

## Effect of Fish Oil on Oxidative Stress and Monoamine of Brain Tissue in STZ Mice

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**Abstract:** Diabetic peripheral neuropathy is a multi-factorial disorder. Increased oxidative stress damage due to increased reactive oxygen species is directly neurotoxic leading to nerve functional deficits. There are no much studies dealing with the effect of fish oil on brain function and oxidative stress. The aim of the present research was to illustrate the changes in brain tissue neurotransmitters as relevant to oxidative stress that induced in STZ rats. The aim included studying the effect of fish oil administration on ameliorating these changes. Animals were divided into four groups: control group, control received fish oil, diabetic group and diabetic group received fish oil for 8 weeks. Daily dose was 1.2mg/kg/day. At the end of treatment, animals were sacrificed, brain was removed and frozen at -80°C, the levels of neurotransmitters were assayed by HPLC and oxidative stress by colorimetric methods. Results revealed that STZ diabetic rats demonstrated significant increase in the extracted brain tissue Malondialdehyde (MDA), Nitric oxide (NO) nor epinephrine, dopamine and serotonin. Administration of fish oil for 8 weeks resulted in significant decrease of the above mentioned biomarkers as compared to diabetic group. Correlation coefficient between NO and MDA, norepinephrine were positively significant ( $r=0.622$ ,  $r=0.550$ ) respectively. Correlation coefficient between MDA and nor epinephrine ( $r=0.588$ ), NO ( $r=0.622$ ) and dopamine ( $r=0.684$ ) respectively were positively significant. In conclusion: Fish oil may exert some protective and anti-oxidative influence on brain neurotransmitters and may provide resistance to free radical thus the therapeutic potential of oxidative stress inhibition to prevent or reverse brain disorders.

**Key word:** Fish Oil- Nor adrenaline • Serotonin • Dopamine • STZ • MDA • NO

### INTRODUCTION

The past decades have been a period of rapid expansion in the scientific knowledge of W-3 polyunsaturated fatty acids (PUFA). A number of investigations have demonstrated that diet supplemented with fish oil enriched in omega-3 fatty acids especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has profound beneficial health effects against various diseases [1-10].

Polyunsaturated omega 3 fatty acids affect on the structure, biochemistry and physiological function of the brain. All cells and organelles in the brain are very rich in W3 polyunsaturated fatty acids on the glycerol backbone position 2 of phospholipids especially DHA and EPA [11].

The alpha linolenic acid can control certain neurosensory and higher functions such as learning. A quantitative decrease of these fatty acids in the brain results in impairment of membrane function

activity of enzymes, receptors and transporters [12] Docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) effect expression level of number of genes in brain such as synaptic plasticity, cytoskeleton and membrane association, signal transduction ion channel formation, energy metabolism and regulatory protein [13, 14].

Docosahexaenoic acid (DHA), a component of fish oil is a powerful therapeutic agent that can protect brain tissue that would have died, its repair mechanism rendered some areas indistinguishable from normal tissue [15] In diabetics, diabetic peripheral neuropathy is a multifactor disorder, attributable to the reversible metabolic consequences of hyperglycemia, insulin deficiency to a spectrum of metabolic and vascular abnormalities including an increase of the polyol pathway, abnormalities in lipid metabolism, advanced glycation end product formation, increased oxidative stress damage, defect in growth factors and endoneurial hypoxia [16] that are thought to induce further neuro

chemical, neurotropic or neurovascular defects in the peripheral nerve system. Accumulating evidence implicates increased oxidative stress in turn increased ROS are directly neurotoxic promoting neuronal apoptosis [17] and may inhibit mitochondrial respiratory enzymes, leading to deficits of nerve energy production and nerve functional deficits.

Diabetes impairs essential fatty acid metabolism by decreasing activities of delta 6 and delta 5 which desaturase enzymes that convert dietary linoleic acid and alpha linolenic acid to long chain polyunsaturated fatty acids (PUFA), including gamma linolenic acid arachidonic acid (AA), (DHA) and (EPA) [18]. As a result, AA and DHA levels are reduced in membrane phospholipids of several tissues including erythrocytes and sciatic nerve, in diabetic animals [19,20].

Due to decrease in desaturation of enzyme more unsaturated fatty acid in the omega 6 path way decrease syntheses. This leads to diabetic neuropathy [21] Insulin deficiency can also promote alteration of fatty acid metabolism, via blockade of the conversion of gamma linoleic acid to gamma linolenic acid [18] imiting the formation of arachidonate and thereby perturbing the production of vasodilation.

Glucose-related or 'glucotoxic' pathogenic mechanism in experimental diabetes include nonenzymatic glycation of proteins [22], auto oxidation of glucose [23] and activation of the aldose reductase path way [24]. Activation of the aldose reductase path way alters cellular redox couples, exacerbates oxidative stress [25,26] activation of the aldose reductase path way promotes intracellular sorbitol and fructose accumulation in nerve with effects on signal transduction pathway and alteration in vasoactive agents, including endothelium-derived nitric oxide (NO) [27, 28] Insulin deficiency can also promote alteration of fatty acid metabolism, especially oxidation where MAD is the index of PUFA oxidation.

Present work was designed mainly to illustrate any changes in brain neurotransmitter pattern as relevant to oxidative stress in diabetic rats and the modulation of these neurotransmitters by fish oil.

## MATERIALS AND METHODS

Forty adult male albino rats weighing 150-170 g were randomly chosen from the animal house of the National Research Center, Cairo- Egypt. The animals were maintained on standard laboratory diet ad libitum. All animals received professional humane care in compliance with the guidelines of the Ethical Committee of the

National Research Center, Cairo- Egypt. They were housed in standard cages and left to acclimatize for 7 Days to laboratory condition before the commencement of the experiment

**Design of the Experiment:** The animals were randomly distributed into the following groups:

- Normal control rats, which were fed on basal diet.
- Diabetic rats, which were fed on basal diet.
- Diabetic rats, which were fed fish oil (1.2mg/kg/day) [29] orally and served as treated group.
- Non-diabetic rats, which were fed on basal diet and fish oil (1.2 mg/kg/day) and served as treated control group.
- Fish oil is purchased from (market).
- All rats were sacrificed at eight weeks after induction of diabetes

**Induction of Diabetic:** This was done by intraperitoneal administration of streptozotocin (STZ), purchased from Sigma-Aldrich Chemie (Deishofen, Germany ), dissolved in citric acid buffer (pH 4.2) at a dose level 60 mg /body weight. Blood glucose was determined spectrophotometrically as described by Trinder [30] and rats with a blood glucose level more than 200 mg/dl were selected for study.

**Extraction of the Brain Tissue:** Rats were scarified by decapitation, brains were removed, then washed with ice-cold saline solution (0.9% NaCl), weighed less than one gram and stored at -80°C for the biochemical analyses. The brain was homogenized with 0.1 M phosphate buffer saline at pH 7.4, to give a final concentration of 10% w/v, centrifuged at 4000 r.p.m for 15 min at 4°C. Put in eppendorf and centrifuged again at 5000 for 10 min at 4°C [31] Supernatant was collected and stored at -80C until assay of bio- markers. For the determination of monoamine neurotransmitters, frozen samples were homogenized in cold 0.1 N-perchloric acid.

## Biochemical Studies:

**Determination of Glucose:** Glucose was measured in supernatants of brain homogenates by a standard glucose oxidase method according to Trinder [30].

Glucose in the presence of glucose oxidase is converted to peroxide and gluconic acid. The produced hydrogen peroxide reacts with phenol and 4-amino-antipyrine in the presence of peroxidase to yield a colored quinonemine, which is measured spectro-photometrically.

**Determination of Lipid peroxidation:** Measuring thiobarbituric acid-reactive substances (TBARS) including lipid hydroperoxides and aldehydes, in biological samples is a method widely used for screening and monitoring lipid peroxidation. Malondialdehyde (MDA) forms a 1:2 adduct with thiobarbituric acid which can be measured by spectrophotometry. In practice, TBARS are expressed in terms of malondialdehyde (MDA) equivalents [32] Malondialdehyde was determined by measuring thiobarbituric-reactive species using the method of Ruiz-Larrea *et al* [33] in which the thiobarbituric acid-reactive substances react with thiobarbituric acid to produce a red-colored complex having peak absorbance at 532 nm.

**Determination of Nitric Oxide Metabolites:** Nitric oxide has a short biological half-life and is rapidly converted into its stable metabolites, nitrite and nitrate. Determination of nitrite and nitrate (NOx) in body fluids and tissues is widely used as a marker of NO● production. Nitric oxide measured as nitrite was determined by using Griess reagent, according to the method of Moshage *et al.* [34] where nitrite, stable end product of nitric oxide radical, is mostly used as an indicator for the production of nitric oxide.

**Determination of Brain Monoamines:** Determination of brain serotonin, noradrenaline and dopamine was carried out using high performance liquid chromatography (HPLC) system, Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model). Separation was achieved on ODS-reversed phase column (C18, 25 9 0.46 cm i.d. 5 lm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3

(v/v) and was delivered at a flow rate of 1 ml/min. UV detection was performed at 270 nm and the injection volume was 20µl. The concentration of both catecholamine and serotonin were determined by external standard method using peak areas. Serial dilutions of standards were injected and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve [35].

**Statistical Analysis:** The data analysis was carried out using the statistical package for social science (SPSS software version 16, Chicago, Illinois). All numeric variables were expressed as mean ± standard deviation (SD). Statistical comparison was performed using one way analysis of variance (ANOVA) test followed by Post Hoc LSD multigroup comparison. Pearson's correlation test was used for correlating parametric variables. For all tests a probability (p<0.05) was considered significant.

## RESULTS

STZ diabetic rats demonstrated significant increase in extracted brain tissue MAD, NO, nor epinephrine, dopamine and serotonin. Administration of fish oil for 8 weeks resulted in a significant decrease of the above mentioned biomarkers. as compared to diabetic group (Table 1). Correlation coefficient between NO and MDA, nor epinephrine were positively significant (r= 0.622, r=0.550) respectively. Correlation coefficient between MAD and norepinephrine (r=0.588), NO (r=0.622) and dopamine (r=0.684) respectively were positively significant (Table 2).

Table 1: Brain neurotransmitter, lipid per-oxidation and nitric oxide in different groups

Parameters	Control	Diabetic	Control and Fish Oil	Diabetic and Fish Oil
Noradrenaline.ng/gm tissue	730±125.21	938±32.19	766±72.85	792±151.14
Dopamine.ng/gm tissue	30.83±4.91	50.83±9.1	33.66±4.08	43.50±7.20
Serotonin.ug/gm tissue	3.75±0.765	4.5 0±0.48	3.81±1.58	3.95±0.43
MDA nano mol/g tissue	5.60±5.7	62.83±17.1	1.16±0.15	19.16±5.49
NO2 micro mol/g tissue	0.069±0.008	0.85±.007	.035±0.15	0.048±0.019

Table 2: Pearson's cCorrelation coefficients between the neurotransmitter and the oxidant parameters in the different studied groups.

Parameters	Noradrenalin	Dopamine	Serotonin	NO	MDA
MAD	0.588**	0.684**	0.293	0.622**	1
NO	0.550**	0.318	0.202	1	0.622**
Serotonin	0.322	0.113	1	0.202	0.293
Noradrenalin	1	0.390	0.322	0.588**	0.550**
Dopamine	0.390	1	0.113	0.318	0.684**

Correlation coefficient,r. \*\* is significant at p<0.01 \* is significant at p< 0.05

## DISCUSSION

A great number of anatomical, functional and biochemical alterations have been described in the nervous system of diabetic animals [36, 37]. This variety of alterations (generally named as diabetic neuropathy) affects the brain, spinal cord and peripheral nerves. They were reported many years ago as degenerative changes in the autonomic nervous system of diabetic rats, with widespread degeneration of ganglionic tissue, reduction of axonal caliber and demyelination [38]. In the central nervous system, diabetes reduces brain weight and neocortical volume, which is associated with a reduction of the number of cortical neurons [39]. All these central and peripheral changes are consistent with decreased neuronal activity.

The present study demonstrated a neuroprotective effect of fish oil enriched with omega-3 on diabetic rats. The analyzed data found that diabetic rats group had significantly high brain levels of norepinephrine, serotonin and dopamine as compared to the control group, a finding which is in agreement with Marco *et al.* [40].

The authors explained that the severity and duration of diabetic effect on neurotransmitter synthesis only and reduced release from nerve with increase storage probably. The present study also showed a significant increase in the mean brain levels of MDA and NO<sub>2</sub> in diabetic rat group as compared to the control group. Abebe and Mozaffari [41] and Elseweidy *et al.* [42] joined between this increment in each of MDA and NO and the level of hyper-glycaemia in STZ rats indicating an increased oxidative stress [43].

In the present work we investigated the effect of fish oil on diabetic neuropathy. The mean levels of brain parameters in diabetic group who modulated with fish oil orally were significantly decreased as compared to the diabetic group but still not reached to the levels of the control group except that of NO. This is in agreement with El-Seweidy *et al.* [42]. Thus may enhance resistance to free radical attack [44]. Oxidative stress plays a central role in the pathogenesis of metabolic diseases like diabetes mellitus and its complication (like peripheral neuropathy) due to its great oxygen consumption, high lipid and poor antioxidants defence [45]. Oxygen free radical attack objects on the polyunsaturated components of membranes and may cause a serious organizational dysfunction within cells and tissues [46]. It has been suggested that the use of omega-3 PUFAs may have ameliorating effect on such damage by two possible ways: First, omega-3 PUFA may increase the levels of catalase

within the peroxisome and in the cytoplasm resulting in enhanced defense against free oxygen radicals. Second, omega-3 PUFAs, which has been supplemented, may be replaced with Polyunsaturated fatty acid components of the membranes that had been attacked by oxygen free radicals such as super-oxide anions, hydrogen peroxide and hydroxyl radicals [47,48]. High oxidative stress due to hyperglycaemia promotes free radicals generation evidence based mainly on increased lipid peroxidation [49] leading to membrane damage thus transformation of NO<sub>2</sub> diminishes the capacity of endothelial cells to generate bioactive useful NO, causing endothelial sclerosis [50] leading to insulin resistance and possible vice versa [51] and alteration in membrane ion transport and permeability [52,53]. NO, a lipid soluble ROS, is generated by the action of nitric oxide synthases (NOS). All isoforms of NOS can be expressed in the CNS. Neurons express primarily neuronal NOS (nNOS), but a subset of neurons have been identified which express endothelial NOS. Because NO is formed by the stoichiometric conversion of L-arginine to L-citrulline, decreased NO level in CS upon omega-3 EFA supplementation may suggest the decreased entrance of L-arginine to the neuronal cell as a unique substrate of nNOS. Additionally, *in vitro* biochemical studies indicate that nNOS can be phosphorylated by calcium/calmodulin-dependent protein kinase and protein kinase C. Phosphorylation of nNOS by all of these enzymes decreases NOS catalytic activity *in vitro* [54]. Therefore, it can be speculated that omega-3 EFA has probably *in vivo* regulatory action on nNOS activity by using secondary messenger pathways or some other direct effects on enzyme structure as well as expression process of the enzyme protein, neurochemical, neurophysiologic and behavioural modification as well as cerebrovascular disturbances in the brain, impairing its function and structural integrity [55], thus the therapeutic potential of oxidative stress inhibition to prevent or reverse the diabetic brain disorders is a promising strategy [56].

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