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Vitamin E-sensitive genes in the developing rat fetal brain: a high-density oligonucleotide microarray analysis

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Abstract Vitamin E (tocopherols and tocotrienols) is essential for normal neurological function. Recently we have reported that the neuroprotective properties of tocotrienols are much more potent than that of the widely studied tocopherols (Sen, C.K., Khanna, S., Roy, S. and Parker, L. (2000) J. Biol. Chem. 275, 13049–13055). The objective of this study was to evaluate whether (i) oral supplementation of tocotrienols during pregnancy is bioavailable to fetal and mother brains; (ii) short-term change in dietary vitamin E levels of pregnant rats influences gene expression profile of developing fetal brains. We report that dietary tocotrienol is bioavailable to both mother and fetal brains. The enrichment is more in fetal brain tissue. Using a GeneChip microarray expression profiling approach we have identified a specific set of vitamin E sensitive genes in the developing rat fetal brain.

Key words: Fetus; Pregnancy; Diet; Microarray; Tocotrienol; Tocopherol

1. Introduction

Vitamin E is essential for normal neurological function [1,2]. Based on symptoms of primary vitamin E deficiency, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function [2]. Most of the vitamin E sensitive neurological disorders are associated with elevated levels of oxidative damage markers. This has led to the popular hypothesis stating that the neuroprotective effects of vitamin E are mediated by its antioxidant property [3]. Vitamin E is a generic term for all tocopherols and their derivatives having the biological activity of RRR-α-tocopherol [4,5]. In nature, eight substances have been found to have vitamin E activity: α-, β-, γ- and δ-tocopherol; and α-, β-, γ- and δ-tocotrienol. Often, the term vitamin E is synonymously used with α-tocopherol. While D-α-tocopherol (RRR-α-tocopherol) has the highest bioavailability and represents the standard against which all the others must be compared, it is only one out of eight natural forms of vitamin E.

Tocotrienols, formerly known as ζ-, ε- or η-tocopherols, are chemically similar to tocopherols except that they have an isoprenoid tail with three unsaturation points instead of a saturated phytyl tail. While tocopherols are predominantly found in corn, soybean and olive oils, tocotrienols are particularly rich in palm, rice bran and barley oils [4,5]. Tocotrienols have been long known to possess powerful antioxidant, anti-cancer and cholesterol-lowering properties. Tocotrienols are thought to have more potent antioxidant properties than α-tocopherol [6,7]. The unsaturated side chain of tocotrienol allows for more efficient penetration into tissues that have saturated fatty layers such as the brain and liver [8]. Superior antioxidant, free radical scavenging effects of tocotrienol compared to that of tocopherol appear to be due to their better distribution in the fatty layers of the cell membrane [8]. While tocotrienols have shown better beneficial effects than α-tocopherol in a limited number of situations indicated above, little is known about the exact mechanism of action. Micromolar amounts of tocotrienols, but not tocopherols, have been shown to suppress the activity of hydroxy-3-methylglutaryl coenzyme A reductase, the hepatic enzyme responsible for cholesterol synthesis [9,10]. We have observed that α-tocotrienol is much more potent than α-tocopherol in inhibiting glutamate-induced signal transduction pathways leading to neurodegeneration [11]. Previously it has been suggested that dietary α-tocotrienol does not reach the brain [12]. The objective of this study was two-fold. First, to confirm whether tocopherol and tocotrienol fed to pregnant rats reach the mother and fetal brains using a HPLC-CoulArray technique recently developed in our laboratory [13]. Second, we utilized a high-density oligonucleotide microarray approach to screen the developing (day 17 of gestation) fetal brain transcriptome for vitamin E sensitive genes.

2. Materials and methods

2.1. Animals and supplementation protocol

Pregnant (3 days) rats (10 weeks old; Sprague–Dawley, Harlan, Indianapolis, IN, USA) were randomly divided into following two groups: (i) E^+ group – fed a standard rat chow that is enriched in α-tocopherol (~200 nmol/g diet). Additionally, this group was supplemented for 2 weeks with a daily gavage of tocotrienol rich fraction (TRF) suspended in vitamin E-stripped corn oil (Harlan). A mixture of 110 mg α-tocopherol and 119 mg of α-tocotrienol contained in 1 g TRF was fed to pregnant rats on a per kg body-weight basis. TRF was provided in the form of Tocomin® 50% provided by Carotech Sdn Bhd (Perak, Malaysia); (ii) E^- group – fed a vitamin E deficient diet (TD88163, Harlan; α-tocopherol/tocotrienol levels below detec-

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Abbreviations: apoB, apolipoprotein B; DMT, Data Mining Tool 2.0; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; HO, hemeoxygenase; HMG2, high-mobility group protein 2; MAS, Affymetrix Microarray Suite 4.0; NOPP140, nuclear phosphoprotein p130; PKC, protein kinase C; RT-PCR, reverse transcription-polymerase chain reaction; TRF, tocotrienol rich fraction

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tion limits) and supplemented with a matched volume of vitamin E-stripped corn oil. All rats were maintained under standard conditions at 22 ± 2°C with 12:12 h dark:light cycles. All animal protocols were approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) of the Ohio State University, Columbus, OH, USA. *Sample collection.* On 17th day of gestation, body weights of each rat were recorded. Rats were killed. Mother and fetal brains were removed, rinsed in ice-cold phosphate-buffered saline, pH 7.4 (PBS) and snap frozen in liquid nitrogen. Samples were briefly stored in −80°C.

2.2. Vitamin E extraction and analysis

Vitamin E extraction and analysis from mother and fetal brains was performed as described previously using a HPLC-coulometric electrode array detector (Coularray Detector – model 5600 with 12 channels; ESA Inc., Chelmsford, MA, USA) [13]. This system uses multiple channels with different redox-potentials. α-, γ- and δ-tocopherols and tocotrienols were detected on channels set at 200 mV, 300 mV, and 400 mV, respectively.

2.3. Affymetrix GeneChip probe array analysis

Total RNA was extracted by pulverizing the fetal brains in liquid N_2 followed by extraction using Trizol (Gibco BRL) [14,15]. A further clean up of RNA was performed using the RNeasy kit (Qiagen). Targets were prepared for microarray hybridization according to previously described protocols [14]. To assess sample quality the samples were hybridized for 16 h at 45°C to GeneChip Test-2 arrays. Satisfactory samples were hybridized to Rat Genome arrays (U34A). The arrays were washed, stained with streptavidin–phycoerythrin and were then scanned with the GeneArray scanner (Agilent Technologies) in our own facilities.

Raw data were collected and analyzed using Affymetrix Microarray Suite 4.0 (MAS) and Data Mining Tool 2.0 (DMT) software. The following two approaches were utilized to identify differentially expressed genes: (i) using *comparison analysis* in MAS, six pair-wise comparisons were generated from replicates of both E^+ and E^- groups. Average fold-changes were calculated for both up- or down-regulated genes. Genes for which the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets; (ii) *T*-test was performed using DMT, and genes that significantly ($P < 0.05$) changed (increased or decreased) in the E^+ group compared to the E^- group were selected. The average difference values of selected genes were loaded into the Cluster and TreeView software [16]. The data was adjusted according to the median center for a clear graphic display of vitamin E sensitive genes.

2.4. Reverse-transcription and polymerase chain reaction (RT-PCR)

Expression levels of hemeoxygenase 3 (HO-3), cyclin D1, high-mobility group protein 2 (HMG2), nucleolar phosphoprotein p130 (NOPP140) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were independently determined using RT-PCR as described previously [17]. In brief, the total RNA (1 μg) was transcribed into cDNA using oligo-dT primer and Superscript II. RT-generated cDNA were amplified by PCR using gene-specific primers as described in Table 1. PCR reaction products were electrophoresed in a 1% agarose gel containing 0.25 μg/ml ethidium bromide. The gel was digitally imaged under conditions of ultraviolet transillumination. Quantification of band intensity was performed using the Scion Image (Scion Corporation) that is based on NIH Image software.

Table 1
Primers used for RT-PCR

mRNA	Primer sequence 5′ to 3′
Cyclin D1	CTG CAT GTT CGT GGC CTC TAA GAT
	CCA GAA GGG CTT CAA TCT GTT CCT
GAPDH	TAT GAC TCT ACC CAC GGC AAG TTC A
	CAG TGG ATG CAG GGA TGA TGT TCT
HMG2	TCC TCC CAA AGG TGA TAA GAA AGG A
	TGG CAC GGT ATG CAG CAA TA
HO-3	ATG GCA TCA GAG AAG GAA AAC CAT T
	CCC ATC AAG TAT TGA GAG CCC ATT C
NOPP140	TCA GTG CCA CCA AGA GTC CCT TAA
	CTT CTT CAC TGG AAT CTT CGG AGG A

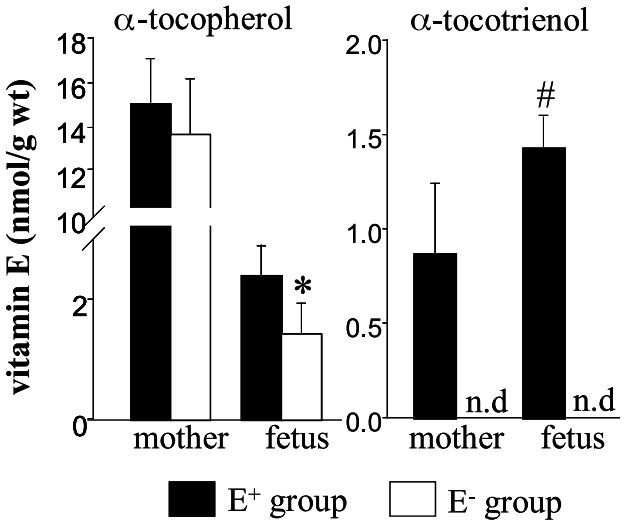


Fig. 1. Vitamin E levels in fetal and mother rat brains. Pregnant (3 days) rats were randomly divided into (i) E^+ group – fed a standard rat chow that is enriched in α-tocopherol. Additionally, this group was supplemented for 2 weeks with a daily gavage of TRF suspended in vitamin E-stripped corn oil; and (ii) E^- group – fed a vitamin E deficient diet and supplemented with a matched volume of vitamin E-stripped corn oil. On the 17th day of gestation, brains were collected and vitamin E analysis was performed using HPLC. $^*P < 0.05$ significantly different compared to the E^+ group. $\#P < 0.05$ significantly different compared to mother brain. n.d., not detected.

3. Results

3.1. Vitamin E levels in mother and fetal brains

α-Tocopherol level in the fetal brain was multi-fold lower than that observed in the mother brain (Fig. 1A). Compared to E^+ group, feeding a vitamin E deficient diet for only 2 weeks during pregnancy did not significantly decrease the α-tocopherol levels in the adult mother brain. However, under similar conditions, feti from the mothers of E^- group had significantly lower α-tocopherol levels in brain compared to the feti from E^+ group (Fig. 1A). α-Tocotrienol was below detection limits in the brains of mothers as well as feti of the E^- group. Oral supplementation of TRF for 2 weeks to mothers during pregnancy resulted in delivery of α-tocotrienol to the mother as well as fetal brains. Importantly, incorporation of tocotrienol in the fetal brain was significantly higher compared to that in the mother brain (Fig. 1B). Of interest, short-term vitamin E deficiency in pregnancy diet did not influence vital parameters of pups such as weight or general health (our unpublished observations).

3.2. Transcriptome profiling

The transcriptomes of developing fetal brains from E^+ and E^- groups were compared using the U34A rat genome high-density oligonucleotide GeneChip array. This array analyzes approximately 7000 full-length sequences and approximately 1000 EST clusters. Using raw data from all replicates available from both groups, a total of six pair-wise comparisons were generated. The average (six pair-wise comparisons) fold-changes of all the genes that were differentially expressed were calculated. Data indicated that a majority of genes remained unchanged (Fig. 2). A total of 645 (7.3%) genes were up-regulated in vitamin E^+ group compared to the E^- group. Out of which 416 genes increased by a magnitude of two-fold or

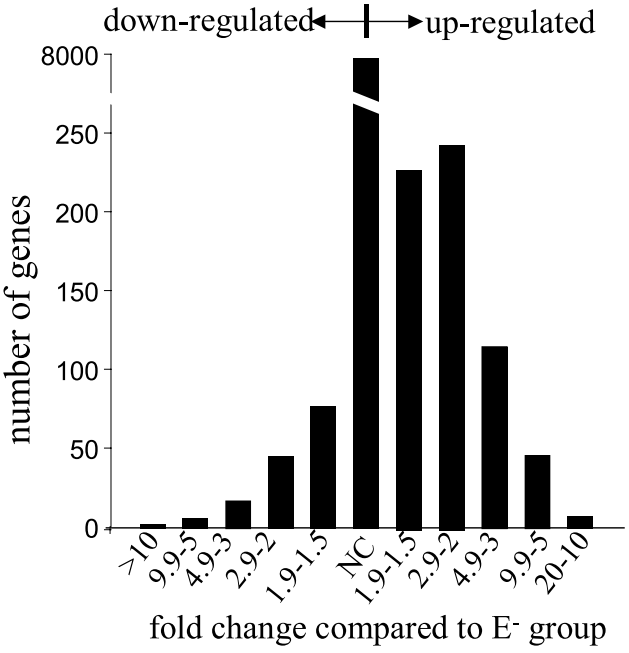


Fig. 2. Range of the average fold changes of differentially expressed genes in E^+ and E^- groups. Six pair-wise comparisons were generated from replicates of both E^+ and E^- groups using *comparison analysis* in MAS. Average fold changes were calculated for both up- or down-regulated genes in response to dietary vitamin E. NC = no charge.

more. On the other hand 152 (1.7%) of the genes were down-regulated with 74 of them lowered by two-fold or more (Fig. 2). Using the *t*-test analysis (see Section 2 for details), a total 144 genes were observed to have changed significantly ($P < 0.05$) in vitamin E deficiency group compared to the supplemented group. The data was adjusted according to the median center for a clear graphic display of vitamin E sensitive genes (Fig. 3A,B). Next, genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. Using this approach of data analysis, a total of 19 probe sets were found to be up-regulated and 34 repressed in E^+ group compared to E^- group (Tables 2 and 3). Among the up-regulated genes, two probe sets targeting HO-3 were increased by 3.9 and 3.1 folds, respectively (Fig. 4). In contrast, the expression of maspin, GAPDH, apolipoprotein B (apoB) and G protein beta1 subunit (rGb1) genes was highly (three- to five-fold) repressed in response to dietary vitamin E.

3.3. Validation of GeneChip data using RT-PCR

Select vitamin E sensitive genes identified by the GeneChip approach were verified using conventional semi-quantitative RT-PCR (Fig. 4). The band intensity of the PCR products was quantified and fold change for each gene in E^+ group compared to E^- group was calculated. Data showed that fold change detected using both GeneChip or RT-PCR approaches for genes up-regulated E^+ vs. E^- groups were comparable (Fig. 4). For GAPDH, both microarray as well as RT-PCR data indicated a decrease in expression in E^+ group compared to E^- group. However, the fold-change in GAPDH expression was much higher in the microarray data compared to the RT-PCR data (Fig. 4).

4. Discussion

We present first evidence showing that consumption of a vitamin E deficient diet for only 2 weeks during pregnancy can substantially lower the vitamin E levels of fetal brain while not affecting the vitamin E levels of adult brain underscoring the importance of proper levels of this vitamin in the diet during pregnancy. Compared to an adult brain, a higher uptake of the α-tocotrienol form of vitamin E by fetal brain was also observed. Results of this study provide the first global assessment of vitamin E sensitive genes in a developing fetal brain. Of the 8000 genes surveyed, only 17 genes displayed an increase in gene expression levels in fetal brain as a result of vitamin E feeding to mothers, whereas 34 displayed a decrease

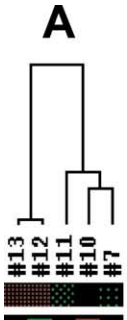
Table 2
Genes up-regulated in fetal brains of E^+ group

Affymetrix accession number	Description
M13100cds#1_at	HO-3 or RATLIN3A
X05472cds#2_at	RNREP24R, rat 2.4 kb repeat DNA right terminal region
X07686cds_s_at	rat L1Rn B6 repetitive DNA element
M13100cds#1_g_at	HO-3 or LINE3 (L1Rn)
rc_AA800912_g_at	GTF2I repeat domain-containing 1 putative ortholog
rc_AA924591_at	Cyp4a locus, encoding cytochrome P450 (IVA3)
X62951mRNA_s_at	mRNA (pBUS19) with repetitive elements
rc_AI231445_at	unr protein
rc_AA852046_s_at	VL30 element mRNA
AB010154_at	PKN mRNA for serin/threonine protein kinase expressed in hippocampus
rc_AI231257_at	cyclin D1
X53581cds#3_f_at	long interspersed repetitive DNA containing seven ORFs
AF055714UTR#1_at	hypertension-regulated vascular factor-1C-4
X05472cds#3_f_at	RNREP24R rat 2.4 kb repeat DNA right terminal region
S76466_at	type I serine-threonine kinase receptor
S78556_at	75 kDa glucose-regulated protein
rc_AA998882_s_at	NOPP140
D84418_r_at	chromosomal protein HMG2
U35244_g_at	vacuolar protein sorting homolog r-vps33a

Six pair-wise comparisons among brains obtained from individual feti, the mothers of whom were fed vitamin E^+ and vitamin E^- diet during pregnancy for 2 weeks. Genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. ESTs for which no description is available were excluded.

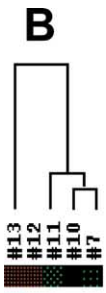
in expression indicating that a highly specific set of genes are sensitive to the vitamin E levels in a developing fetal brain.

Based on symptoms of primary vitamin E deficiency in adults, it has been demonstrated that vitamin E has a central role in maintaining neurological structure and function [2]. However, efforts to systematically evaluate the molecular basis of vitamin E action on the brain are lacking. Our data show that α-tocopherol level in the fetal brain was multifold lower than that observed in the mother brain. This data is in accordance with a previous study where α-tocopherol levels in fetal brain were lower compared to that of the brains of 21-day-old rats [18]. Furthermore, in humans, the serum α-tocopherol levels in full-term neonates are known to be several folds lower (0.212 ± 0.127 vs. 1.160 ± 0.513 mg/dl)



Fructose-6-phosphate-2-kinase / fructose 2-6 biphosphatase3	Metabolism / Biosynthesis
EST, similar to hypothetical protein MGC 14833 [H. sapiens]	Unknown
F-spondin	Neuron
EST, weakly similar to 810024JJ URF 4 [H. sapiens]	Unknown
Protein tyrosine phosphatase, no-receptor type substrate 1	Immune response signaling
PAM COOH-terminal interactor protein 1	Sensing / signaling / Cell communication
Rat mixed-tissue library Rx03955	Unknown
Cortactin-binding protein 1/ Proline rich synapse associated protein 1	Neuron
Tyrosine kinase receptor ligand 2 (RETL 2)	Neuron
Rat mixed-tissue library Rx04977	Unknown
14-3-3 protein beta-subtype	Neuron
Aldolase A, fructose biphosphate	Metabolism / Biosynthesis
Neural visinin-like protein	Neuron
Heart myosin light chain	Metabolism / Biosynthesis
Potassium voltage-gated channel, subfamily H (age related), member 3	Metabolism / Biosynthesis
Quinoid dihydropteridine reductase	Neuron
Signal transducer and activator of transcription 6	Sensing / signaling / Cell communication
Pro-protein convertase 5 isoform B	Neuron & Development
EST, moderately similar to Y053 human hypothetical protein KIAA0053 [H. sapiens]	Unknown
EST, weakly similar to JC 5238 galactosylceramide-like protein, GCP [H.sapiens]	Neuron
ADP-ribosylation factor related protein 1	Sensing / signaling / Cell communication
G protein coupled receptor (RTA)	Metabolism / Biosynthesis
Putative G-protein coupled receptor RA1c	Olfaction (Brain)
Developmentally-regulated cardiac factor	Vascular
Sulfonylurea receptor	Metabolism / Biosynthesis
Non-selective type endothelin receptor	Vascular
Adenylyl cyclase type VIII	Neuron
EST, moderately similar to protein kinase inhibitor alpha	Sensing / signaling / Cell communication
Nucleolar phosphoprotein of 140 kD	Cell cycle
Mu opioid receptor	Neuron
EST, highly similar to rat pyruvate dehydrogenase E1 component, beta subunit	Metabolism / Biosynthesis
Rat mixed-tissue library Rx03063	Unknown
Uncoupling protein-3 (nuclear gene encoding mitochondrial protein)	Metabolism / Biosynthesis
Chondroadherin	Development
EST, highly similar to cyclin M4 [H. sapiens]	Cell cycle
Malic enzyme 1	Metabolism / Biosynthesis
Arylalkylamine N-acetyltransferase	Neuron
Pancreatic islet	Immune response

Fig. 3. Cluster image illustrating the genes differentially expressed in fetal brains of E^+ group. For a clear graphic display of vitamin E sensitive genes, *t*-test was performed on replicate samples of E^+ group (animals #7, #10, #11) and E^- group (animals # 12 and #13) that significantly ($P < 0.05$) changed (increase or decrease) in the E^+ group as compared to the E^- group. The average difference values of selected genes were loaded into the Cluster and TreeView software where the data was adjusted according to the median center. Red to green gradation in color represent higher to lower expression signal compared to median center. In some cases due to very high expression levels the signal is saturated and shows red in all samples. The scale is digitally adjustable. A medium intensity (not optimal for all data presented) scale is shown. For details of vitamin E feeding and tissue collection, see legend of Fig. 1. A: Up-regulated; or (B) down-regulated genes in E^+ group compared to E^- group.



Actin-filament binding protein Frabin	Metabolism / biosynthesis
RB109 (Brain specific protein)	Unknown
26 S proteasome	Metabolism / biosynthesis
Rat mixed-tissue library Rx02831	Unknown
Keratinocyte islet cell line	Neuron
Pheromone receptor VN5	Sensing / signaling / Cell communication
Rat mixed-tissue library Rx02341	Unknown
GTP cyclohydrolase	Metabolism / biosynthesis
Lysosomal ATPase ortholog	Metabolism / biosynthesis
Putative transmembrane ortholog	Secretory
Choline kinase R1	Cancer /biosynthesis
Angiotensinogen	Vascular
Cytochrome P450 IIE1	Electron transfer
Calmodulin-dependent protein kinase II-delta	Neuron
Serine proteinase inhibitor-like protein	Vascular
NTR2 receptor	Neuron
Alcohol dehydrogenase	Metabolism / biosynthesis
Gap junction structural protein	Sensing / signaling / Cell communication
Type II iodothyronine deiodinase	Humoral
Rat mixed-tissue library Rx01430	Unknown
Liver nuclear protein p47	Cell cycle
70 kd heat-shock like protein	Sensing / signaling / Cell communication
EST,weakly similar to MMT18 DNA repair/ transcription protein MET18[S.cerevisiae]	Transcription
LTBP-2 like protein	Immune response
Zinc finger binding protein 148	Transcription
Rabaptin	Neuron
Rat mixed-tissue library Rx00215	Unknown
Heat shock 27 kDa protein	Sensing / signaling / Cell communication
Nuclear factor kappa B p105 subunit	Immune response
Wistar orphan receptor COUP-TFI	Humoral
EST, DLC-1 (Arhgap 7) [M. musculus] ortholog	Cancer
GABAA receptor alpha-4 subunit	Neuron
Cytochrome P450 IIIA9	Electron transfer
EST, MGC6279 hypothetical protein [M. musculus] ortholog	Unknown
T-complex 1	Metabolism / biosynthesis
EST, mitochondrial ribosomal protein 64 [M. musculus] ortholog	Translation
CCAAT binding factor CBF-C/NFY-C	Transcription
Na-K ATPase alpha-1 subunit	Metabolism / biosynthesis
Mast cell protease 3	Secretory
Rat mixed-tissue library Rx044402	Unknown
C2-HC type zinc finger protein r-MvT13	Transcription
Rps2r2	Immune response
EST, lymphocyte antigen 117 [H.sapiens] putative ortholog	Unknown
Carcinoembryogenic antigen-related protein	Cancer /biosynthesis
Prostatein subunit C3	Immune response
Thyroid hormone responsive protein	Humoral
Racgap1 Rac GGTPase-activating protein 1	Metabolism / biosynthesis
Coupling factor 6 of mitochondrial ATP synthase	Unknown
Activin type IIB receptor	Metabolism / biosynthesis
Ribosomal protein S4	Development
Rat mixed-tissue library Rx016777	Translation
Calmodulin	Sensing / signaling / Cell communication
D-3-phosphoglycerate dehydrogenase	Translation
EST, weakly similar to T46904 hypothetical protein	Metabolism / biosynthesis
EST, highly similar to WN5A_rat WNT-5A protein precursor [R. norvegicus]	Unknown
T-cell receptor beta chain	Immune response
D-dopachrome tautomerase	Immune response
Major histocompatibility complex class II-H alpha	Immune response
EST, highly similar to CY_human cytochrome C1, heme protein precursor	Electron transfer
Ribosomal protein L15	Translation
Nucleoporin Nup 84	Immune response
Ribosomal protein S8	Translation
Apolipoprotein B	Vascular
Rat mixed-tissue library rx00639	Unknown
Phosphatidate phosphohydrolase type 2	Metabolism / biosynthesis
Ribosomal protein L5	Translation
Interleukin 2	Immune response
6-phosphofructo-2-kinase	Metabolism / biosynthesis

Fig. 3 (*Continued*).

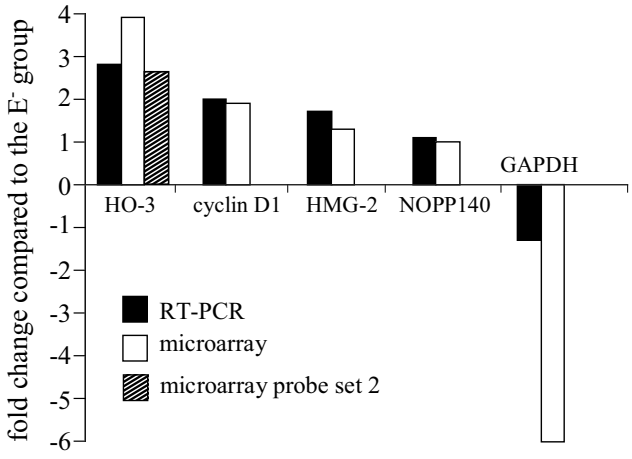


Fig. 4. RT-PCR validation of GeneChip microarray expression analysis. Expression levels of were independently determined using RT-PCR. The following genes identified as differentially expressed in E^+ group compared to E^- group using GeneChip microarray analysis were verified using RT-PCR: HO-3, cyclin D1, HMG2, NOPP140 and GAPDH. The band intensity of the PCR products was quantified and fold change for each gene in E^+ group compared to E^- group was calculated (solid bars). For comparison, fold changes observed in the expression of a specific gene using GeneChip microarray analysis (one or more probe sets) was also plotted (empty and hatched bars).

compared to that of their mothers [19]. We have previously shown that compared to α-tocopherol, tocotrienols are strikingly more potent in protecting neuronal cells against glutamate-induced degeneration [11]. However, in vivo data demonstrating the availability of dietary tocotrienols in the brain was lacking. The present study provides first evidence that dietary supplementation of TRF during pregnancy leads to a significant enrichment of α-tocotrienol in both maternal and fetal brains. Dietary vitamin E is absorbed in the intestine and carried by lipoproteins to the liver. In the liver, the α-tocopherol fraction is incorporated into very low-density lipoprotein (VLDL) by a α-tocopherol transfer protein and then secreted into the bloodstream [20]. A recent study shows that scavenger receptor class B type I (SR-BI), which mediates cellular selective cholesteryl ester uptake from lipoproteins, facilitates efficient transfer of α-tocopherol from high-density lipoprotein (HDL) to cultured cells [21]. Furthermore, in SR-BI-deficient mutant mice, relative to wild-type control animals, there was a significant increase in plasma α-tocopherol levels (1.1- to 1.4-fold higher) that was mostly due to the elevated α-tocopherol content of their abnormally large plasma HDL-like particles [21]. Mechanisms of uptake and transport of tocotrienols in organs and tissues are poorly understood in adults and more so in fetal tissues.

HO-3 was one of the few vitamin E sensitive genes up-

Table 3
Genes down-regulated in fetal brains of E^+ group

Affymetrix accession number	Description
U58857_at	maspin
AFFX_rat_GAPDH_M_st	GAPDH
M27440_at	apoB
U88324_g_at	rGb1
AFFX_rat_beta-actin_3_st	cytoplasmic beta-actin
rc_AI045858_at	weakly similar to T14794 hypothetical protein DKFZp586P1522
rc_AI008852_at	eukaryotic translation elongation factor
U46118_at	cytochrome P450 3A9
L27112_s_at	stress-activated protein kinase alpha II
rc_AA965261_at	H2A histone family, member Y
AF091563_r_at	QIL-LD1 olfactory receptor
AB013454_at	NaPi-2 beta
M27467_at	cytochrome oxidase subunit VIc
U50412_at	phosphoinositide 3-kinase regulatory subunit p85alpha
U88324_at	rGb1
M32474_at	carcinoembryonic antigen-related protein
AF037071_at	carboxyl-terminal PDZ ligand of neuronal NO synthase
AB010436_at	cadherin-8
L03386_i_at	zinc finger protein
rc_AI072341_at	vacuolar protein sorting homolog r-vps33b
rc_AI073204_at	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein
rc_AI103498_at	ribosomal protein L5
D16308_at	cyclin D2
rc_H31847_at	dynein light intermediate chain
D17521_at	PKC-regulated chloride channel
D90038_at	70 kDa peroxisomal membrane protein
rc_AI171630_s_at	p38 mitogen-activated protein kinase
AF083330_at	kinesin-like protein KIF3C
M65251_s_at	rat angiotensinogen gene-inducible enhancer-binding protein 1
rc_AA892378_at	AF151893 1 CGI-135 protein
rc_AA800194_at	similar to T08812 probable succinate-CoA ligase
AF090867_g_at	guanosine monophosphate reductase mRNA
D14045_s_at	DNA topoisomerase IIA
rc_AI179150_s_at	CDK110

Six pair-wise comparisons among brains obtained from individual feti, the mothers of whom were fed vitamin E^+ and vitamin E^- diet during pregnancy for 2 weeks. Genes for those the concordance exceeded 50% in pair-wise comparisons were selected, especially if the gene was detected with redundant probe sets. ESTs for which no description is available were excluded.

regulated in fetal brains. HO isozymes, HO-1, HO-2 and HO-3, are heat shock protein 32 protein cognates with a known function of catalyzing the isomer-specific oxidation of the heme molecule, including that of NO synthase [22]. HO-1 is highly inducible, whereas HO-2 and HO-3 are constitutively expressed. These proteins play a central role in the cellular defense mechanisms. HO activity is responsible for the production of equimolar amounts of CO, biliverdin and free Fe [22,23]. Recent findings with the HOs suggest that these proteins may serve as an intracellular 'sink' for NO [24]. LINE1 was identified to be another vitamin E sensitive transcript. The LINE-1, or L1 family of interspersed repeats accounts for at least 10% of the mammalian genome. Like other interspersed repeat DNA families in genomes of other organisms, L1 is dispersed and amplified throughout the genome by a series of duplicative transposition events. Due to the high copy number of L1 sequences in the genome, L1 is abundantly represented in the RNA population of most cells. However, most of the transcripts that contain L1 are the result of fortuitous transcription and are not intermediates in L1 retrotransposition. This high background of L1-containing transcripts, many of which are truncated and rearranged, makes it difficult to distinguish the transcript encoded by an active L1 element(s) [25]. ApoB mRNA was one of top candidates that were lower in E^+ group compared to the E^- fetal group. ApoB plays a central role in lipoprotein metabolism and exists in two isoforms in plasma, apoB-100 and apoB-48 [26]. High levels of apoB and LDL cholesterol have been associated with an increased risk for coronary heart disease [27]. An earlier study has shown that administration of TRF (100 mg/day) decreases serum apoB [28]. Tocopherol has been shown to inhibit protein kinase C (PKC) activity in cells [29]. PKC-regulated chloride channel was one of the genes that were suppressed in the E^+ group.

Taken together, these results provide evidence that compared to the adult brain, fetal brain is deficient in vitamin E and that fetal brain vitamin E status is tightly linked to the dietary vitamin E intake of the mother. Dietary tocotrienol is bioavailable to the brain. Gene expression patterns in response to dietary vitamin E suggest that vitamin E in pregnancy diet favorably influences the gene expression profile of the developing fetal brain.

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