Journal of Nutrition & Intermediary Metabolism 2 (2015) 2–7

Contents lists available at ScienceDirect

Journal of Nutrition & Intermediary Metabolism

journal homepage: http://www.jnimonline.com/

Effects of dietary supplementation with docosahexaenoic acid (DHA) on hippocampal gene expression in streptozotocin induced diabetic C57Bl/6 mice☆

Jency Thomas [a, c, d], Manohar Lal Garg [a, c, *, 1], Doug William Smith [b, c, 1]

[a] Nutraceuticals Research Group, University of Newcastle, Callaghan, NSW 2308, Australia
[b] Priority Research Centre for Translational Neuroscience and Mental Health, University of Newcastle, Callaghan, NSW 2308, Australia
[c] School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia
[d] Human Biosciences, Faculty of Health, La Trobe University, Victoria 3083, Australia

ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form
27 March 2015
Accepted 6 April 2015
Available online 27 May 2015

Keywords:
Diabetes
Hippocampus
DHA
Cognitive function
Synaptic plasticity
Inflammation

ABSTRACT

A body of evidence has accumulated indicating diabetes is associated with cognitive impairments. Effective strategies are therefore needed that will delay or prevent the onset of these diabetes-related deficits. In this regard, dietary modification with the naturally occurring compound, docosahexaenoic acid (DHA), holds significant promise as it has been shown to have anti-inflammatory, anti-oxidant, and anti-apoptotic properties. The hippocampus, a limbic structure involved in cognitive functions such as memory formation, is particularly vulnerable to the neurotoxic effects related to diabetes, and we have previously shown that streptozotocin-induced diabetes alters hippocampal gene expression, including genes involved in synaptic plasticity and neurogenesis. In the present study, we explored the effects of dietary supplementation with DHA on hippocampal gene expression in C57Bl/6 diabetic mice. Diabetes was established using streptozotocin (STZ) and once stable, the dietary intervention group received AIN93G diet supplemented with DHA (50 mg/kg/day) for 6 weeks. Microarray based genome-wide expression analysis was carried out on the hippocampus of DHA supplemented diabetic mice and confirmed by real time polymerase chain reaction (RT-qPCR). Genome-wide analysis identified 353 differentially expressed genes compared to non-supplemented diabetic mice. For example, six weeks of dietary DHA supplementation resulted in increased hippocampal expression of *Igf II* and *Sirt1* and decreased expression of *Tnf-α*, Il6, *Mapkapk2* and *ApoE*, compared to non-supplemented diabetic mice. Overall, DHA supplementation appears to alter hippocampal gene expression in a way that is consistent with it being neuroprotective in the context of the metabolic and inflammatory insults associated with diabetes.

Introduction

While a decline in cognitive function and memory is considered to be a normal consequence of ageing [1], disease conditions such as diabetes also lead to the development of cognitive dysfunction and memory impairments [2–5]. Both type I and II diabetes are associated with memory deficits, and the hippocampus is particularly vulnerable, with structural and functional alterations being caused by the disease processes [6–8]. For example, morphological changes, such as dendrite atrophy (reduced dendritic length and number of branch points), have been observed in experimental models of diabetes [9,10]. Diabetes also leads to abnormal neurotransmitter release in the hippocampus [11]. Lastly, neurogenesis, the processes through which new neurons are generated and integrated into existing circuits, and that is thought to be crucial for normal cognitive functions, is impaired in animal models of diabetes [12]. Indeed, impaired hippocampal synaptic plasticity and neurogenesis appear to be major factors in the cognitive deficits associated with diabetes [13]. While the underlying cellular mechanisms for these impairments remain to be fully elucidated, we have started to characterise these mechanisms and

☆ The present study was supported by a grant from the Priority Research Centre for Physical Activity and Nutrition, University of Newcastle, Australia.
* Corresponding author. School of Biomedical Sciences & Pharmacy, University of Newcastle, Callaghan, NSW 2308, Australia.
E-mail address: manohar.garg@newcastle.edu.au (M.L. Garg).
[1] Denotes co-senior authors.

http://dx.doi.org/10.1016/j.jnim.2015.04.001

previously reported diabetes causes alterations in the hippocampal expression of genes that are important for both synaptic plasticity and neurogenesis [14].

The restoration of normal hippocampal synaptic plasticity and neurogenesis is therefore an important goal for the prevention of, or recovery from, diabetes associated cognitive decline. Of the various substances that have been shown to promote hippocampal and overall cognitive function, docosahexaenoic acid (DHA) is showing considerable promise [15]. DHA is a long chain omega-3 polyunsaturated fatty acid (LnC-PUFA) and an integral component of neural membrane phospholipids. DHA influences membrane fluidity, modulates signal transduction and also receptor affinity, and thereby affects multiple brain functions [16]. Several epidemiological studies have shown a close relationship between DHA and cognition, with low plasma DHA levels associated with cognitive deficits in healthy elderly subjects and also in patients suffering from neurodegenerative disorders such as Alzheimer's disease (AD) [17–20]. Additionally, preclinical studies have shown that DHA enhances performance in learning and memory tasks in aged animals [21], and it also improves cerebral blood flow [22].

DHA is highly concentrated in the brain and has been shown to have anti-oxidant, anti-inflammatory, and anti-apoptotic properties [23]. Importantly, a high dietary intake of DHA leads to accumulation of DHA in brain membrane phospholipids [21]. The potential beneficial effects of DHA to cognitive function have been reported [24–27], however, there are no studies exploring the effects of DHA on hippocampal gene expression in diabetes. This type of study could provide insight into the underlying molecular mechanism by which DHA could confer its beneficial effects. In the present study we employ streptozotocin (STZ) induced diabetic animals to study the effect of dietary supplementation with DHA on hippocampal gene expression.

Materials and methods

Subjects

Seven week-old, male C57BL/6 mice (University of Newcastle; weighing 20–25 g upon arrival) were individually housed in a temperature and humidity controlled environment under a 12 h light/dark cycle, with food and water available *ad libitum*. All procedures were performed in strict accordance with protocols approved by University of Newcastle Animal Care and Ethics Committee, the New South Wales Animal Research Act and Regulations, and the Australian code of practice for care and use of animals for scientific purposes.

Methods and treatment

After one week of acclimatization, animals received daily injections of streptozotocin (STZ; Sigma; 50 mg/kg i.p.) freshly dissolved in 100 μl of 0.5 M sodium citrate buffer for five consecutive days as previously described [14]. Blood glucose levels were determined from saphenous vein blood using an Accu-Check Integra (Roche Diagnostics Mannheim, Germany). After three weeks of STZ administration, animals exhibiting blood glucose levels ≥15 mM were considered to be diabetic. Following confirmation of a diabetic state, animals were put on a semi-pure diet (AIN93G; Specialty Feeds Western Australia), which satisfies all nutritional requirements. For DHA supplementation (EPAX, 1050TG), the animals received 50 mg/kg/day of DHA mixed with AIN93G diet every day for a period of 6 weeks. Blood glucose levels and body weight were monitored weekly for six weeks. Food intake was monitored and recorded every week, and all animals had *ad libitum* access to drinking water.

Animal sacrifice and tissue harvesting

At the end of the six-week period of stable diabetes and dietary supplementation, animals were overdosed with pentabarbitone and the brains were rapidly removed, immediately cooled in ice-cold PBS and frozen in dry-ice chilled isopentane. Tissue was stored at −80 °C until required. Ten μm thick, coronal hippocampal sections were obtained by cryosection, mounted on nuclease-free glass microscope slides and stored at −80 °C until further use. Blood was drawn from saphenous vein and processed for gas chromatography to analyse erythrocyte fatty acid analysis (see Table 1).

Erythrocyte fatty acid analyses

The fatty acid composition of erythrocytes was determined according to modified Lepage and Roy (1986) method where acetyl chloride methylation procedure is appropriated. Fatty acid methyl esters were quantified using gas chromatography. The identity of each fatty acid peak was ascertained by comparison of the peak's retention time with the retention times of synthetic standards of known fatty acid composition (Nu Check Prep, Elysian, MN, USA). The relative amount of each fatty acid was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids. Fatty acid results are reported as percentage of total fatty acids.

RNA extraction

Sections were rinsed in ice-old PBS (DEPC treated) and then fixed in ice-cold ethanol (100%) for 3 min. Whole hippocampi were dissected from sections at approximately 250 μm intervals through the rostrocaudal extent of the hippocampus. Total RNA was extracted from hippocampal tissue pooled for each animal. Contaminating genomic DNA (gDNA) removed in solution by DNase-I digestion using Qiagen's RNeasy mini kit and DNase reagents, according to the manufacturer's instructions. The concentration of DNase treated RNA was determined by UV absorbance spectrophotometry (Thermo Scientific Nanodrop 1000).

Microarray profiling

Gene expression profiling was carried out by the Australian Genome Research Facility (AGRF, Australia). Approximately 200 ng of total RNA was prepared for hybridization to Illumina Mouse WG-6 v2.1 Expression microarrays. Expression data was first analysed using Illumina's Genome studio 2010.2 software and incorporated

Table 1
Fatty acid levels in the erythrocytes of DHA supplemented diabetic group and non-supplemented diabetic group.

Common name	Lipid name	Diabetic ($n = 10$)	DHA ($n = 10$)	*P* value*
Palmitic acid	C16:0	23.8 ± 0.9	32.9 ± 2.7	<0.0001
Palmitoleic acid	C16:1n-7	0.9 ± 0.3	1.6 ± 0.8	0.0366
Stearic acid	C18:0	12.1 ± 1.2	12.2 ± 1.8	0.846
Oleic acid	C18:1n-9	21.9 ± 1.9	20.6 ± 1.9	0.346
cis-vaccenic acid	C18:1n-7	2.4 ± 0.3	3 ± 0.5	0.531
Linoleic acid	C18:2n-6	8.4 ± 0.8	5.8 ± 0.3	0.0024
Arachidonic acid	C20:4n-6	21.3 ± 0.9	6.2 ± 0.9	<0.0001
Eicosapentaenoic acid	C20:5n-3	0.124 ± 0.02	4.4 ± 0.76	<0.001
Docosahexaenoic acid	C22:6n-3	5.8 ± 0.4	9 ± 1.7	0.0264
Lignoceric acid	C24:0	1.1 ± 0.1	1.7 ± 0.6	0.112
Nervonic acid	C24:1n-9	2.1 ± 0.2	1.6 ± 0.6	0.048

*P values were obtained using independent samples *t*-test for diabetic and DHA supplemented diabetic group.

normalisation by averaging. Fold-change in either direction of ≥1.2, average signal intensity of ≥100, and statistical significance as determined by ANOVA, were criteria used to identify genes of interest.

Reverse transcription and q RT-PCR

Reverse transcription

Reverse transcription was performed using Superscript III (Invitrogen, Australia), according to manufacturer's instructions. Briefly, 200 ng of total RNA, 1 μl oligo(dT_{18}) primer, 1 μl of 10 mM dNTP, and molecular grade water to 13 μl, were mixed and heated for 5 min at 65 °C for 5 min, the chilled on ice for 1 min. Next 4 μl 5× first strand buffer, 1 μl of 0.1m DTT, 1 μl RNaseOUT (40 U/μl) and 1 μl of superscript, were added and the mixture incubated for 60 min at 50 °C, followed by a 70 °C enzyme inactivation step for 15 min. Reverse transcription, without reverse transcriptase (RT−), was also performed to assess gDNA contamination.

Real-time qPCR

All gene transcript information was obtained from Ensembl (www.ensembl.org). qPCR primers (Table 4) were designed using NCBI's primer design tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). qPCR volumes were 12 μl, contained 1 ng cDNA, and were run in triplicate on an ABI 7500 real time PCR system (Applied Biosystems, USA). qPCR data was analysed using Applied Biosystems 7500 sequence Detection software (version 1.4). For each gene, all samples, including water controls, were run on a single qPCR plate. For each animal, the delta Ct (ΔCt, threshold cycle) was determined for the gene of interest relative to the average of two house-keeping genes, succinate dehydrogenase (SDH) and beta actin. For each gene, the ΔΔCt method was used to compare diabetic and diabetic plus DHA group [28].

Statistics

All statistical analyses were performed for qPCR were performed using PASW statistics software (version 18; SPSS Inc., Chicago, IL USA). Statistical significance was assessed using one-way analysis of variance (ANOVA). Results were considered statistically significant if the observed significance level was ≤0.05. As we have previously reported findings for the control and diabetic conditions [14], comparisons in the present study are between the diabetes plus DHA group and the previous diabetes group [14].

Results

Blood glucose and body weight

The diabetic plus DHA group showed an increase in blood glucose levels three weeks after STZ administration. All animals in the group that exhibited blood glucose levels ≥15 mM were considered diabetic. The average (±SEM) blood glucose levels in the diabetes plus DHA group was 18.7 ± 1.47 mM. There was not a significant difference in blood glucose levels between the diabetes and the diabetes plus DHA group ($P = 0.609$). Also, there was no significant difference in body weight between the diabetes and the DHA supplemented diabetic group ($P = 0.800$).

Erythrocyte fatty acid

In response to DHA supplementation, erythrocyte fatty acid analysis showed significant increase in DHA ($P = 0.0264$) and EPA ($P < 0.001$) in the DHA supplemented group. A significant reduction in arachidonic acid was seen in the DHA supplemented group as opposed to the non-supplemented diabetic group ($P < 0.0001$) (see Table 1).

Genome wide gene expression analysis

Using the criteria described above, 353 genes were differentially expressed in the hippocampus of the DHA supplemented diabetic group compared to the non-supplemented diabetic group. Twenty-one genes had increased expression and 332 (Supplementary table) had decreased expression. Genes that were significantly differentially expressed in the hippocampus of diabetic animals compared to non-diabetic controls, and that had this diabetes-induced change in expression either normalized or exacerbated by DHA, are shown in Table 2.

Pathway analysis

Pathways associated with diet induced gene expression changes

To garner a better understanding of diet-induced changes in hippocampal gene expression, and to assess potential biological function, the list of differentially expressed genes obtained from microarray analysis was subjected to pathways analysis using Database for Annotation, Visualization, and Integrated Discovery (DAVID) [29]. DAVID analyses gene lists to statistically determine whether there is enrichment for genes that belong to *a priori*-defined gene sets. This analysis found 5 significantly (<0.05) enriched pathways (see Table 3), using Kyoto Encyclopedia of Genes and Genomes (KEGG). The most significantly enriched pathways in the DHA supplemented group were the Mapk and Jak/Stat signalling pathways.

Confirmation by RT-qPCR

A subset of genes was chosen for RT-qPCR confirmation. Significant increases in *Igf II* (3.31 fold) and *Sirt1* (1.81fold) and decreases in expression of *ApoE* (−1.38 fold), and *Tnf-α* (−1.76 fold) were confirmed (Fig. 1).

Table 2
List of genes whose expression was affected by diabetes relative to non-diabetic controls, and that were either partly or wholly normalised or exacerbated, by DHA supplementation.

Gene names	Symbol	Fld change DHA/Diab
Apolipoprotein E	ApoE	↓1.2
Insulin like growth factor 2	Igf2	↑1.6
Sirtuin 1	Sirt1	↑1.5
Tumour necrosis factor- alpha inducing protein 1	Tnfaip1	↓1.6
Mitogen activated protein kinase activating protein kinase 2	Mapkapk2	↓1.6
Interleukin 1 beta	Il1b	↓1.8
Interleukin 20	Il20	↓1.5
Interleukin 6 signal transducer	Il6st	↓1.6
Phosphoinositide-3-kinase, regulatory subunit 2	Pi3kr2	↑1.6

Table 3
List of pathways identified by KEGG pathway analysis, showing those pathways that were enriched in the hippocampus of the DHA supplemented diabetic group, relative to the non-supplemented diabetic group.

Pathways	*P*-value*	Fold enrichment	Benjamani	Genes
MAPK signalling pathway	0.0011	4.1	0.084	Mapkapk2, Dusp9, fgf2, Grb2, Il1b, Mef2c, Ntrk1, Ptpn7, Max
Jak-STAT signalling pathway	0.0012	5.6	0.049	Csf2rb2, Grb2, Ifnb1, Il13ra2, Il20, Il6st, Stat3
Pathways in cancer	0.013	3	0.31	asp9, Fgf2, Grb2, Ntrk1, Ptgs2, Max, Stat3, Wnt5a
Alzheimer's disease	0.015	4	0.26	ApoE, Bad, Casp9, Il1, CxI, CxIV
Apoptosis	0.032	5.6	0.42	asp9, Csf2rb2, Il1b, Ntrk1

Table 4
List of qPCR primers.

Target gene	Forward (5′–3′)	Reverse (5′–3′)
Succinate dehydrogenase	GCAGAGCCTGTGCCCTGAGC	CCCACACGGAACACTGCAGCA
Beta actin	TGTGCTGCTCACCGA	TGGCTACGTACATGG
Sirt1	GCAGATTAGTAAGCGGCTTGAGGGT	TGGCATGTGCCACTGTCACTGTT
Apolipoprotein E	GCTGCAGAGCTCCCAAGTCACAC	TTCCTCCAGCTCCTTTTTGTAAGCC
Igf2	CGCCGGCTTCCAGGTACCAAT	ACACGGCGAAGGCCAAAGAGATG
Tnf-α	CAGAGGTGTGCTGCACCTCGAT	CGGGCCTCTGGGAATTCCACCT

Discussion

The current study shows that dietary supplementation with DHA can alter the molecular profile of the hippocampus in diabetic mice in a way that is consistent with DHA being neuroprotective. Previous studies have shown that dietary supplementation with DHA has beneficial effects on cognition and memory and it also promotes neuronal survival [30,31]. In the present study, mice that were already diabetic had their diet supplemented with DHA for six weeks. This dietary intervention resulted in an increase in hippocampal insulin like growth factor II (*Igf II*) and *Sirt1* expression and a decrease in hippocampal expression of pro-inflammatory *Tnf-α*, *Il6* and *ApoE* lipoprotein, relative to non-supplemented diabetic mice.

Igf II is a mitogenic polypeptide that is relatively abundant in the hippocampus and is thought to be involved in cognitive functions. Indeed, hippocampal Igf II levels decline with ageing, in parallel with age-related cognitive decline [32–35]. Schmeisser et al., 2012 demonstrated the presence of Igf II and Igf II receptors in synaptic elements of hippocampal neurons, which is consistent with the notion that Igf II has a role in synaptic plasticity and cognitive functions [36]. Wuarin et al., 1996, showed that *Igf II* expression was decreased in the brains of both insulin dependent and independent diabetic animals [37] and consistent with this, we found by qPCR analysis that STZ-induced diabetes caused a reduction in *Igf II* ($P = 0.025$). An increase in *Igf II* expression in the DHA supplemented diabetic group is particularly encouraging as Igf II has been shown to activate Igf I and Igf II receptors to mediate memory enhancement [34]. Additionally, Bracko et al., 2012 and Agis-Balboa et al., 2011 have demonstrated that Igf II is a regulator of adult hippocampal neurogenesis [38,39]. The neuroprotective effects of the Igfs are thought to be due to the involvement of PI3k/Akt signalling pathways [40]. In this regard, our DHA supplemented diabetic group showed a 1.6 fold increase in expression of Pi3kr2, also known as P85-β, a regulatory subunit of PI3k [41], which has been shown to be crucial for PI3k-dependent memory consolidation processes in the hippocampus [42].

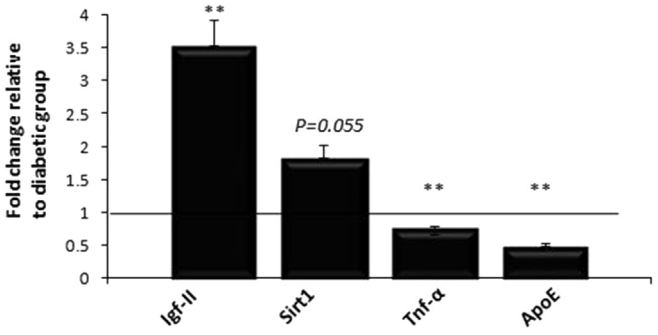

Fig. 1. Confirmation of gene expression by RT-qPCR. Increased Igf-II and Sirt1, and decreased Tnf-α and ApoE expression in the hippocampus of DHA (50 mg/kg/day) supplemented STZ-induced diabetic C57BL/6 mice. Once diabetes was established and stable, mice were fed either a control diet or one supplemented with DHA. Data are presented as means ± SEM, relative to control diet diabetic mice. $^{**}P \leq 0.05$ ($n = 6$/group).

The most significantly enriched pathways, as determined by KEGG analysis, in the DHA supplemented group, were the Mapk and JAK/STAT signalling pathways. These pathways are involved in pro-inflammatory responses and enhance apoptosis in several neurodegenerative conditions [24]. Notably, microarray analysis indicated a number of the pro-inflammatory genes in these pathways (*Mapkapk2*, *Il1b*, *Il20*, *Il13ra2*, *& Il6st*) were significantly down regulated in the hippocampus of DHA supplemented diabetic mice. Previously, it was shown that Mapkapk2 deficiency reduced neurodegeneration and neuro-inflammation in a Parkinson's disease model [43], further demonstrating a role for Mapkapk2 in neuroinflammation. We also found a decrease in *Tnf-α* expression in the DHA supplemented diabetic group. *Tnf-α* is another pro-inflammatory factor and can directly trigger the death of neurons by inhibiting Igf survival responses [44]. There was also a trend for a decrease in expression of the pro-inflammatory cytokine, *Il6* (*data not shown*). Taken together, these data indicate DHA supplementation induces an anti-inflammatory effect in the context of diabetes. Increased expression of pro-inflammatory mechanisms in STZ-induced diabetes has been previously reported [45,46] and our findings support a case for DHA in limiting the potential damage cause by inflammation in this type of metabolic insult.

Another gene of interest that showed a significant increase in expression in the DHA supplemented group was *Sirt1* (silent information regulator 1). *Sirt1* is a member of the sirtuin family that encodes a class III histone deacetylase [47]. *Sirt1* is expressed in hippocampal neurons and has been shown to improve learning and memory by activating the brain derived neurotropic factor (BDNF) gene [48–50]. Sirt1 has been previously shown to be decreased in the brains of old rodents [51], indicating a role for Sirt1 in age

related cognitive decline. Increased expression of Sirt1 in the hippocampus of DHA supplemented diabetic group as seen in the present study may be one of the mechanisms by which DHA could lead to cognitive improvement in diabetic condition. Both Igf and Sirt1 are involved in hippocampal neurogenesis and synaptogenesis, although the mechanisms that underlie this involvement are complex and require further clarification [48,51]. Notwithstanding this lack of mechanistic detail, our finding that DHA supplementation increased the hippocampal expression of both *Igf and Sirt1* in diabetic but not in normal mice [27], further underscores its potential as a therapeutic agent.

Lastly, we also found a decrease in *ApoE* expression in the DHA supplemented, relative to non-supplemented, diabetic group. Previously, we reported an increase in *ApoE* expression in the hippocampus of STZ induced diabetic mice [14] and an increase in *ApoE* expression has been suggested to be indicative of ongoing neuronal dysfunction [52]. In the CNS, ApoE is mainly produced by astrocytes and transports cholesterol to neurons via ApoE receptors, a process that is crucial for synaptogenesis [53]. The decrease in *ApoE* expression could be of clinical importance as *ApoE* is involved in key immune responses, including the production of pro-inflammatory cytokines such as *Il1b*. Interestingly, *Il1b* was one of the down-regulated genes (−1.8 fold) in the DHA supplemented diabetic group. Liu et al. (2011) demonstrated that neurons exposed to *Il1b* express elevated levels of ApoE, a finding that is similar to that observed in neurodegenerative diseases such as AD [54]. Therefore, a significant decrease in *ApoE* expression in the hippocampus of the DHA supplemented diabetic group is yet another indicator of the potential beneficial effects of DHA and warrants further investigation.

Overall, the present study helps to elucidate the molecular changes in the hippocampus of diabetic mice following dietary supplementation with DHA. Our findings provide an important framework for future studies to further investigate the effects of nutritional intervention on the molecular mechanisms that cause diabetes related cognitive dysfunction. Furthermore, our findings underscore the potential of naturally occurring compounds such as DHA.

Authors' contribution

All authors participated in the conception and study design. JT and DWS carried out the animal work. JT performed qRT-PCR and data analysis. JT, DWS and MLG prepared the manuscript. All the authors contributed to the revisions and subsequent drafts and reviewed the final version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jnim.2015.04.001.

References

[1] Yurko-Mauro K, McCarthy D, Rom D, Nelson EB, Ryan AS, Blackwell A, et al. Beneficial effects of docosahexaenoic acid on cognition in age-related cognitive decline. Alzheimers Dement 2010;6(6):456–64.
[2] Revsin Y, Rekers NV, Louwe MC, Saravia FE, De Nicola AF, de Kloet ER, et al. Glucocorticoid receptor blockade normalizes hippocampal alterations and cognitive impairment in streptozotocin-induced type 1 diabetes mice. Neuropsychopharmacology 2009;34(3):747–58.
[3] Beauquis J, Roig P, Homo-Delarche F, De Nicola A, Saravia F. Reduced hippocampal neurogenesis and number of hilar neurones in streptozotocin-induced diabetic mice: reversion by antidepressant treatment. Eur J Neurosci 2006;23(6):1539–46.
[4] Li ZG, Zhang W, Grunberger G, Sima AA. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res 2002;946(2):221–31.
[5] Huber JD. Diabetes, cognitive function, and the blood-brain barrier. Curr Pharm Des 2008;14(16):1594–600.
[6] Chen RH, Jiang XZ, Zhao XH, Qin YL, Gu Z, Gu PL, et al. Risk factors of mild cognitive impairment in middle aged patients with type 2 diabetes: a cross-section study. Ann Endocrinol Paris 2012;73(3):208–12.
[7] Alvarez EO, Beauquis J, Revsin Y, Banzan AM, Roig P, De Nicola AF, et al. Cognitive dysfunction and hippocampal changes in experimental type 1 diabetes. Behav Brain Res 2009;198(1):224–30.
[8] Trudeau F, Gagnon S, Massicotte G. Hippocampal synaptic plasticity and glutamate receptor regulation: influences of diabetes mellitus. Eur J Pharmacol 2004;490(1–3):177–86.
[9] Magarinos AM, McEwen BS. Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. Proc Natl Acad Sci U S A 2000;97(20):11056–61.
[10] Reagan LP, Magarinos AM, McEwen BS. Neurological changes induced by stress in streptozotocin diabetic rats. Ann N Y Acad Sci 1999;893:126–37.
[11] Yamato T, Misumi Y, Yamasaki S, Kino M, Aomine M. Diabetes mellitus decreases hippocampal release of neurotransmitters: an in vivo microdialysis study of awake, freely moving rats. Diabetes Nutr Metab 2004;17(3):128–36.
[12] Beauquis J, Homo-Delarche F, Revsin Y, De Nicola AF, Saravia F. Brain alterations in autoimmune and pharmacological models of diabetes mellitus: focus on hypothalamic-pituitary-adrenocortical axis disturbances. Neuroimmunomodulation 2008;15(1):61–7.
[13] Stranahan AM, Arumugam TV, Cutler RG, Lee K, Egan JM, Mattson MP. Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. Nat Neurosci 2008;11(3):309–17.
[14] Thomas J, Garg ML, Smith DW. Altered expression of histone and synaptic plasticity associated genes in the hippocampus of streptozotocin-induced diabetic mice. Metab Brain Dis 2013;28(4):613–8.
[15] He C, Qu X, Cui L, Wang J, Kang JX. Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. Proc Natl Acad Sci U S A 2009;106(27):11370–5.
[16] Salem Jr N, Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids 2001;36(9):945–59.
[17] Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes – the EVA Study. Am J Clin Nutr 2003;77(4):803–8.
[18] Beydoun MA, Kaufman JS, Satia JA, Rosamond W, Folsom AR. Plasma n-3 fatty acids and the risk of cognitive decline in older adults: the Atherosclerosis Risk in Communities Study. Am J Clin Nutr 2007;85(4):1103–11.
[19] Conquer JA, Tierney MC, Zecevic J, Bettger WJ, Fisher RH. Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment. Lipids 2000;35(12):1305–12.
[20] Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, et al. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol 2006;63(11):1545–50.
[21] Petursdottir AL, Farr SA, Morley JE, Banks WA, Skuladottir GV. Effect of dietary n-3 polyunsaturated fatty acids on brain lipid fatty acid composition, learning ability, and memory of senescence-accelerated mouse. J Gerontol A Biol Sci Med Sci 2008;63(11):1153–60.
[22] Tsukada H, Kakiuchi T, Fukumoto D, Nishiyama S, Koga K. Docosahexaenoic acid (DHA) improves the age-related impairment of the coupling mechanism between neuronal activation and functional cerebral blood flow response: a PET study in conscious monkeys. Brain Res 2000;862(1–2):180–6.
[23] Crupi R, Marino A, Cuzzocrea S. n-3 Fatty acids: role in neurogenesis and neuroplasticity. Curr Med Chem 2013.
[24] Feng Z, Zou X, Jia H, Li X, Zhu Z, Liu X, et al. Maternal docosahexaenoic acid feeding protects against impairment of learning and memory and oxidative stress in prenatally stressed rats: possible role of neuronal mitochondria metabolism. Antioxid Redox Signal 2012;16(3):275–89.
[25] Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, et al. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest 2005;115(10):2774–83.
[26] Pongrac JL, Slack PJ, Innis SM. Dietary polyunsaturated fat that is low in (n-3) and high in (n-6) fatty acids alters the SNARE protein complex and nitrosylation in rat hippocampus. J Nutr 2007;137(8):1852–6.
[27] Thomas J, Garg ML, Smith DW. Dietary supplementation with resveratrol and/or docosahexaenoic acid alters hippocampal gene expression in adult C57Bl/6 mice. J Nutr Biochem 2013;24(10):1735–40.
[28] Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008;3(6):1101–8.
[29] Dennis Jr G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 2003;4(5):P3.
[30] Akbar M, Calderon F, Wen Z, Kim HY. Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. Proc Natl Acad Sci U S A 2005;102(31):10858–63.
[31] Labrousse VF, Nadjar A, Joffre C, Costes L, Aubert A, Grégoire S, et al. Short-term long chain omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice. PLoS ONE 2012;7(5):e36861.

[32] Kitraki E, Bozas E, Philippidis H, Stylianopoulou F. Aging-related changes in IGF-II and c-fos gene expression in the rat brain. Int J Dev Neurosci 1993;11(1):1–9.
[33] Russo VC, Gluckman PD, Feldman EL, Werther GA. The insulin-like growth factor system and its pleiotropic functions in brain. Endocr Rev 2005;26(7):916–43.
[34] Chen DY, Stern SA, Garcia-Osta A, Saunier-Rebori B, Pollonini G, Bambah-Mukku D, et al. A critical role for IGF-II in memory consolidation and enhancement. Nature 2011;469(7331):491–7.
[35] Kar S, Seto D, Doré S, Hanisch U, Quirion R. Insulin-like growth factors-I and -II differentially regulate endogenous acetylcholine release from the rat hippocampal formation. Proc Natl Acad Sci U S A 1997;94(25):14054–9.
[36] Schmeisser MJ, Baumann B, Johannsen S, Vindedal GF, Jensen V, Hvalby ØC, et al. IkappaB kinase/nuclear factor kappaB-dependent insulin-like growth factor 2 (Igf2) expression regulates synapse formation and spine maturation via Igf2 receptor signaling. J Neurosci 2012;32(16):5688–703.
[37] Wuarin L, Namdev R, Burns JG, Fei ZJ, Ishii DN. Brain insulin-like growth factor-II mRNA content is reduced in insulin-dependent and non-insulin-dependent diabetes mellitus. J Neurochem 1996;67(2):742–51.
[38] Agis-Balboa RC, Arcos-Diaz D, Wittnam J, Govindarajan N, Blom K, Burkhardt S, et al. A hippocampal insulin-growth factor 2 pathway regulates the extinction of fear memories. Embo J 2011;30(19):4071–83.
[39] Bracko O, Singer T, Aigner S, Knobloch M, Winner B, Ray J, et al. Gene expression profiling of neural stem cells and their neuronal progeny reveals IGF2 as a regulator of adult hippocampal neurogenesis. J Neurosci 2011;32(10):3376–87.
[40] Zheng WH, Kar S, Quirion R. Insulin-like growth factor-1-induced phosphorylation of transcription factor FKHRL1 is mediated by phosphatidylinositol 3-kinase/Akt kinase and role of this pathway in insulin-like growth factor-1-induced survival of cultured hippocampal neurons. Mol Pharmacol 2002;62(2):225–33.
[41] Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet 2006;7(8):606–19.
[42] Kim JI, Lee HR, Sim SE, Baek J, Yu NK, Choi JH, et al. PI3Kgamma is required for NMDA receptor-dependent long-term depression and behavioral flexibility. Nat Neurosci 2011;14(11):1447–54.
[43] Thomas T, Timmer M, Cesnulevicius K, Hitti E, Kotlyarov A, Gaestel M. MAPKAP kinase 2-deficiency prevents neurons from cell death by reducing neuroinflammation – relevance in a mouse model of Parkinson's disease. J Neurochem 2008;105(5):2039–52.
[44] Venters HD, Tang Q, Liu Q, VanHoy RW, Dantzer R, Kelley KW. A new mechanism of neurodegeneration: a proinflammatory cytokine inhibits receptor signaling by a survival peptide. Proc Natl Acad Sci U S A 1999;96(17):9879–84.
[45] Kumar B, Gupta SK, Srinivasan BP, Nag TC, Srivastava S, Saxena R, et al. Hesperetin rescues retinal oxidative stress, neuroinflammation and apoptosis in diabetic rats. Microvasc Res 2013;87:65–74.
[46] Cai D. Neuroinflammation in overnutrition-induced diseases. Vitam Horm 2013;91:195–218.
[47] Tang BL. Sirt1's systemic protective roles and its promise as a target in anti-aging medicine. Transl Res 2011;157(5):276–84.
[48] Pfister JA, Ma C, Morrison BE, D'Mello SR. Opposing effects of sirtuins on neuronal survival: SIRT1-mediated neuroprotection is independent of its deacetylase activity. PLoS One 2008;3(12):e4090.
[49] Kim D, Nguyen MD, Dobbin MM, Fischer A, Sananbenesi F, Rodgers JT, et al. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. Embo J 2007;26(13):3169–79.
[50] Gao J, Wang WY, Mao YW, Gräff J, Guan JS, Pan L, et al. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 2010;466(7310):1105–9.
[51] Koltai E, Zhao Z, Lacza Z, Cselenyak A, Vacz G, Nyakas C, et al. Combined exercise and insulin-like growth factor-1 supplementation induces neurogenesis in old rats, but do not attenuate age-associated DNA damage. Rejuvenation Res 2011;14(6):585–96.
[52] Revsin Y, Saravia F, Roig P, Lima A, de Kloet ER, Homo-Delarche F, et al. Neuronal and astroglial alterations in the hippocampus of a mouse model for type 1 diabetes. Brain Res 2005;1038(1):22–31.
[53] Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 2013.
[54] Liu L, Aboud O, Jones RA, Mrak RE, Griffin WS, Barger SW. Apolipoprotein E expression is elevated by interleukin 1 and other interleukin 1-induced factors. J Neuroinflammation 2011;8:175.