnarborg SW, Chang A, Zhang CL, et al. (2003)Defective valvulogenesis in HB-EGF and TACE-null mice is associated with aberrant BMP signaling. Development 272: 2704–2716.

142. Felbor U, Kessler B, Mothes W, Goebel HH, Ploegh HL, et al. (2002)Assembly of the ADAM33 gene with asthma and bronchial hyperresponsiveness. Nature 418: 426–430.

143. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Haitchi HM, et al. (2006)Understanding the pathobiology of severe asthma to generate new therapeutic opportunities. J Allergy Clin Immunol 117: 496–506, quiz 507.

144. Hayashida S, Chen H, Peschon J, Shi W, Zhang Y, et al. (2003)Pulmonary hypoplasia in mice lacking tumor necrosis factor-alpha converting enzyme indicates an indispensable role for cell surface protein shedding during embryonic lung morphogenesis. Dev Biol 232: 204–218.

145. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Wilson SJ, et al. (2005)ADAM33 expression in asthmatic airways and human embryonic lungs. Am J Respir Crit Care Med 171: 168–170.

146. Wolfsberg TG, Cooper LA, Takenawa T, Guan JL (2004)Focal adhesion kinase regulation of N-WASP subcellular localization and function. J Biol Chem 279: 35275–35281.

147. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Wilson SJ, et al. (2005)ADAM33 expression in asthmatic airways and human embryonic lungs. Am J Respir Crit Care Med 171: 168–170.

148. Wolfsberg TG, Cooper LA, Takenawa T, Guan JL (2004)Focal adhesion kinase regulation of N-WASP subcellular localization and function. J Biol Chem 279: 35275–35281.

149. Haitchi HM, Powell RM, Shaw TJ, Howarth PH, Wilson SJ, et al. (2005)ADAM33 expression in asthmatic airways and human embryonic lungs. Am J Respir Crit Care Med 171: 168–170.

150. Wolfsberg TG, Cooper LA, Takenawa T, Guan JL (2004)Focal adhesion kinase regulation of N-WASP subcellular localization and function. J Biol Chem 279: 35275–35281.

151. Wolfsberg TG, Cooper LA, Takenawa T, Guan JL (2004)Focal adhesion kinase regulation of N-WASP subcellular localization and function. J Biol Chem 279: 35275–35281.

152. Wolfsberg TG, Cooper LA, Takenawa T, Guan JL (2004)Focal adhesion kinase regulation of N-WASP subcellular localization and function. J Biol Chem 279: 35275–35281.

153. Wolfsberg TG, Straight PD, Gruen RL, Huwiler AJ, Primaolok P, et al. (1995)Adam, a Widely Distributed and Developmentally-Regulated Gene Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain. Developmental Biology 169: 378–383.

154. Wolfsberg TG, Primaolok P, Mules DG, White JM (1993)Adam, A Novel Family of Membrane-Proteins Containing a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

155. Wolfsberg TG, Straight PD, Gruen RL, Huwiler AJ, Primaolok P, et al. (1995)Adam, a Widely Distributed and Developmentally-Regulated Gene Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain. Developmental Biology 169: 378–383.

156. Wolfsberg TG, Primaolok P, Mules DG, White JM (1993)Adam, A Novel Family of Membrane-Proteins Containing a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

157. Wolfsberg TG, Primaolok P, Mules DG, White JM (1993)Adam, A Novel Family of Membrane-Proteins Containing a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

158. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

159. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

160. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

161. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

162. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

163. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.

164. Madisen L, Neubauer M, Plowman G, Rosen D, Segarini P, et al. (1990)Family Encoding Membrane-Proteins with a Disintegrin and Metallolproteinase Domain-Multipotential Functions in Cell-Cell and Cell-Matrix Interactions. Journal of Cell Biology 131: 275–278.
161. Moskovitz J, Weissbach H, Brot N (1996) Cloning the expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins. Proc Natl Acad Sci U S A 93: 2095–2099.

162. Moskovitz J, Jenkins NA, Gilbert DJ, Copeland NG, Jentsky F, et al. (1996) Chromosomal localization of the mammalian peptide-methionine sulfoxide reductase gene and its differential expression in various tissues. Proceedings of the National Academy of Sciences of the United States of America 93: 3203–3208.

163. Moskovitz J (2005) Methionine sulfoxide reductases: ubiquitous enzymes involved in antioxidant defense, protein regulation, and prevention of aging-associated diseases. Biochimica Et Biophysica Acta-Proteins and Proteomics 1703: 213–219.

164. Moskovitz J, Bar-Noy S, Williams WM, Berlet BS, Stadtmann ER (2001) Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. Proceedings of the National Academy of Sciences of the United States of America 98: 12929–12934.

165. Levine RL, Moskovitz J, Stadtmann ER (2000) Oxidation of methionine in proteins: Roles in antioxidant defense and cellular regulation. Isbhub Life 50: 301–307.

166. Picot CR, Petropoulos I, Perichon M, Moreau M, Nizard C, et al. (2005) Structural and functional diversity. Curr Opin Struct Biol 11: 39–46.

167. Laity JH, Lee BM, Wright PE (2001) Zinc finger proteins: new insights into antioxidant defense and mitochondrial reactive oxygen species. Molecular and Cellular Biology 23: 6520–6530.

168. Storz P, Doppler H, Toker A (2005) Protein kinase D mediates mitochondria-to-nucleus signaling and detoxification from mitochondrial reactive oxygen stress. Free Radic Biol Med 39: 1332–1341.

169. Storz P, Doppler H, Toker A (2005) EGFR signaling is required for the differentiation and maintenance of neural progenitors along the dorsal midline of the Drosophila embryonic head. Development 132: 3417–3426.

170. Kornblum HI, Hussain R, Wiesen J, Miettinen P, Zurcher SD, et al. (1998) Abnormal astrocyte development and neuronal death in mice lacking the epidermal growth factor receptor. J Neurosci Res 53: 697–717.

171. Winston JT, Koepp DM, Zhu CH, Elledge SJ, Harper JW (1999) A family of yeast F-box proteins: Roles in antioxidant defense and cellular regulation. Iubmb Life 50: 1032–1037.

172. Lapillonne A, Clarke SD, Heird WC (2004) Polyunsaturated fatty acids and human liver. Am J Pathol 163: 2303–2317.

173. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, et al. (2005) Modification by docosahexaenoic acid of age-induced alterations in gene expression by dietary n-3 fatty acids. Proc Natl Acad Sci U S A 102: 11321–11326.

174. Berger A, Match DM, German JS, Roberts MA (2002) Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. Lipids Health Dis 1: 2.

175. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, et al. (2005) Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids. Proc Natl Acad Sci U S A 102: 11321–11326.

176. Deutscher M, Zvara A, Hackler L Jr, Barcelo-Coblijn G, et al. (2002) The role of n-3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by dietary n-3 fatty acids. Proc Natl Acad Sci U S A 99: 2619–2624.

177. Barcelo-Coblijn G, Hugnes E, Kitajka K, Puskas LG, Zvara A, et al. (2003) Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids. Proc Natl Acad Sci U S A 100: 11321–11326.

178. Bergner A, Match DM, German JS, Roberts MA (2002) Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. Lipids Health Dis 1: 2.

179. Seth D, Leo MA, McGuinness PH, Läbcher CS, Brennan Y, et al. (2003) Gene expression profiling of alcoholic liver disease in the baboon (Papio hamadryas) and human liver. Am J Pathol 163: 2303–2317.

180. Cox LA, Schlaibert-Loutsevich N, Hubbard GB, Nijland MJ, McDonald TJ, et al. (2006) Gene expression profile differences in left and right liver lobes from mid-gestation fetal baboons: a cautionary tale. J Physiol 572: 59–66.

181. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, et al. (2005) Modification by docosahexaenoic acid of age-induced alterations in gene expression and molecular composition of rat brain phospholipids. Proc Natl Acad Sci U S A 102: 11321–11326.

182. Berger A, Match DM, German JS, Roberts MA (2002) Dietary effects of arachidonate-rich fungal oil and fish oil on murine hepatic and hippocampal gene expression. Lipids Health Dis 1: 2.