Effect of Omega-3 Fatty Acids on Erythrocyte Membrane in Diabetic Rats

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Abstract

Background: Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, almost always with a major contribution from insulin resistance which may be affected by cell membrane fatty acids and phospholipids fractions.

Aim: To evaluate the effects of omega-3 fatty acids on erythrocyte membrane and also in decreasing oxidative stress in diabetic rats.

Materials and Methods: Sixty healthy male albino rats weighting 180-200 g divided into five groups included: control, Omega-3, diabetic, prophylactic and treated groups. Fasting blood samples were collected from all groups for determination of fasting blood sugar, plasma insulin, erythrocyte membrane lipid profile, phospholipids fractions by HPLC, plasma and erythrocyte membrane lipid peroxidation product (malondialdehyde) and reduced glutathione.

Results: Fasting blood sugar and plasma insulin sensitivity were improved by Omega-3 administration, also reduced glutathione improved by Omega-3 in both erythrocyte membrane and plasma, while, malondialdehyde decreased indicated the decrease of oxidative stress by Omega-3 administration.

Conclusion: Omega-3 supplementation increased insulin sensitivity by improving cell membrane contents especially phospholipids fractions.

Introduction

Diabetes mellitus is a complex of metabolic disease characterized by hyperglycemia, diminished insulin production, impaired insulin action, or a combination of both resulting in the inability of glucose to be transported from the blood stream into the tissues, which in turn results in high blood glucose levels and excretion of glucose in the urine [1]. Cell functions involved in the action of insulin receptor binding enzyme and transporter activities are controlled by membrane properties [2]. It is well known that hyperglycemia in diabetes results in excessive protein glycation and the production of reactive oxidants, which leads to oxidative damage in organs [3]. In addition, oxygen free radicals exert their cytoplasmic effect by peroxidation of membrane phospholipids, which leads to changes in the permeability and loss of membrane integrity [4]. Hyperlipidemia has also been reported as one of the causative factors for increased lipid peroxidation in diabetes [5], thus the free radicals attack the polyunsaturated fatty acids in membranes to produce lipid peroxides leading to decrease in membrane permeability [6].
Fatty acids (FA) composition of membrane phospholipids such as sphingomyelin (SM), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are tissue specific [7] but are affected by the composition of the dietary fat [8]. Changes in the fatty acids composition of erythrocyte membrane, which are easily accessible cells, reflects changes in that of membrane phospholipids of less accessible tissues [9]. The fatty acids composition of cell membrane can influence membrane associated phenomena such as the interaction between insulin and its receptors [10].

It was found that, fatty acids composition of the membrane phospholipids of insulin target tissues, such as liver and skeletal muscle, is a critical factor that influences both insulin secretion and its biological actions [11], thus membranes enriched in unsaturated fatty acids tend to bind more insulin than membrane enriched in saturated fatty acids [12].

Erythrocytes are particularly sensitive to oxidative damage due to the presence of fatty acids content in their membranes and high cellular concentrations of oxygen and hemoglobin [13]. Erythrocyte damage includes changes in membrane protein and lipid structure, which in turn induced alterations in external surface of the cell [14].

Thus it is from interest to study the relationship between cell membrane phospholipids fractions and their role in insulin action, also to study the role of Omega-3 fatty acids in reducing oxidative stress in diabetic rats.

**Materials and Methods**

**Chemicals**

Streptozotocin (STZ), 5.5 dithiobis-2-nitrobenzioc acids (DTNB) were purchased from Sigma Chemicals Co. (St.louis, MO, U.S.A.), Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and sphingomyelin(SM) from bovine sources as phospholipids standards were purchased from Sigma Chemicals Co. (Munih,Germany). Thiobarbituric acid (TBA) was obtained from MERCK. Omega-3 plus (Ω-3) was purchased from SEDICO Pharmaceutical Company 6 October City, Egypt).

**Experimental animals**

Male albino rats (*Sprague Dawely strain*) weighting 180-200 g were obtained from the animal house of National Research Center, Giza, Egypt. The animals were housed in individual suspended stainless steel cages in a controlled environment (22-25°C) and 12 hour light, 12 hour dark with food and water freely available. The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of NRC.

**Induction of diabetes mellitus**

Streptozotocin (STZ) was dissolved in 50 mM sodium citrate solution (pH 4.5) containing 150 mM NaCl. The solution (6.0 mg/0.5 ml/100 g body weight) was subcutaneously administrated in rats; fasting blood sugar was estimated after 3 days to confirm the development of diabetes mellitus [15].

**Experimental design**

Rats were divided into five groups, 12 rats in each group. Group I: Control group in which healthy rats received isotonic saline (500 μg/kg/day). Group II: Omega-3 group in which healthy rats received Omega-3 (500 μg/kg/day) [16]. Group III: Diabetic group received isotonic saline (500 μg/kg/day). Group IV: Treated group in which diabetic rats received Omega-3 (500 μg/kg/day). Group V: Prophylactic group in which healthy rats received Omega-3 (500 μg/kg/day) orally for thirty days then received STZ for the induction of diabetes.

**Blood sampling and biochemical analysis**

After the experimental period (30 days), animals were kept fasting for 12 hours, blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes, blood was collected into tubes contain sodium floride for blood glucose estimation and heparinized tubes for other biochemical parameters. Blood was centrifuged at 4000 rpm for 10 minutes. Plasma was separated and immediately kept at -20°C until used. Packed RBCs was used for determination of cell membrane parameters.

Fasting blood sugar was measured with commercially available enzymatic kits [17]. Plasma insulin level was determined using BioSoure INS-EASIA Kit according to the method described before [18]. Insulin resistance was calculated from the equation:

\[
\text{Insulin resistance} = \frac{\text{fasting glucose (mg dl}^{-1}) \times \text{fasting insulin (μIU ml}^{-1})}{405}
\]

The method used for erythrocyte ghost preparation is based on the hemolysis of RBCs in hypotonic solution for removal of hemoglobin [20].
Reduced glutathione (GSH) and malondialdehyde (MDA) were estimated in both plasma and erythrocyte membrane according to the methods described before [21, 22].

Extraction of erythrocyte membrane lipids was carried out by chloroform/methanol method [23].

Erythrocyte membrane total lipids, total cholesterol, total phospholipids and triglycerides were estimated by enzymatic colorimetric method using Centronik kit, GERMANY.

**Estimation of phospholipids fractions by HPLC**

Fractionation of phospholipids was carried out by High Performance Liquid Chromatography (HPLC) [24]. Agilent HPLC system (1100 series). The separation was achieved on a stainless steel phenomenx column with 300x390x mm silica particles. The mobile phase was acetonitrile-methanol-85%phosphoric acid (1000:40:0.4) v/v. It was delivered to the column at flow rate of 1.5 ml/min at room temperature (25°C). Photodiode array UV-visible detector was used and set at 203 nm.

The sample was dissolved in 2 ml (n-hexane / 2 propanol) (3:1) v/v. 20 μl of the standard and each sample was injected and the phospholipids area resulted in a graph.

**Statistical analysis**

Results are expressed as mean ± standard error. Data were analyzed by independent sample t test (SPSS) version 15 followed by (LSD) test to compare significance between groups. Difference was considered significant when P value <0.05.

**Results**

Fasting blood glucose and insulin resistance were elevated in diabetic group compared to control, while it returned to decrease again by Omega-3 administration in treated group, it also decreased in prophylactic group compared to diabetic group (Table 1).

Erythrocyte membrane total lipids was significantly increased in diabetic group, although it decreased again in treated group and also in prophylactic one as compared to diabetic group that may be due to the concomitant decrease in membrane cholesterol and phospholipids (Fig. 1).

**Table 1: Fasting blood sugar and plasma insulin in different studied groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Fasting blood sugar (mg/dl)</th>
<th>Plasma insulin (μU/ml)</th>
<th>Insulin resistance (mgs/min/mU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Range</td>
<td>72-69</td>
<td>8.40-15.40</td>
<td>1.06-2.60</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>84.6 ± 2.57</td>
<td>11.45 ± 1.46</td>
<td>2.33 ± 0.11</td>
</tr>
<tr>
<td>Omega-3</td>
<td>Range</td>
<td>(95-93)</td>
<td>(9.90-15.90)</td>
<td>(2.17-2.67)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>94.5 ± 1.76</td>
<td>11.3 ± 1.5</td>
<td>2.43 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>0.47%</td>
<td>-1.3%</td>
<td>0.42%</td>
</tr>
<tr>
<td>% change</td>
<td>-61.76%</td>
<td>32.94%</td>
<td>-69.1%</td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>Range</td>
<td>220-234</td>
<td>7.40-10</td>
<td>4.60 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>221 ± 0.73</td>
<td>8.83 ± 0.41</td>
<td>0.90%</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>102%</td>
<td>-20%</td>
<td>97.4%</td>
</tr>
<tr>
<td>% change</td>
<td>175.18%</td>
<td>3.69 ± 0.05</td>
<td>3.40-3.90</td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>Range</td>
<td>179 ± 0.06</td>
<td>6.4 ± 0.59</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>179 ± 0.06</td>
<td>6.4 ± 0.59</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>112%</td>
<td>-26%</td>
<td>59.5%</td>
</tr>
<tr>
<td>% change</td>
<td>-119%</td>
<td>-1.1%</td>
<td>-11.9%</td>
<td></td>
</tr>
<tr>
<td>Prophylactic</td>
<td>Range</td>
<td>(160-165)</td>
<td>(6.60-14.30)</td>
<td>(3.75-4.25)</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>160 ± 1.11 ± 0.77</td>
<td>9.9 ± 0.84</td>
<td>3.95 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>% change</td>
<td>65%</td>
<td>-14%</td>
<td>69%</td>
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<tr>
<td>% change</td>
<td>28.62%</td>
<td>16.15%</td>
<td>-14.1%</td>
<td></td>
</tr>
</tbody>
</table>

Significant P value <0.05; N.S., not significant; Pa value vs. control; Pb value vs. diabetic; Pc value treated vs. prophylactic; %change* Percent of change from control group; %change† Percent of change from diabetic group.

Figure 1: Erythrocyte membrane lipids in different studied groups. a, significant change compared to control group; b, significant change compared to diabetic group.

Phospholipids fractions (PE, PC and SM) were significantly increased in diabetic group compared to control group and decreased again by Omega-3 administration, while the mean value of PS was not changed during the experimental period (Fig. 2).

Plasma malondialdehyde was insignificantly changed in different studied groups compared to diabetic group except in the treated group which was significantly decreased, while in erythrocyte membrane it was significantly increased in diabetic group compared to the
control group, although it tended to decrease again by Omega-3 administration in treated group (Fig. 3).

Discussion

In the current study, the mean value of fasting blood sugar and insulin resistance were increased in diabetic group compared to the control group while it tended to decrease again by Omega-3 administrations in treated group and also in prophylactic one. In agreement, it was indicated that Omega-3 Polyunsaturated fatty acids may have a tissue-specific impact in restoring insulin sensitivity [25].

The measurement of membrane cholesterol gives information about the fluidity or rigidity of cell membrane, which may regulate certain structural and rheological properties of membrane essential for cell viability [26]. In this study, erythrocyte membranes had higher concentrations of cholesterol and triglycerides in diabetic group while Omega-3 administration prevented their elevation.

Enrichment of cholesterol in the membrane lowers the passive permeability of solutes and depletion of cholesterol enhances glucose permeability. This indicates reduced glucose permeability of diabetic erythrocytes is due to enhanced cholesterol content in their membranes and thus the diabetic cells might have starved from glucose [27].

In addition, TG might influence the binding of insulin to its receptor or interfere with early post binding steps [28], higher serum triglycerides leads to a resistance to the antilipolytic effect of insulin, therefore, a reduction in serum TG levels might improve insulin sensitivity [29].

In this study, membrane phospholipids increased in diabetic group and decreased again by Omega-3 administration, this result was in agreement with another study which indicated that the amount of dietary fat as well as the nature of fatty acids regulate various steps in the biosynthesis of membrane phospholipids, thus, total PL, PC, PE and SM in rats fed a diet high in saturated fat were 1.7, 1.5, 2 and 5 fold respectively higher than in rats fed on an unsaturated high fat diet [30].

Diabetes mellitus has shown to be a state of increased free radical formation [5], the oxidative damage is occur both in protein and lipid components of the
membrane [31]. Some authors have reported that the increase in lipid peroxides renders the RBC membrane rigid by altering the amino-phospholipids organization [32]. Furthermore, malondialdehyde (MDA) resulting from lipids peroxidation has been shown to affect the intrinsic mechanical properties of the RBCs membrane resulting in decreased deformability [33]. In this study Omega-3 fatty acids supplementation lead to a significant decrease in MDA, this may be due to the suggestion that Omega-3 fatty acids up regulate gene expression of antioxidant enzymes and down regulate genes associated with production of reactive oxygen species [34].

This study showed a positive correlation between insulin resistance and erythrocyte membrane SM, PE and PC which may be due to SM pathway is linked with insulin signaling through the insulin dependent glucose transporter (GLUT-4) [35]. In addition, the high membrane PE content is closely correlated with decreased protein kinase C activity, a key enzyme in insulin action [2].

We concluded that, Omega-3 fatty acids administration has a beneficial effect on increasing insulin sensitivity in diabetic rats through the improving of cell membrane content especially phospholipids fractions.

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References


