Oral supplementation of the Extract of Fish oil to reduce fasting blood Glucose and Endothel damage but not Malondialdehyde level in diabetic male Wistar Rat (Rattus norvegicus)

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Abstract. The main target of hyperglycaemia is endothelial dysfunction involving pathways; protein kinase activation, hexosamine activation, polyol activation, and Advanced Glycation End Products (AGEs) formation, trigger reactive radical superoxide (O2•-) to stress oxidative. Malondialdehyde (MDA) is an end product of lipid peroxidation in body and is an indicator of oxidant-antioxidant level in diabetic patients. Fish oil composing mostly omega 3 as an antioxidant can reduce oxidative stress and hyperglycaemic condition. This study aimed to investigated the effects of omega-3-rich fish oil in lowering blood sugar levels, inhibiting oxidative stress and aortic endothelial cell damage in diabetic rat models. This study was an experimental study using post-test only control group design. Thirty-two rats divided into two study groups (n = 16 individuals per group), including the diabetic rat’s group (as control) and the diabetic rats group given fish oil doses of 300 mg/kilogram body weight/day. Provision of fish oil was performed for 28 days used Blackmores® fish oil. Blood sugar and malondialdehyde levels were analyzed by spectrophotometric method. The number of aortic endothelial cells was analyzed by haematoxylin-eosin staining. Comparability test showed that the average number of fasting blood glucose level after treatment in both groups showed highly significant differences (p=0.00). Although MDA level was reduced in treatment group than control group, but statistically not significantly difference, p=0.43. Comparability test showed that average of endothelial cell between control and treatment group significantly different (p=0.00). It was concluded that fish oil supplementation containing omega-3 in diabetic rats can lower blood glucose level and can inhibit endothelial cell damage.

1. Introduction
Hyperglycemia can trigger inflammation process that cause aging and many complications such as microvascular and macrovascular. As a result, this complication and mortality cause socio-economic burden and public financing system [1]. The main target of hyperglycemia is endothelial cell, trigger endothelial dysfunction and atherosclerosis. Endothelial dysfunction is inability to control vascular homeostasis [2,3]. Many evidences declare that endothelial dysfunction happen on type 1 and 2 diabetics. The main target of hyperglycemia is endothelial dysfunction involving pathways; protein kinase activation, hexosamine activation, polyol activation, and Advanced Glycation End Products (AGEs) formation, trigger reactive radical superoxide (O2•-) to stress oxidative. Besides that, path,
reactive oxygen was produced from mitochondria chain respiration [4], eNOS uncoupled [5], NADPH oxidase [6], and xanthine oxidase. Thus, reactive oxygen trigger oxidative stress.

Malondialdehyde (MDA) is an end product of lipid peroxidation in body and is an indicator of oxidant-antioxidant level in diabetic patients [7]. Previous studies proof increased of lipid peroxidation in diabetic compared to control [8-10]. There is positive correlation between MDA and blood glucose in diabetic patients [11]. Lipid peroxidation in diabetic accompanied with increase of endothelial circulating cells as a marker of endothel that released from vascular to circulation [12]. Endothelial cell yang mature juga known as Circulating endothelial cells (CECs), as a mature endothel, approximately 15-50 μm in diameter, which are found in the blood. Believed to be endothelial cells released from the intima after vascular damage. Plasma level of CECs increased in vascular disease and are believed to reflect the degree of endothelial damage/stress [13]. Endothelial cells and CECs analyzed with histopathological Hematoxylin-Eosin (HE) staining, light-microscopy, fluorescent microscopy, and immunoassay (flow cytometry) [2,14-17].

Antioxidant needed to reduce oxidative stress and hyperglycemic condition. Fish oil composing mostly omega-3 as an antioxidant. Omega 3 contain of docohexanoic acid (DHA) and eicosapentanoic acid (EPA), have been proof as an antioxidant to many cells. DHA suppress transcription activation of oxidant. DHA supplementation also suppress MDA production MDA and increase catalase in pancreas acinus cell through PPAR γ enzyme thus decrease ROS production. DHA-EPA supplementation inhibit membrane lipid peroxidation. Many studies have been proof DHA-EPA combination repair of endothelial dysfunction to people and animals [18-20]. Omega-3 supplementation 2 gr/day 12 weeks on metabolic syndrome shows decrease of IL-6, triglyceride level, total cholesterol, fasting blood glucose and endothelial dysfunction which measured with FMD (Flow mediated Dilatation) [21,22]. Omega-3 supplementation decrease MDA production as a marker of oxidative stress through endogen antioxidant upregulation [23]. Omega-3 1000 mg/day 12 weeks significantly decrease AGEs serum level in nephropathy diabetic patients compared with control [24].

2. Method
This study is in vivo experimental posttest only control group design to analyze fish oil effect for fasting blood glucose, aorta endothelial cells damage, and peroxidation lipid in diabetic rats. 36 rats divided 2 groups (n=18 each groups). Diabetic rats with placebo as control groups, and diabetic rats with omega-3 300mg/kg/bb/day as an experimental group. Fish oil was given for 28 days [25]. Fasting blood glucose, MDA level, and endothelial cell was measured.

2.1. Statistical analysis
Saphiro wilk were used to analyze normality test and Lavene’s for homogeneity test. Independent T test was used to compare the differences among groups. Values of P<0,05 were considered significant.

3. Result
Normality test of FBG, MDA level, and average of endothelial cells were test with saphiro wilk. The result showed normal distribution in all groups (p>0,05) which shown in table 1.

| Variable                  | Subjects | N   | P       |  
|---------------------------|---------|-----|---------|
| Fasting blood glucose (mg/dl) | P0   | 16  | 0,610   | Normal |
|                           | P1   | 16  | 0,088   | Normal |
| MDA (μM)                  | P0   | 16  | 0,106   | Normal |
|                           | P1   | 16  | 0,718   | Normal |
| Endothelial cells average (cells/4 field of view) | P0   | 16  | 0,417   | Normal |
|                           | P1   | 16  | 0,825   | Normal |

n = samples, p = significance
Homogeneity test of FBG, MDA level, and endothelial cell average between groups were analyze with Lavene’s test. The result showed that data variance was homogeny (p>0.05) which shown in table 2.

Table 2. Homogeneity variables result between groups.

| Variable                      | N   | P    |
|-------------------------------|-----|------|
| Fasting blood glucose (mg/dl) | 32  | 0.226|
| MDA (µM)                     | 32  | 0.092|
| Endothelial cells average (cells/4 field of view) | 32  | 0.992|

Comparability test of FBG, MDA, and endothelial cells average between groups shown in table 3 (p<0.05).

Table 3. Comparability test between groups.

| Variable                      | Groups | average | SD    | T   | P    |
|-------------------------------|--------|---------|-------|-----|------|
| FBG (mg/dl)                   | P0     | 261.56  | 37.284| 7.910| 0.000|
|                              | P1     | 163.94  | 31.976|     |      |
| MDA (µM)                     | P0     | 80.68   | 2.183 | 0.787| 0.437|
|                              | P1     | 75.68   | 1.482 |     |      |
| Endothelial cells average     | P0     | 14.54   | 2.72  | -4.096| 0.000|
| (cells/4 field of view)       | P1     | 18.54   | 2.79  |     |      |

SD = Deviation standard; t = t distribution p = significance

3.1. Discussion

This study showed that fish oil contains omega-3 significantly decrease FBG (p<0.000) compared to control group. It showed omega-3 as hypoglycemic agent. Hypoglycemic mechanism of omega-3 through increase of insulin sensitivity. Omega-3 can modify incorporation phospholipid membrane that inhibit proinflammation cytokine and NF-κβ from B protein cell and regulate insulin sensitivity and insulin receptor. NF-κβ inhibition could inhibit the death of β cell pancreas, thus increase insulin production. EPA/DHA inhibit release of NF-κβ from IK-β in cytoplasm. EPA/DHA also inhibit inflammation pathway through TGF-β inhibition, NLRP3 and Smad 2/3 to nucleus. EPA/DHA activated PPARγ, thus increase insulin sensitivity through increases of GLUT (protein transporter). Omega 3 increase GLUT4, glucose periphery transporter in muscles and adipose [26-27]. High level of GLUT4 trigger glycogen synthesis and prevent glucose oxidation.

The role of omega 3 in preventing the decrease of GLUT4 prevent polyol pathway, which prevent buildup fructose in the body. Polyol pathway involved aldo-keto reductase enzyme and NADPH that changed glucose to sorbitol. Sorbitol will be oxidized become fructose by sorbitol dehydrogenases enzyme with NAD+ as a co-factor. Hyperglycemia trigger stress oxidative because of due to high usage NADPH to polyol pathway, which NADPH was needed for GSH regeneration [28].

This study showed MDA average in experimental groups (P1) lower than control groups (P0), but comparability test Independent T Test not significantly different between groups (p>0.437). Omega-3 supplementation as an antioxidant cannot reduce MDA level significantly in diabetic rats [29].

This meaningless of MDA level between groups, could be due to several things. Dose, and duration could be one of this cause. Omega 3 inhibit RAGE and activated signaling ROS post-receptor [30]. AGEs have an activity as transcription intracellular factor of NF-κβ which initiate intracellular signaling cascade. NF-κβ activate protein kinase C, sorbitol, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). This activation produced ROS to DM and caused oxidative
stress. Other mechanism due to omega-3 incorporation to cell membrane and regulate NADPH oxidase expression and activation, thus prevent release of NF-κB to nucleus [31,32].

The other factor of this meaningless of MDA level due to endogen antioxidant compensation. Thus need further more research of doses and duration to know the effect of fish oil to MDA level. MDA evaluation should be check serially to know exactly oxidative stress development in diabetic.

Omega-3 supplementation increase endothelial cells amounts in diabetic rats vascular. This indicated omega-3 repair endothelial damage in diabetic rats. This mechanism due to endothelial progenitor cells modulation besides hypoglycemic effects that happened in this study. This result expands previous findings that omega-3 supplementation increases endothelial progenitor cells to cardiovascular disease [36]. Increase of endothelial cells due to raft lipid modification through cell migration [33]. Other mechanism through omega-3 incorporation cell membrane that regulate expression and activation of NADPH oxidase that caused increase of survival rates endothelial progenitor cells [31]. EPA and DHA (EPA: DHA = 0.9:1.5; 9 μM EPA plus 15 μM DHA) increase function and bioavailability endothelial progenitor cells and trigger neovascularogenic [34,35]. EPA decrease cells death of endhotel through reactive oxygen inhibition, activation of NADPH oxidase, and upregulation of inducible nitric oxide synthase [36]. DHA protect endothelial cells from oxidative stress and apoptosis [37]. EPA protect endothelial cells in rat’s aorta in vitro, and protect endothelial cells against anoikic through cFLIP repair expression [38]. Endothelial cell average of experimental groups higher than control groups. Thus, couldn’t confirm that higher average of endothelial cells was because of repair endothelial dysfunction mechanism from fish oil or because of endothelial damage did not occur. Thus, need further research to know about it.

4. Conclusion
Based on this study, it was concluded that fish oil supplementation containing omega-3 in diabetic rats can lower blood glucose level and can inhibit endothelial cell damage.

References
[1] International Diabetes Federation 2015 IDF Diabetes Atlas 7th ed. [Online] Retrieved from: http://www.idf.org/diabetesatlas. Accessed on 20 September 2017.
[2] S Xie, N Xie, Y Li, P Wang, C Zhang, Q Li, X Liu, J Deng, C Zhang and C Lv 2012 Upregulation of TRB2 induced by miR-98 in the early lesions of large artery of type-2 diabetic rat Mol Cell Biochem 361(1-12) 305–314.
[3] A C Roberts and K E Porter 2013 Cellular and molecular mechanisms of endothelial dysfunction in diabetes Diabetes & Vasc Dis Res 10(6) 472-482.
[4] M E Widlansky and D D Gutterman 2011 Regulation of endothelial function by mitochondrial reactive oxygen species Antioxid Redox Signal 15 1517–1530.
[5] T S Schmidt and N J Alp 2007 Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease Clin Sci (Lond) 113 47–63.
[6] R Damico, J J Zulueta and P M Hassoun 2012 Pulmonary endothelial cell NOX Am J Respir Cell Mol Biol 47 129–139.
[7] S M Eraky, N Abdel-Rahman and L A Eissa 2018 Modulating effects of omega-3 fatty acids and pioglitazone combination on insulin resistance through toll-like receptor 4 in type 2 diabetes mellitus Prostaglandins, Leukotrienes and Essential Fatty Acids 136 123-129.
[8] P B Pal, K Sinha and P C Sil 2014 Mangiferin attenuates diabetic nephropathy by inhibiting oxidative stress mediated signaling cascade, TNF-α related and mitochondrial dependent apoptotic pathways in streptozotocin-induced diabetic rats PloS ONE 9(9), e107220.
[9] M Shokrzadeh, S Sadat-Hosseini, M Fallah and F Shali 2017 Synergism effects of pioglitazone and Urtica dioica extract in streptozotocin-induced nephropathy via attenuation of oxidative stress Iran J Basic Med Sci 20 497-502.
[10] C A Pieme, J A Tatangmo, G Simo, P C B Nya, W J A Moor, B M Moukette, F T Nzufo, B L N
Nono and E Sobngwi 2017 Relationship between hyperglycemia, antioxidant capacity and some enzymatic and non-enzymatic antioxidants in African patients with type 2 diabetes BMC Res Note 10 141.

[11] S Mishra and B B Mishra 2017 Study of lipid peroxidation, nitric oxide end product, and trace element status in type 2 diabetes mellitus with and without complication Int J Appl Basic Med Res 7(2) 88-93.

[12] B Setiawan, N Kania, D Nugrahenny, N Nurdiana and M A Widodo 2012 Sub-chronic inhalation particulate matter 10 of coal dust induces atherosclerosis in aorta of diabetic and non diabetic rats Biomarker & Genomic Med 6 77-73.

[13] D Burger and R Touyz 2012 Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells J Am Soc Hypertens 6(2) 85-99.

[14] S Nistri, L Mazetti, P Faili and D Bani 2002 High yield method for isolation and culture of endothelial cells from rat coronary blood vessels suitable for analysis of intracellular calcium and nitric oxide biosynthetic pathways Biol Proceed Online 4(1) 32-37.

[15] P K Y Goon, G H Y Lip, C J Boos, P S Stonelake and A D Blann 2006 Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer Neoplasia J 8(2) 79-88.

[16] A Sokoli, K Groebel, K Hoelzle, W M Amselgruber, J M Mateos, M K J Schneider, U Ziegler, K M Felder and L E Hoelzle 2013 Mycoplasma suis infection results endothelial cell damage and activation: new insight into the cell tropism and pathogenicity of hemotrophic mycoplasma Biomol J Prev Med 44(1) 6.

[17] R Maramis, M Kaseke and G N Tanudjadj 2014 Gambaran histologi aorta tikus wistar dengan diet lemak babi setelah pemberian ekstrak daun sirsak (anmona muricata L) Jurnal e-Biomedik (e-BM) 2(2).

[18] A Ishikado, K Morino, Y Nishio, F Nakagawa, A Mukose, Y Sono, N Yoshioka, K Kondo, O Sekine and T Yoshizaki et al. 2013 4-hydroxy hexenal derived from docosahexaenoic acid protects endothelial cells via Nrf2 activation PLoS ONE 8 e69415.

[19] T Sawada, H Tsubata, N Hashimoto, M Takabe, T Miyata, K Aoki, S Yamashita, S Oishi, T Osue, K Yokoi, Y Tsuchihira, T Onishi, A Shimane, Y Taniguchi, Y Yasaka, T Ohara, H Kawai and M Yokoyama 2016 Effects of 6-month eicosapentaenoic acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. An open label, single blinded, prospective randomized controlled trial Cardiovasc Diabetol 15(11) 121.

[20] S Yagi, K Aihara, D Fukuda, A Takashima, T Hara, J Hotchi, T Ise, K Yamaguchi, T Tobiume, T Iwase, H Yamada, T Soeki, T Wakatsu, M Shimabuku, M Akaikie and M Sata 2016 Effects of docosahexaenoic acid on the endothelial function in patients with coronary artery disease J. Atheroscler Thromb 22(5) 447–454.

[21] A Ahmadi, M Gharipour, G Arabzadeh, P Moin, M Hashemipour and R Kelishadi 2014 The effects of vitamin E and omega-3 PUFAs on endothelial function among adolescents with metabolic syndrome Biomed Res Int.

[22] D Tousoulis, A Platiras, G Siasonos, E Oikonomou, A Varyeniotis, E Kokkou and K Maniatis 2013 Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome Biomol J Int 232 10-16.

[23] M Zararsiz, S Meydan, M Sarsilmaz, A Songur, O A Ozen and S Sogut 2011 Protective effects of omega-3 essential fatty acids against formaldehyde-induced cerebellar damage in rats Toxicol Ind Health 27(6) 489-495.

[24] S M Mirhashemi, F Rahimi, A Soleiman and Z Asemi 2016 Effects of omega-3 fatty acid supplementation on inflammatory cytokines and advanced glycation end products in patients with diabetic nephropathy: a randomized controlled trial Iranian J Kidney Dis 10(4) 197-204.

[25] M Zarei, S Fakher, S M B Tabei, M H Javanbakht, H Derakhshanian, P Farahbakhsh-Farsi, M R
Sadeghi, E Mostafavi and M Djalali 2016 Effects of vitamin A, C and E, or omega-3 fatty acid supplementation on the level of paraoxonase and aryesterase activity in streptozotocin-induced diabetic rats: an investigation of activities in plasma, and heart and liver homogenates Singapore Med J 57(3) 153-156.

[26] J Delarue 2007 Inflammation and long chain n-3 poly unsaturated fatty acids Danone Institue: Objective Nutrition 82.

[27] W Widowati 2008 Potensi antioksidan sebagai antidiabetes JKM 7(2).

[28] F Giacco and M Brownlee 2010 Oxidative stress and diabetic complications Circ Res 107(9) 1058-1070.

[29] K Palanisamy, R Krishnaswamy, P Paramasivan, H Chih-Yang and V P Vishwanadha 2015 Eicosapentaenoic acid prevents TCDD-induced oxidative stress and inflammatory response by modulating MAP kinases and redox-sensitive transcription factors Br J Pharmacol 172 4726-4740.

[30] A M de Assis, A Rech, A Longoni, M da Silva Morrone, M A de Bittencourt Paquali, M L S Perry, D O Souza and J C F Moreira 2015 Dietary n-3 polyunsaturated fatty acids revert renal responses induced by a combination of 2 protocols that increase the amounts of advanced glycation end product in rats Nutr Res 35 512-522.

[31] P Balakumar and G Taneja 2012 Fish oil and vascular endothelial protection: Bench to bedside Free Radic Biol Me 53 271–279.

[32] J Endo and M Arita 2016 Cardioprotective mechanism of omega-3 polyunsaturated fatty acids J Cardiol 67 22-27.

[33] S Bodin and M D Welch 2007 Plasma membrane organization is essential for balancing competing pseudopod- and uropod-promoting signals during neutrophil polarization and migration Mol Biol Cell 16 5773–5783.

[34] S C Chiu, E P I Chiang, S Y Tsai, F Y Wang, M H Pai, J N Syu, C C Cheng, R L Rodriguez and F Y Tang 2014 Eicosapentaenoic acid induces neovascularogenesis in human endothelial progenitor cells by modulating c-kit protein and PI3-K/Akt/eNOS signaling pathways J Nutr Biochem 25 934–945.

[35] S Devaraj, A Chien, B Rao, X Chen and I Jialal 2013 Modulation of endothelial progenitor cell number and function with n-3 polyunsaturated fatty acids Atherosclerosis 228 94–97.

[36] C H Lee, S D Lee, H S Ou, S C Lai and Y J Chen 2014 Eicosapentaenoic acid protects against palmitic acid-induced endothelial dysfunction via activation of the AMPK/eNOS pathway In J Mol Sci 15 10334-10349.

[37] C A Pfrommer, W Erl and P C Weber 2006 Docosahexaenoic acid induces cip1 mRNA and protects human endothelial cells from stress-induced apoptosis Am J Physiol Heart Circ Physiol 290(6) H2178-2186.

[38] T Suzuki, K Fukuo, T Suhara, O Yasuda, N Sato, Y Takemura, M Tsubakimoto and T Oghara 2003 Eicosapentaenoic acid protects endothelial cells against anoikis through restoration of cFLIP Hypertension 42(3) 342-348.