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Review Article

THE ROLE OF OMEGA-3 POLYUNSATURATED FATTY ACIDS ON BRAIN COGNITIVE FUNCTION - REVIEW OF STUDIES ON LABORATORY ANIMALS

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OVERVIEW

Fatty acids are essential components of the diet and sources of food energy. A study carried out by Burr and Burr, (1929) first acknowledged that specific components of fatty acids may be necessary for the proper growth and development of animals and humans. They proposed that clinical signs of essential fatty acid (EFA) deficiency was correlated with cardiovascular disease and compromised immune system. In mammals, the fatty acids have been recognized as important nutrition factors for brain cognitive performance. Nutrient supplementation such as omega-3 (ω-3) fatty acids are widely accepted to improve diets, and contribute to the maintenance of good health, especially on the cardiovascular system and other aspects of health, such as cognition throughout life. Fish oil from tuna and salmon are the richest source of docosahexaenoic acid (DHA) that affects the cognitive function of the brain. This could indicate why fish has been called "brain food" and why DHA deficiencies can have a life-long impact on intelligence. Thus, knowledge of health benefits fatty acid as a dietary trend to increase good health status. In fact, world health organizations such as the UK Food Standards Agency and the Japan Society for Lipid Nutrition, advise that more people should aim to increase their intake of ω-3 fatty acid supplementation. As these fatty acids have an essential role during brain development, the first section of this review examines the importance of ω-3 fatty acid in relation to the metabolism. This will be followed by a review of the role of fatty acids on spatial recognition memory such as neural membrane function, cholinergic neurotransmitter and animal behavior studies. Experimental works in mice and rats have shown that ω-3 fatty acid supplementation can increase levels of synaptic vesicles, neuron dendrites and cholinergic neurotransmitters. The mechanism physiological of brain gene expression and cognitive function by ω-3 fatty acid also will be examines in this section. Thus, this review is focused on dietary ω-3 fatty acid supplementation on brain cognitive function and the mechanism interaction between ω-3 fatty acid and genetic functions on laboratory animals.

Fatty acids

Fat is a substance containing one or more fatty acids bound to a glycerol backbone (Rudin and Felix, 1996; for review see Sprecher, 2000). Fats play major roles in the metabolic, storage and protective functions of the mammalian body (Gur and Harwood, 1991). Practically, fatty acids can be from four to twenty-eight carbons in the chain, with can be classified as short-, medium-, or long-chained. In addition, fatty acids can also be classified as saturated fatty acid (SFA) and unsaturated fatty acid (UFA) (Rudin and Felix, 1996). In SFA, the carbons in the chain are completely saturated with hydrogen atoms. The results are a dense and solid fat such as the white fat in beef and lamb produced butter that does not melt at room temperature (Rudin and Felix, 1996). However with UFAs, especially polyunsaturated fatty acids, the carbons carry less hydrogen.

Polyunsaturated fatty acids (PUFAs) are essential for normal growth and development. The PUFAs are classified according to the position of the first double bond from the methyl terminal end. The first double bond in ω-3 is found at the third carbon atom from the methyl terminal, whereas in ω-6 the first double bond is located after the sixth carbon atom from the methyl terminal. The precursors of two families of PUFAs namely, linoleic acid (LA, ω-6) and α-linolenic acid (ALA, ω-3) are termed essential because they are cannot be produced by the animal or human body and must be supplied from the diet (Birberg-Thornberg *et al*., 2006). Sources of ω-6 PUFAs and LA are found mainly in vegetable products such as soybean, corn, nut, and sunflower oils (Bouziane *et al*., 1992; Madani *et al*., 1998). However, sources of ω-3 fatty acids are based on fish (menhaden, mackerel, herring, and salmon) and vegetable (rapeseed, soybean and nut) oils (Aid *et al*., 2005). However, the ALA is also found in the chloroplast of green leafy vegetables, such as spinach, seeds of flax and linseed (Kitessa *et al*., 2003).

A significant proportion of the fatty acids are present as PUFA derivatives of the two parent essential fatty acids, ALA and LA. The precursor essential fatty acids ALA and LA are metabolized by a process of desaturation and chain elongation. These precursors undergo sequential desaturation through the addition of double bonds and elongation by addition of carbon atoms. The DHA is synthesized from ALA by the addition of a double bond by a Δ6-desaturase to form stearidonic acid (SDA, C18:4n-3). The elongation of SDA forms eicosatetraenoic acid (ETA, C20:4n-3) and the addition of another double bond by a Δ5-desaturase produces eicosapentaenoic (EPA, C20:5n-3). The elongation of EPA forms docosapentaenoic (DPA, C22:5n-3), and the final addition of a double bond produces DHA (for review see Sprecher *et al*., 1995). The long chain ω-6 fatty acid synthesis such as the elongation of LA to DPA

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occurs via the same alternating desaturation and elongation steps.

Role of ω-3 fatty acids on spatial recognition memory

ω-3 fatty acids and neural membrane function

ω-3 fatty acids are the major structural components of membrane phospholipids (Bertrand *et al*., 2006). They influence membrane fluidity and ion transport across cell membranes (Ehringer *et al*., 1990). Among the long chain fatty acids, DHA is important structural components of the highly lipid biomembrane of neuron cell (for review, see Lauritzen *et al*., 2001). Previous studies report that DHA deficiency caused a reduction in the size of neurons of the brain region in the hippocampus (Ahmad *et al*., 2002). These authors showed that neuron size in the hippocampus, hypothalamus and parietal cortex are decreased in rats which are fed a DHA deficient diet containing 1.9gm/100gm safflower oil compared to those fed on a DHA adequate diet (Flaxseed oil, 0.48gm/100gm). The authors also reported that the brains of these rats exhibited a nearly 90% decrease of DHA. Thus, these results indicate that membrane neuron cell depends on DHA content for giving an optimal function in neurons such as signal transduction and synaptic activity.

Further evidence that ω-3 fatty acids promotes neurite growth in hippocampal neurons comes from the previous works reporting that DHA supplementation of about 2.6% in culture increased the population of neurons with longer neurite length per neuron and with a higher number of branches (Calderon and Kim, 2004). The author suggested that rats containing a lower level of DHA (about 0.1%) showed decreased neurite length, branches and neuron population, and consequently impaired their cognitive performance. This was supported by previous studies reporting that the DHA promotes neurite growth and survival in hippocampal neurons (Cao *et al*., 2005). These findings suggest that an ω-3 fatty acid family, especially DHA, selectively promotes the development of hippocampal neurons, which in turn affects the number and quality of synaptic connections during brain function.

Another approach valuable for the evaluation of the role of ω-3 fatty acids comes from the previous works reporting that an ω-3 fatty acid sufficient diet can promote structural changes in hippocampal neurons (Yoshida *et al*., 1997). The studies explained that rats with an ω-3 fatty acid deficient diet containing safflower oil show a 30% decreased density of synaptic vesicles in the terminal of the brain region compared with those in the DHA group (perilla oil). These results suggest that an ω-3 fatty acid deficient diet decrease lead to synaptic vesicle functions such as synthesis movement to releasable sites and fusion with synaptic membrane in the hippocampal region. Additionally, ω-3 fatty acid supplementation increased the number of synaptic vesicles (Weisinger *et al*., 1995) and improved fluidity of the synaptic membrane which improved interneuron communication and signal transduction (Ahmad *et al*., 2002; Calderon and Kim, 2004; Yoshida *et al*., 1997). Functionally, the fluidity of neuronal membranes affects the signal processing properties of neurons and can improve neural performance (Ehringer *et al*., 1990). Overall, it can be suggested that ω-3 fatty acid is essential in normal neurogenesis and synaptogenesis, and is also linked to improvement in learning and memory function.

ω-3 fatty acids and cholinergic neurotransmitter

Acetylcholine (ACh) is a neurotransmitter which is found in both the peripheral nervous system and central nervous system (CNS) in many organisms (Mathew *et al*., 2007). This neurotransmitter is released from the brain neurons to extracellular fluids and plays important roles in various biological processes such as cognitive functioning, memory and emotion (Zhang *et al*., 2002). In addition, central cholinergic activity facilitates human and animal cognitive function (Harmon and Wellman, 2003). A disturbance in the central cholinergic systems such as decreased ACh levels are partly responsible for the decline in cognitive functions in Alzheimer's patients (Bennett *et al*., 2007) and aging humans (Leung *et al*., 2003). Previous studies report that cholinergic activity facilitates long-term potentiation in various areas of the brain such as cerebral regions and cerebrospinal fluid levels of choline and acetylcholine (Dash *et al*., 2007). Thus, this could indicate that high level of cholinergic neurotransmitter such ACh is important for the improvement cognitive and memory functions.

It is known that ω-3 fatty acids can modulate the ACh in the brain by diet with ω-3 supplementation in rats (for review, see Young and Conquer, 2005). Previous studies report that an increase ACh cerebral levels following administration of dietary 5% DHA are correlated with an improvement performance in passive avoidance tasks in a rats (Minami *et al*., 1997). This theory is supported by the finding that ω-3 fatty acid sufficiency from tuna oil acts to enhance the stimulated synaptic release process of ACh in the hippocampus, which consequently contributes to the improvement of learning and memory performance in rats (Aid *et al*., 2005; Aid *et al*., 2003). This could indicate that ω-3 fatty acid plays a key role in ACh function via changes in the brain phospholipids composition and may consequently improve learning and memory function.

A previous study reported that the behavioral and cognitive changes in rats induced by ω-3 fatty acid deficiency could be due to changes in cholinergic neurotransmitters (Delion *et al*., 1994). Similarly, cholinergic neurotransmission in the hippocampus is specifically affected by a diet-induced lack of neuronal ω-3 fatty acid (Aid *et al*., 2003). Furthermore, there are functional interactions between cholinergic and monoaminergic systems that are altered by an ω-3 fatty acid

deficient diet (Delion *et al*., 1994). Both systems contribute to the impairment of hippocampal function and induce behavior performance disturbances in rats. Thus, the hippocampal cholinergic system plays a major role in the regulation of cognitive functions and its modification might contribute to the cognitive and behavioral disturbances that occur in ω-3 fatty acid deficient diet.

ω-3 fatty acids and cognitive functions

A diet with ω-3 fatty acid especially DHA maintains a fluid synaptic membrane and consequently improves the ability of learning and memory function in mammals (Suzuki *et al*., 1998). Previous studies have shown that ω-3 fatty acid deficient mice demonstrated impaired learning in the memory version of the Barnes circular maze as they spent more time and made more errors in search of an escape tunnel (Fedorova *et al*., 2007). In the brain's fatty acid profiles, this study found a 51% loss of total brain DHA in mice with an ω-3 fatty acid deficient diet compared to ω-3 fatty acid sufficient mice. Similarly, previous studies report that the level of brain DHA decreased about 50% in mice on an ω-3 fatty acid deficient diet compared to control group on an ω-3 fatty acid sufficient diet (Carrie *et al*., 1999). These studies suggest that the ω-3 fatty acid deficient diet significantly decreased learning performance and retinal DHA level in adult mice. In a follow up study, the authors confirmed that an ω-3 fatty acid deficient di*et al*tered fatty acid composition in brain regions and significantly reduced spatial learning as well as mice behavior (Carrie *et al*., 2000).

Further evidence that ω-3 fatty acid is required for cognitive function comes from the previous work reporting that ω-3 fatty acid deficient diet significantly decreased learning performance in adult mice (Umezawa *et al*., 1999). It was observed that mice which were fed on ω-3 fatty acid deficient diet consisting of 250.3g/100g safflower oil had significantly reduced learning performance compared with mice with ω-3 fatty acid sufficient diet (Perilla oil; 0.24g/100g). Deficiency of ω-3 fatty acid in the di*et al*so leads to reduced brain DHA levels in rats (Moriguchi and Salem, 2003). The reduced levels of DHA lead to a loss in brain function as reflected in poorer spatial task performance. However, the spatial task performance of DHA deficient rats can be normalized after dietary ω-3 fatty acid supplementation for 13 weeks to restore brain DHA. Thus, this could indicate that learning and memory performance are correlated with the brain level of ω-3 fatty acid especially DHA.

Many studies have shown that ω-3 fatty acids from fish oil are essential for normal neurological development associated in learning and memory function (Carrie *et al*., 2000; Chung *et al*., 2008; Joshi *et al*., 2004). One example of such evidence comes from previous work reporting that a fish oil diet from sardine oil (100g/kg) induced a significant increase in exploratory activity and learning ability in young mice (Carrie *et al*., 2000). A similar effect suggested that adults mice which were fed on the sardine oil diet for a long period maintain higher levels of DHA brain phospholipids and improved learning ability (Suzuki *et al*., 1998). This was in line with the reports that fish oil (70g/kg; cod liver oil) supplementation during pregnancy improved cognitive performance in dams and their offspring (Chung *et al*., 2008; Joshi *et al*., 2004). In addition, fish oil supplements might reduce the risk of memory loss or Alzheimer disease in human populations (Cole *et al*., 2005). Overall, it is clear that dietary fish oil is important in neurological development associated with an improvement in brain cognitive function.

ω-3 fatty acids and animal behavior task

Previous studies have shown that there are several maze tasks that assess cognitive function including the Morris water maze (MWM), Y-maze and elevated plus maze (Abumrad *et al*., 2005; Astur *et al*., 2004). Dietary ω-3 fatty acid deficiency over three generations disrupted learning and memory performance in the MWM task by adult rats (Moriguchi and Salem, 2003). In the case of the MWM task, the ω-3 fatty acid deficient group showed longer escape latency and delayed acquisition of this task compared with the ω-3 fatty acid sufficient group. Previous studies report that rats with DHA (1%) and LNA (3.1%) dietary supplementation have a shorter escape latency in the MWM task compared to the rats in DPA (1%) and LA (1%) dietary supplementation (Lim *et al*., 2005; Lim *et al*., 2005). However, dietary fish oil induced a significant increase in exploratory and locomotor activity in the MWM test in young mice (Carrie *et al*., 2000).

Another approach valuable for evaluation of the role of ω-3 fatty acid in neuronal and cognitive function comes from the works reported previously (Bluthe, 2005). This study demonstrated that mice were fed ω-3 fatty acid deficient diet containing peanut oil and the control-lipid diet containing a mixture of peanut and rapeseed oil (1200 mg LA and 300mg ALA per 100g diet). The spatial task performance of these animals was then compared using a two-trial recognition task in the Y-maze. The outcome of that study indicated that during the acquisition phase, the activity (number of visits/2-open arms) was the same whatever the dietary treatment administered. However, during the restitution phase, 45 min after the end of the acquisition phase or when the three arms were all open for exploration, animals in ω-3 fatty acid deficient group were less able to recognize the new arm compared to the mice fed the control-lipid diet. Based on these findings, it is quite evident that ω-3 fatty acid family (especially ALA) provided in the diet plays a key role in cognitive functions and is required for optimal brain function.

In the elevated plus maze, a test of anxiety, the time spent on open arms of the maze was significantly lower in ω-3 fatty acid deficient mice compared to the sufficient mice which received rapeseed oil 30g/kg and peanut oil 30g/kg (Carrie *et al*., 2000). Similarly, the time spent in open arms as well as the frequency of entry into the open arms tended

to be higher in the ω-3 fatty acid sufficient mice compared to deficient mice in the elevated plus maze of anxiety protocol (Nakashima *et al*., 1993). The findings of increased anxiety in ω-3 fatty acid deficient animals are supported by previous studies which also showed that ω-3 fatty acid deficient rats spent less time in the open arms compared to the sufficient group, signaling an anxiogenic response. However, after one week of supplementation with ω-3 fatty acid, the rats demonstrated a significant improvement in terms of the number of entries into the open arms. This condition may explain the improved behavior performance of anxiety protocol in the elevated plus maze as a result of the ω-3 fatty acid sufficient diet.

Effect of ω-3 fatty acids on brain gene expression

Fatty acid regulation of gene expression occurs in unicellular and complex organisms. Fatty acid also plays a role in controlling gene expression in a variety of tissues such as nerves and brain tissues (Barcelo-Coblijn *et al*., 2003). It has become evident that ω-3 fatty acid can also act as signaling molecules involved in regulating gene expression, eicosanoid synthesis and membrane structure (Duplus *et al*., 2000). Previous studies have report that several genes are activated by dietary ω-3 fatty acid and some gene products have beneficial effect on brain functions such as learning and memory (Kitajka *et al*., 2002; Kitajka *et al*., 2004; Puskas *et al*., 2003). Further to this, DNA microarray technology is a good approach for identifying changes in transcription of multiple genes in certain brain regions such as hippocampus (Puskas *et al*., 2003) and dentate gyrus (Burger *et al*., 2007).

As has been reported previously, 55 genes were detected as overexpressed and 47 were suppressed in rats fed ω-3 fatty acid supplementation (Kitajka *et al*., 2004). These finding shows that the expression of genes can be altered by DHA supplementation, using cDNA microarray analysis. Several genes such as transthyretin participating in signal transduction processes were overexpressed in rat brains receiving a DHA-enriched diet for one month (Barcelo-Coblijn *et al*., 2003). Similarly, transthyretin gene was expressed by fish oil supplementation in a rat's brain hippocampus over one month (Puskas *et al*., 2003). This gene binds thyroid hormones and plays an important role in cognitive function. In such studies, thyroid hormone deficiency during brain development impairs performance of cognitive function (Wilcoxon *et al*., 2007). This finding was similar in previous studies reporting that a low level of calmodulin-dependent protein kinase-II activation in a transgenic mouse model, resulted in enhanced performance in cognitive function which was associated with an increased transthyretin transcription (Butler *et al*., 1995). Thus, these could indicate that transthyretin gene has a marked influenced in synaptic plasticity, learning and memory.

Further evidence that ω-3 fatty acid can modulate the suppression and enhancement of expression genes comes from the previous studies reporting that ω-3 fatty acid induces various genes involved in diverse functions in different brain regions (Kitajka *et al*., 2004). These findings proposed an experimental feeding protocol containing perilla oil, which is rich in ALA (39% ALA/29% LA) and fish oil rich in DHA (27% DHA/23% LA/3% ALA/12% EPA). This resulted in gene encoding synuclein α and γ which were over-expressed. These genes participated in signal transduction processes, synaptosomes and ion channel formation. In addition, synuclein possibly related to cognitive functions in young rats receiving ω-3 fatty acid from conception until adulthood (Barcelo-Coblijn *et al*., 2003). The synuclein is shown to accumulate in the brain of song birds during the period of song learning (Recchia *et al*., 2004). In has been shown that over-expression of synucleins appear to be associated with the development and maturation of neurons and neurotransmission (Eslamboli *et al*., 2007). Furthermore, genes participating in signal transduction processes such as calmodulins also were up-regulated by the dietary of high LNA (perilla oil; 8%) or high EPA + DHA (fish oil; 8%) (Kitajka *et al*., 2002). Interestingly, calmodulins may enhance communication between neurons during signal transduction process and have a special role in the stimulant-induced plasticity of the CNS (Jordan *et al*., 2007). Overall, dietary ω-3 fatty acid influences the transcription of key genes involved in cognitive function as well as being important for normal brain function and exerting protection against the incidence of neurodegenerative diseases such as Alzheimer disease.

CONCLUSION

Nutritional status is one of the factors that can influence learning and memory function in mammals. Food restriction is also increased the animal to lead learning and memory deficits. Increasing number of evidence shows that dietary ω-3 fatty acid in brain cell membranes is important to improve learning and memory function. In biochemistry study, ω-3 fatty acids also have important roles to regulate and modulate brain gene expression associated learning and memory function. Therefore, future study needs to address this issue especially dietary ω-3 fatty acid deficiency interacts with neural membrane function and cholinergic neurotransmitter associated with cognitive function. The mechanism by which ω-3 fatty acids modulate the gene expression associated with cognitive function is yet to be explored. It is important to understand the possible effect since this area of study is limited. Thus, a number of experiments need to be conducted to demonstrate the mechanism effects of ω-3 fatty acid in brain gene expression associated with cognitive functions.

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