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Mechanism of Spatial Memory Improvement In Hippocampus Through Docosahexaenoic Acid (DHA) Supplementation In Rat (*Rattus Norvegicus*) Model Of Protein Malnutrition

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Abstract:
Background*: Malnutrition is a complex problem which has a wide impact on general health condition, including the brain. Postnatal protein malnutrition causes a dysfunction in memory consolidation, a decrease in information retention, and an impairment in spatial memory. Docosahexaenoic acid (DHA) may improve the spatial memory. The purpose of this study was to assess the effect of DHA on spatial memory through the FABP7, PPARγ, GFAP, Tuj-1 and BDNF pathways.*
Materials and Methods*: The rats were classified into 3 groups, namely, normal, malnutrition (control), and malnutrition supplemented with DHA (treatment). Protein malnutrition diet (AIN-76A) was given for 50 days since birth, followed by DHA supplementation in the treatment group. At 90 days, spatial memory was assessed, and the rats were terminated to obtain the brain for the measurements of FABP7, PPARγ, GFAP, Tuj-1 expression and BDNF levels.*
Results*:*
Results showed that there was a significant difference in spatial memory (p = 0.000), while there were no significant differences in the FABP7, PPARγ, GFAP, Tuj-1 expression and BDNF levels. Astrocytes (GFAP) in protein malnutrition are reactive, leads to an increase in the total number of astrocytes. This increase affects the expressions of FABP7 and PPARγ. FABP7 is highly selective to DHA. Transient reactive astrocytes may provide a trophic support to the regenerated axon, however, if the dysfunction occurs longer, it may inhibit the regeneration of axon. A chronic dysfunction, including protein malnutrition, may become maladaptive, which leads to astrocyte dysfunction. Protein malnutrition leads to a decrease in neurogenesis of hippocampus, causing a dysfunction in pattern recognition and memory consolidation. DHA supplementation may decrease cognitive dysfunction due to protein limitation and may improve the spatial memory
Conclusion: *DHA supplementation increases the spatial memory in animal model of malnutrition, but the mechanism is not through the PPARγ, GFAP, Tuj-1 and BDN.*
Key Word*: Protein malnutrition,* PPARγ, GFAP, Tuj-1, BDNF, spatial memory

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I. Introduction

Cases of malnutrition in Indonesia are still prevalent. In 2018, the prevalence of severe and moderate malnutrition was 17.7%[1]. Various detrimental effects of malnutrition on the brain have been reported. Postnatal protein malnutrition causes a dysfunction in memory consolidation [2] and interferes with spatial memory [3,4]. This is caused by the dysfunction in the cholinergic fibers in hippocampus[3], a decrease in Brain Derived Neurotrophic Factor (BDNF) level[5], volume deficit of medial prefrontal cortex (mPFC) and average neuronal size deficit[6], lower dendritic spine formation[7] and decreased neurogenesis in hippocampus [8]. The effective solution to overcome the impact of malnutrition in children is by increasing the nutritional status. One of the important nutritional intake is DHA. Fatty acid, including DHA, is functionally bound by fatty acid-binding proteins (FABP). The expression of FABP is triggered by the increase in total fatty acids[9]. One type of FABP is FABP7. FABP7 is highly selective to DHA. In adult brain, FABP7 is expressed by astrocytes [10]. DHA functions in the modulation of learning and memory through the upregulation of Peroxisome Proliferator-Activated Receptors γ (PPARγ) gene in hippocampus[11]. The effect of DHA on astrocytes facilitates the formation of BDNF [12]. BDNF is produced by neurons as well as the astrocytes [13]. In several studies, astrocytes are proven to trigger the proliferation of neural stem cells (NSC) into neural progenitor in the subgranular zone (SGZ) of

hippocampus [14]. The prevention of malnutrition has been conducted through various strategies, but DHA supplementation has not been the primary choice. Nutrition rehabilitation in rats with malnutrition after breastfeeding showed an increase in performance in spatial memory test of the animal model[15]. The brain DHA significantly increases the neurogenesis in hippocampus. The increase in neurogenesis is characterized by the increase in total number of Tuj-1 positive cells[16]. DHA supplementation in children with malnutrition also increases the performance in neuropsychology [17] and increases the DHA status in infants with malnutrition[18]. The current treatment of patients with severe malnutrition has not included the element of DHA supplementation as part of severe malnutrition prevention. Fulfillment of nutrition is focused more on proteins, vitamins and minerals [19]. The previous studies have not explained the mechanism of DHA in increasing the cognitive function in children with malnutrition. The aim of this study was to assess the effect of DHA supplementation on spatial memory through the FABP7, PPARγ, GFAP, Tuj-1 and BDNF pathways.

II. Material And Methods

Treatment

The rats were mated, and when the pups were born, the dams were classified into 3 groups. The normal group (KN) consisted of the breastfeeding dams who were given standard diet, while the malnutrition groups (KK and KP) consisted of breastfeeding dams who were given AIN-76A malnutrition diet after the age of 21 days. The weaned pups were placed in individual cages. At 21 days, KN were given standard diet, while KK and KP were given protein malnutrition diet. Protein malnutrition is a condition where there is a deficient protein level in serum. The malnutrition groups were given Modified AIN-76A Purified Rodent Diet containing 6.0% Casein (Dyets®, USA).

At 50 days, KN was given standard diet and aquadest (placebo) by sondage. In this recovery phase, all animals were given commercial diet[3]. Before DHA administration in KP, all groups were assessed with [3] pretest MWM. Furthermore, KK was given standard diet and aquadest with sondage, and KP was given standard diet and DHA supplementation (1 mg). DHA was diluted with aquadest and given by sondage. DHA was given daily until the age of 90 days or for 6 weeks[20]

Examination of spatial memory

After 8 weeks of treatment, all the rat models were examined with MWM to assess their spatial memory. Spatial memory was compared among the control and treatment groups. All rats were trained (escape latency) by MWM method hidden platform test for 8 days. Everyday, the rats were trained twice a day for a duration of 90 seconds. The time when the rats reached the platform (escape latency) was noted. After that, the rats were dried and rested. If they failed in 90 seconds, they would be directed onto the platform and let them take a rest for 30 seconds. The failed rats were noted as having the time of 90 seconds. In the second examination, they were placed at different locations and they swam to and up onto the platform. The time for reaching the platform was noted (escape latency). After reaching the platform, the rats were rested for 30 seconds. To find out the rat spatial memory, they were examined by probe test. The platform was removed from the water, while the other components were allowed like before. The starting place was chosen randomly. The rats swimmed for 90 seconds and the percentage for swimming to the target quadrant was calculated.

Immunohistochemistry and immunofluorescence examinations

After the measurement of spatial memory, euthanasia was conducted with method that did not induce discomfort, safe for the researchers, and did not change the chemistry and histopathology of the tissues. Animals were decapitated and the brains were obtained. The brains were divided into 2 parts: the right hemispheres provided the hippocampus for ELISA examination by soaking it in phosphate buffer saline (PBS); and the left hemispheres were soaked in formalin buffer for the tissue preparation for immunohistochemistry examination, including immunostaining of Tuj-1 and GFAP, and double staining immunofluorescence examination of PPARγ + GFAP and FABP7 + GFAP. The Tuj-1 was examined in hippocampal dentate gyrus, while GFAP was examined in the CA1 and CA3 layers of hippocampus. The examination of PPARγ and FABP7 was conducted in hippocampus.

Data analysis

The data normality was assessed with Saphiro-Wilk test. If the data were not normally distributed and not homogenous, Kruskal-Wallis test was conducted, and continued with Mann-Whitney test. For normally distributed data, the analysis was conducted with one-way Anova test with 95% confidence level. The posthoc test was conducted with the LSD test.

III. Result

The effect of DHA on FABP7 expression

Total expression of FABP7 in astrocytes was assessed with fluorescence method. The average FABP7 expression in astrocytes in each group is shown in Figure 1.

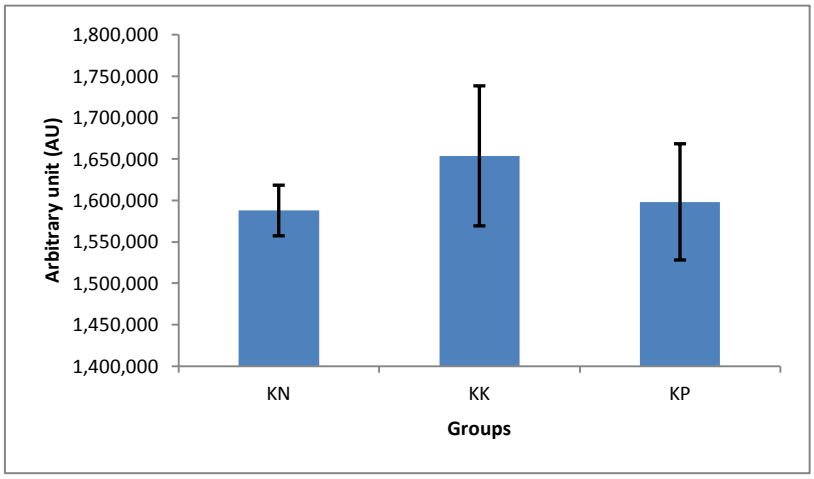

Figure 1: Average expression of FABP7 in hippocampal astrocytes (KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.159).

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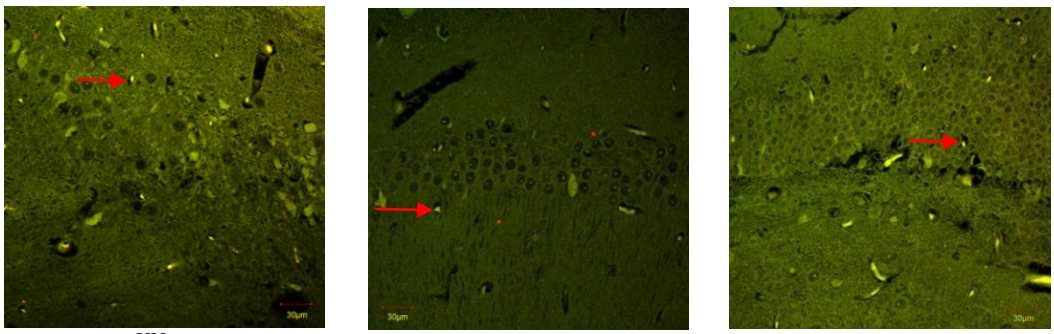


KN KK KP

Figure 2: Expression of FABP7 in hippocampal astrocytes with immunohistochemical staining, 400x magnification, using CLSM. Expression of FABP7 in astrocytes is marked with arrows (KN = normal group, KK = control group, KP = treatment group).

Examination of FABP7 expression in astrocytes showed that the expression of FABP7 in KK was higher than that in the KN and KP. Kruskal-Wallis test provided the p value of 0.159 ($p > 0.05$), which means there was no significant difference in FABP7 expression between groups.

The effect of DHA on PPARγ expression

Total expression of PPARγ in astrocytes was assessed with fluorescence method. The average PPARγ expression in each group is shown in Figure 3.

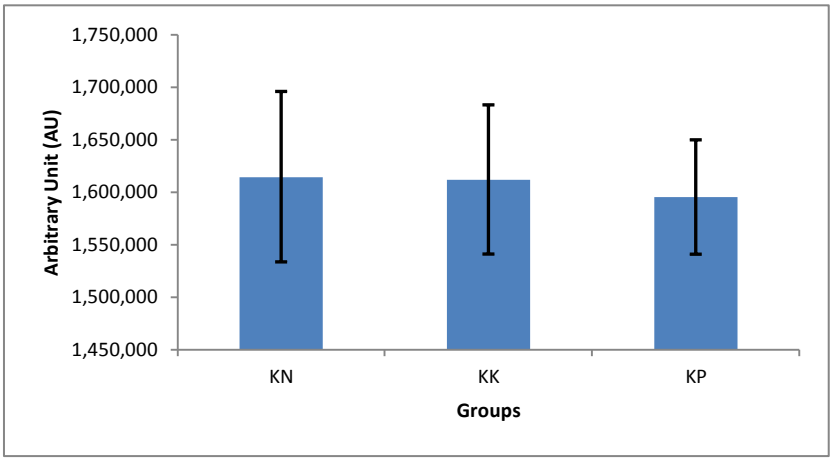
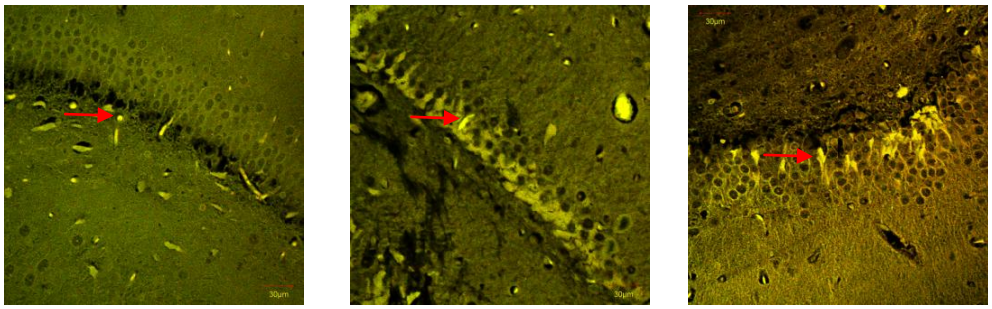


Figure 3: Average expression of PPARγ in hippocampal astrocytes ((KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.826).



KN KK KP

Figure 4: Expression of PPARγ in hippocampal astrocytes with immunohistochemical staining, 400x magnification, using CLSM. Expression of PPARγ in astrocytes is marked with arrows (KN = normal group, KK = control group, KP = treatment group)

Examination of PPARγ expression in astrocytes showed that the expression of PPARγ in KK was higher than that in the KN and KP. Anova test provided the p value of 0.826 ($p > 0.05$), which means there was no significant difference in PPARγ expression between groups.

The effect of DHA on GFAP expression

GFAP expression represents the total number of astrocytes, assessed with cell count method. Th average total count of astrocytes in each group is shown in Figure 5.

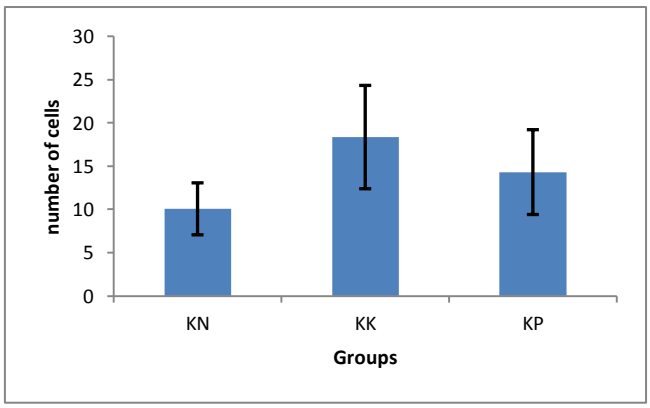


Figure 5: Average GFAP expression (astrocytes) in rat hippocampus after the treatment (KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.003).

Total count of astrocytes, shown by average expression of GFAP, in KK was higher than that in KN and KP. Normality test with Shapiro-Wilk test on GFAP expression data showed that data were normally distributed, and the data were also homogenous. The Anova test was then conducted with $\alpha = 0.05$.

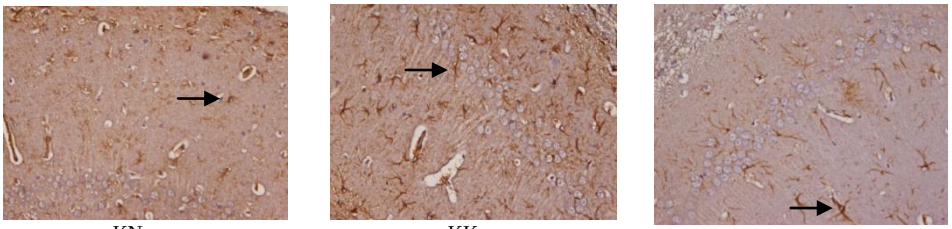


Figure 6: GFAP expression (astrocytes) in the CA1 layer of hippocampus with immunohistochemistry staining, 400x magnification, using Olympus BX 51 microscope and Olympus DP71 camera. Astrocytes are marked with arrows (KN = normal group, KK = control group, KP = treatment group).

The Anova test provided the p value of 0.003 ($p < 0,05$), which means that there was a significant difference in GFAP expression between groups. LSD posthoc test showed differences in GFAP expression between KN, KK and KP. This means that GFAP expression in normal rats was lower than rats with malnutrition and rats with malnutrition and DHA supplementation. The GFAP expression in rats with malnutrition was not different from that in the rats with malnutrition and DHA supplementation.

The effect of on BDNF level

The hippocampal BDNF level was assessed with ELISA method. The average BDNF levels are shown in Figure 7.

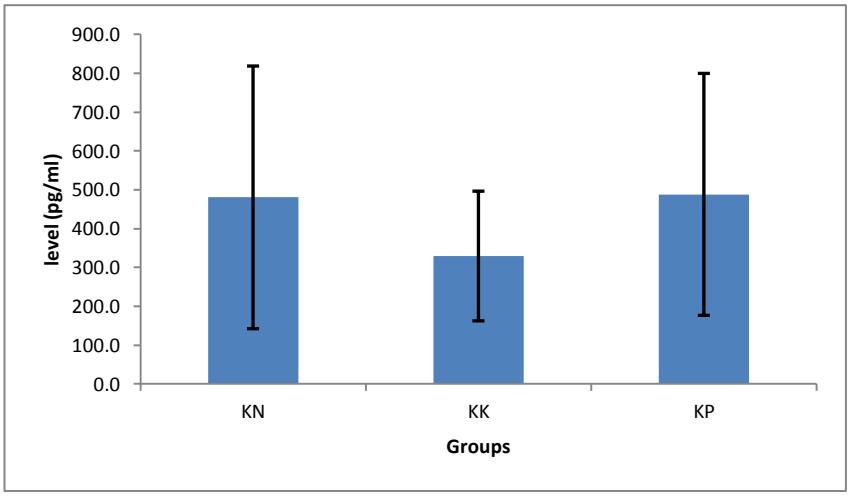


Figure 7: Average hippocampal BDNF level in rats after treatments (KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.727).

In Figure 7, average BDNF level was highest in KP, which was higher than KN and KK. Kruskal-Wallis test provided the p value of 0.727 ($p > 0.05$), which means that there was no significant difference in BDNF level between groups.

Effect of DHA on Tuj-1 expression

Tuj-1 expression represents the total count of NPC, measured with the cell count method. The average total count of NPC in each group is shown in Figure 8.

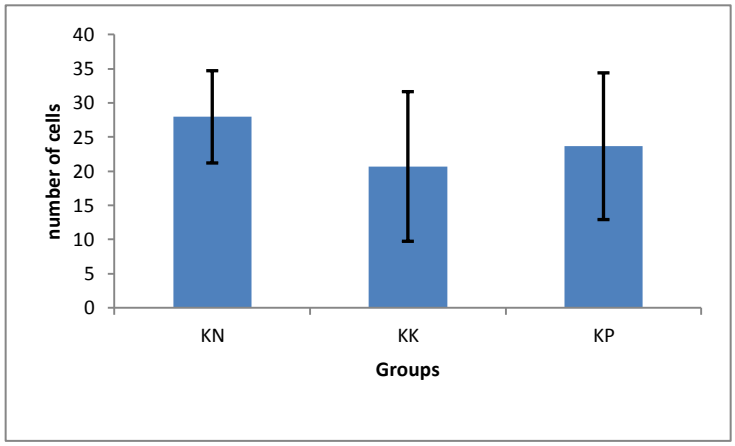


Figure 8: Average Tuj-1 expression in hippocampal dentate gyrus (KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.258).

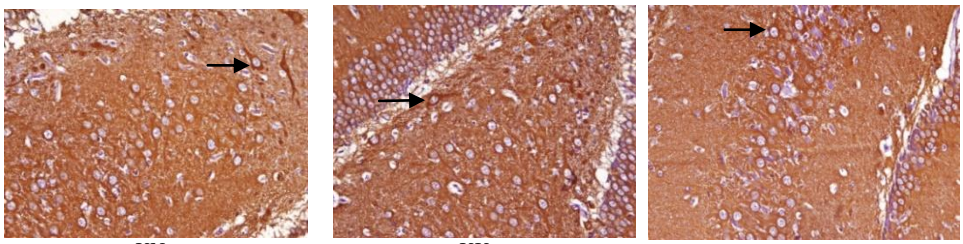


Figure 9: Expression of Tuj-1 (NPC) in hippocampal dentate gyrus with immunohistochemistry staining, 400x magnification, using Olympus BX 51 microscope and Olympus DP71 camera. NPC is marked with arrows (KN = normal group, KK = control group, KP = treatment group).

Results showed that the total count of NPC in KN was higher that that in KK and KP. Anova test gave the p value of 0.258 ($p > 0.05$), which means that there was no significant difference in Tuj-1 expression between groups.

The effect of DHA on spatial memory

Assessment of posttest spatial memory (probe test) was conducted with the Morris Water Maze. The average spatial memory values are shown in Figure 10.

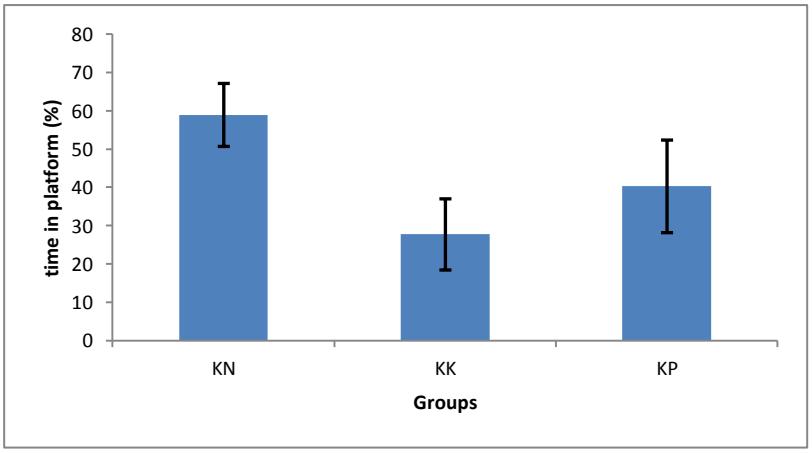


Figure 10: Average spatial memory value (probe test). (KN = normal group, KK = malnutrition group+standard diet, KP = malnutrition group+standard diet+DHA 1 mg; p = 0.000).

The average probe test values showed that spatial memory value in KN was higher than that in KK and KP. Anova test provided the p value of 0.000 ($p < 0.05$), which means there was a significant difference in spatial memory between groups. The difference between groups was assessed with LSD, and the results showed that the significant differences were found between KN and KK, between KN and KP, and between KP and KK. This means that spatial memory values in normal rats were higher than those with malnutrition and those with malnutrition and DHA supplementation. Spatial memory of rats with malnutrition and DHA supplementation was higher than that in rats with malnutrition.

IV. Discussion

The effect of DHA on FABP7 expression

This study showed that the expression FABP7in malnutrition group was higher than that in the other groups, but the difference was not significant. This might be because the astrocytes in protein malnutrition are reactive, leads to the increase in their total count. This increase affects the increased expression of FABP7. Reactive astrocytes in the brain with protein malnutrition express more FABP7 to prevent more severe brain damage. FABP7 controls the function of astrocytic caveolae, one of the membrane microdomain, which is the main source of cellular activities in responding to external stimuli through the regulation of caveolin-1 expression[21]. The external stimulus is protein malnutrition. Until recently, there were no studies evaluating astrocyte FABP7 expression in protein malnutrition. Astrocytes express FABP7 that participates in regulating astrocyte lipid homeostasis, mainly in developing brain.

FABP7 is highly selective to DHA. In adult brain, FABP7 is expressed by astrocytes. FABP7 is not detected in adult neurons, microglias, and oligodendrocytes[10]. DHA has functions in binding FABP7 and controlling development and metabolism of central nervous system from the beginning[22]. DHA supplementation in malnutrition group was expected to increase DHA level in the rat brain. An increase in DHA level will increase the binding to FABP7.

The study by Kagawa *et al*. suggested that without FABP7 (in FABP7-KO group), the response to lipopolysaccharides (LPS) was decreased compared to normal astrocytes. This shows that FABP7-KO astrocyte has a weak response to external stimuli, followed by a decrease in cell activity[21]. Reactive astrocyte is one of the defensive mechanisms to minimize and improve damage due to CNS disorders. FABP7 is mostly expressed in several types of glial cells, such as astrocyte and oligodendrocyte precursor cells. In cases of trauma to CNS and during brain development, FABP7 expression in astrocytes is proven to be significantly increased in rat cortex. The study by Hara *et al*. showed that FABP7 increased significantly in the area of scratch wound compared to the astrocytes in intact areas. Astrocytic dendrites gradually occupy the scratched area, and astrocytes at the margin of the scratched area expressed higher FABP7 level compared to the astrocytes in intact areas[23]. This proved that FABP7 is important in the development of reactive astrocytes in the context of CNS trauma.

The effect of DHA on PPARγ expression

In this study, PPARγ expression in astrocytes of malnutrition group increased. The increase in total astrocytes due to reactive astrocytes caused the increased PPARγ expression in this study. This is similar with the study by Cunha *et al*. showing that there was a significant increase in PPARγ expression in MSC animals with protein malnutrition compared to the normal animals[24]. The results showed that protein malnutrition may change the balance of adipogenesis. Differentiation and maturation of mesenchymal stem cell are controlled by specific signal transduction and certain transcription factor, including PPARγ. In protein malnutrition, astrocyte maturation occurs earlier than usual and there is a dysfunction in astrogenesis[25]. Based on the study by Cunha et al., it can be assumed that PPARγ expression is increased to overcome this dysfunction in astrogenesis.

Modulation of PPARγ and FABP7 mediated by DHA simultaneously affect malignant astroglial cell pathway and brain tumor astrocytoma[26]. Astrocytes have high double unsaturated fatty acid contents acted as the "donor" activator of PPARγ. Astrocytes are the main source for fatty acid synthesis in the brain. This fatty acid will be used by neurons to develop and produce DHA[27].

The administration of omega-3 fatty acid diet increases spatial memory and upregulates PPARγ gene expression in hippocampus[11]. PPARγ is mostly expressed in CNS, particularly brain parts that involved in learning function and memory. The antiinflammatory and antiapoptotic functions of PPARγ are very important to increase brain function if the brain has a dysfunction. In protein malnutrition condition, there is a dysfunction in the brain, including cell morphology. Along with that, PPARγ manages the brain disorder by increasing the expression.

The effect of DHA on GFAP expression

GFAP expression in malnutrition group was higher than that in the normal group and the malnutrition group supplemented with DHA. This study is similar with the study by Sinha *et al*. who also found an increase in total count of astrocytes and the change in morphology of rats with protein malnutrition. One specific marker

usually seen in brain dysfunction is reactive astrocyte (astrogliosis). In astrogliosis, there is an increase in GFAP expression[28], as well as the enlarging and thickening of cell bodies[29]. A previous study found an increase in GFAP expression in cerebral cortex and hippocampus of experimental animals 2 days postnatal who had malnutrition since gestation period, but at 60 days postnatal, there was no difference in GFAP expression. The increase in GFAP expression due to protein malnutrition at 2 days postnatal is due to reactive astrocyte process. The study by Feoli *et al*. found an increase in S100β, which showed that astrocytes matured earlier (precook/premature), and followed by a dysfunction in neuronal development[30].

Different results are found in the study by Mendonca *et al.* who found that there was a decrease in GFAP (astrocytes) in suprachiasmatic nucleus and medial preoptic hypothalamus in groups with malnutrition. This might be caused by the differences in diet for malnutrition. Mendonca study used RBD, a type of diet high in carbohydrates, but low in vitamins, minerals, fat, and protens[31]. This study used low protein diet (AIN-76A Purified Rodent Diet) which contains 6% casein.

Various conditions caused by protein malnutrition, in the forms of delayed GFAP appearance, early maturation and low astrocyte density, have become the factors for cognitive impairment[25]. Due to protein malnutrition, astrocytes will be active by increasing their total count (astrogliosis) and also by changing morphological structure by enlarging cell bodies and processes, the marker of damage or dysfunction of astrocyte[30]. In this study, morphological structure of astrocytes was not observed, so that the authors could not prove the change in morphology.

Reactive astrocyte is a secondary event. This occurs due to a change in homeostasis in the brain. Not only increasing total astrocytes, this compensation mechanism also changes the morphology. In pathological condition, astrocytes show morphological changes, including soma hypertrophy, asymmetrical branching changes, and overlapping with their neighbors. One trigger of reactive astrocyte is non-physiological condition, including environmental issues[32]. Reactive astrocyte can be beneficial and detrimental. Transient reactive astrocyte may provide trophic support for regenerating axon[33], but may also inhibit axon regeneration[34]. Reactive astrocytes regulate genes responsible to induce synaptic formation. These genes include those encoding thrombospondin, which might help in repairing the brain[35], however, this change might also results in unwanted synapses, one of them might cause epilepsy[36]. Astrocyte response to immediate dysfunction is aimed to restore homeostasis in brain function, for example, wound healing. However, if the dysfunction is chronic, it might lead to maladaptation and furthermore astrocyte dysfunction[32].

DHA supplementation in malnutrition group showed a decrease in GFAP expression, even if it was not significantly different with the other group. This might become a preliminary proof that DHA has a potency in the prevention of reactive astrocyte. DHA may act as a controller of rat brain homeostasis with protein malnutrition. The collected data support the contribution of reactive astrocytes in the physiology of CNS, including Alzheimer's disease[37].

The effect of DHA on Tuj-1 expression

In this study, there was no significant difference between groups in total NPC. There is an assumption that high count of astrocytes in malnutrition group affected this cell differentiation process. Astrocyte has an important role in neurogenesis process. A study by Perez-Garcia *et al.* found that the impact of protein malnutrition since gestational period was continued in breastfeeding phase, causing a decrease in total BrdU positive cells compared to control. Aside to affecting the neurogenesis process, protein malnutrition also decreases the proliferation level of neurons and neuronal cell survival[8]. The study by Cruz-Rizzolo *et al*. found that protein malnutrition since gestational period might cause a decrease in neuronal size, but did not affect the total count of neurons[6]. This might be due to a decrease in dendritic branches in neuronal bodies. In contrast, the study by Soares *et al.* proved that the total count of neurons in CA1 layer in hippocampus decreased in rats with postnatal protein malnutrition compared to rats with normal diet, but not the cell soma size[38].

Those studies proved that protein malnutrition affects neurogenesis. Neuronal migration from neurogenesis area to specific area is mainly driven by immunoglobulins and chemokines. A lack of both substances might affect the migration process and low axon growth[39]. In learning process, there is an increase in production of new neurons in hippocampus. Experimental animals having protein limitation have a decrease in hippocampal capacity in producing new neurons. Protein limitation in long term decreases cell ability in proliferation. However, neuron differentiation into new cells in hippocampal stem cell is not affected and neuronal endurance (survival) is not different between protein malnutrition group and control group[8].

In malnutrition group supplemented with DHA, the total expression of Tuj-1 was higher than that in malnutrition group. Although in many studies DHA has an important role in triggering neurogenesis process, in this study DHA did not increase the neurogenesis in brain hippocampal dentate gyrus in rats with malnutrition. Neurogenesis is a process of proliferation, differentiation, and survival of neural stem cells in subgranular zone (SGZ) of dentate gyrus and subventricular zone (SVZ) in lateral ventricle. The increase in brain DHA significantly increases neurogenesis in hippocampus, which is shown by an increase in total neurons that

proliferate and neuritogenesis. Increased neuritogenesis is proven by an increase in dendritic spine density in pyramidal neurons in CA1 layer in hippocampus[40]. DHA also triggers neuronal differentiation and neurite growth[41]. DHA supplementation on neural stem or progenitor cells increases cell proliferation[42]. This neurogenesis process occurs in normal condition, with no impairment affecting neurogenesis process, such as in protein malnutrition.

The effect of DHA on BDNF level

Asessment of BDNF level in hippocampus showed no significant difference between groups, although BDNF level of malnutrition group supplemented with DHA was higher compared to the control group (malnutrition). This is different from the study by Perez-Garcia *et al*. who found that in protein malnutrition there was a significant decrease in BDNF level compared to the normal group. Protein malnutrition occurred since gestational period showed a decrease in neuronal cell proliferation in hippocampus and hippocampal capacity impairment in producing new neurons (neurogenesis), both in basal condition and when the brain was responding to learning task [8].

DHA supplementation can increase BDNF level. BDNF is mainly produced from neurons supplemented by DHA. Although BDNF is mostly expressed by neurons, astrocytes are also able to produce BDNF. BDNF produced by astrocytes also has a role in learning and memory[43]. Protein malnutrition damages neuron, and this has been shown by many studies. Studies on protein malnutrition at the beginning of life showed a loss of intact synapses between mossy fiber and dendrites in neurons in hippocampal CA3 layer[44]. This shows that brain with protein malnutrition will have neuronal damage. Aside from neuronal impairment, astrocytes might be also affected, but the size of damage in astrocytes needs to be studied further.

BDNF particularly regulates neuronal endurance, repair process and neuronal regeneration during development phase, including the synaptic plasticity[45]. Accelerated neuronal regeneration in brain is supported by BDNF. Although BDNF level in treatment group was increased more than BDNF level in the normal group, but due to neuronal and astrocyte impairments, spatial memory values could not be increased. In contextual learning, BDNF expression will be rapidly and selectively upregulated in hippocampus. When BDNF signal is disturbed by obstacles, spatial learning will also be disturbed. In case of protein malnutrition, we need to elucidate which pathway is inhibited that causes the available or newly formed BDNF cannot perform well. This is proven by the poor performance of experimental animals in MWM[46], so that DHA supplementation in animal models could nor increase BDNF level in hippocampus, mainly in animal models of malnutrition supplemented with DHA.

The effect of DHA on spatial memory

Assesment of spatial memory (probe test) in experimental animals showed a signficant difference between normal group (non-malnutrition) and malnutrition groups (control and tretament groups). The treatment group was also different from the control group ($p = 0.013$). A study by He *et al*. showed that rat brain supplemented with DHA during pregnancy has a higher total neuronal cells[47]. This occurred in normal condition (non-malnutrition). In protein malnutrition, there was a possibility of impairment in DHA absorption. Therefore, DHA supplementation was administered together with nutritional improvement in the form of standard diet.

According to Kar *et al*., deleterious effect of malnutrition on cognitive development was due to an impairment in structural and functional maturation process of the neurons in the forms of delayed myelinization and decreased dendritic arborization[48]. From this study, it was shown that malnutrition caused cognitive decline, particularly in spatial memory, and there was an impairment in behavior of experimental animals. This is due to dysfunction in locomotor and exploration activity of the animals. A decrease in total neuron in granular cells also triggers spatial memory dysfunction[49].

The study by Burgos *et al.* showed that the experimental animals with malnutrition had an impairment in neurochemical system and neocortex function. One decreased neurochemical was (3-H)-dihydroalprenolol which bound the β-adrenoceptors in frontal and occipital neocortex. A change in β-adrenoceptor level might cause a change in expression of several proteins involved in trafficking, sorting, and endocytosis in cell membrane[4]. The study by Flores *et al*. proved that β1-adrenoceptor had a role in cognitive function in prefrontal cortex, including working memory and behavior. β1-adrenoceptor along with protein kinase is involved directly in synaptic LTP and memory formation. In animals with malnutrition, the expression of β1-adrenoceptor dan protein kinase signaling in neocortex decreased, so that the synaptic tissue in prefrontal cortex has a dysfunction of LTP[50]. In this study, neurochemical β1-adrenoceptor could not be measured, therefore, we can not elucidate whether this factor causes an impairment in spatial memory in malnutrition group.

In the malnutrition group supplemented with DHA, spatial memory value was higher than in the malnutrition group, but lower than in the normal group. In the group with DHA supplementation, the animals could learn where the platform position was by using visual cues that were part of spatial memory. The study by Avraham *et al*. proved that fish oil supplementation (rich in DHA) might decrease the cognitive dysfunction due

to severe diet limitation, and could repair neurochemical system. In the assessment of cognitive function using 8-arm spatial maze, diet limitation group supplemented with fish oil showed a better performance than the diet limitation group. In neurochemical assessment, serotonin (5-HT) level in experimental animals who had diet limitation decreased compared to control, while the level in the diet limitation group supplemented with fish oil was higher than in the diet limitation group, but lower than in the control group. Dopamine level also showed the same pattern with serotonin level[51]. These results are similar with this study, but neurochemical assessment was not conducted, so that it is not known whether this factor affects the results of MWM.

The effort of animals in learning to find the platform is an art of learning. Learning could increase the production of new neurons in hippocampus. This hippocampus is brain structure involved in spatial change identification of object position and detection related to new things. Protein limitation from the gestational phase causes a decrease in hippocampal neurogenesis, which impairs recognition pattern and memory consolidation[8].

V. Conclusion

DHA supplementation increases the spatial memory in animal models of protein malnutrition, but not through the FABP7, PPARγ, GFAP, BDNF and Tuj-1 pathways

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