Does Omega-3 Fatty Acid Supplementation Have Beneficial Effects on Plasma Homocysteine, Insulin Resistance and Lipid Profile of Type 2 Diabetic Patients? A Randomized Clinical Trial

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**ABSTRACT**

**Background:** This study was conducted to determine the effects of n-3 PUFAs supplementation on plasma homocysteine (Hcy) level, lipid profile and insulin resistance in patients with type 2 diabetes (T2D). **Methods:** This study is a double-blind controlled trial involving 70 patients with T2D selected from Yazd Diabetes Research Center in 2013. Patients were randomly assigned to receive either 2 g/day omega-3 soft gels (OG) or 2 g/day placebo (PG) for 6 weeks. At the beginning and end of the study, Hcy concentration, fasting plasma glucose (FBG), fasting plasma insulin, total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c), HDL-c/LDL-c ratio, insulin resistance (HOMA-IR), insulin sensitivity (IS) and beta-cell function were measured and compared. **Results:** Sixty five participants completed the study. The results of this study showed that omega-3 fatty acid supplementation caused significant increase in Hcy (P = 0.007) and LDLc (P = 0.02), while HDLc and HDLc/LDLc ratio were significantly decreased (P = 0.001 and 0.006, respectively). In both groups, insulin and HOMA-IR were increased, while IS decreased significantly. Beta-cell function was increased only in OG (P = 0.005). There was no significant difference in mean change of any factors. **Conclusion:** The present study found no beneficial effects of 2 g/day omega-3 supplement for 6 weeks on biomarkers of Hcy, FBG, insulin and lipid profile in th T2D patients.

**Key words:** Type 2 diabetes; Homocysteine; Omega-3; Lipid profiles; HOMA-IR.

**Introduction**

Type 2 diabetes (T2D) has been declared “the epidemic of the 21st century” affecting approximately 347 million people worldwide (Danaei et al., 2011). Its rapidly increasing global prevalence is a primary cause of concern (Huang et al., 2012) as it is anticipated to be the 7th leading cause of death in 2030 (WHO, 2011). Diabetes and hyperglycemia cause vascular damage and impaired lipid profile, particularly increased susceptibility to peroxidation. These factors result in...
in atherosclerosis, which can lead to decreased blood flow to the heart muscle or brain (Buse et al., 2007).

Homocysteine (Hcy) is a sulfur containing amino acid [COOCH(CH2CH2SH)NH3] that is produced by the metabolism of methionine in the body and does not participate in the building of proteins (McDowell and Lang, 2000). It is known as an independent risk factor for cardiovascular disease (CVD) (Huang et al., 2012). Recent evidence suggests that high levels of fasting Hcy may be a direct participant of different diseases, including brain atrophy, cognitive impairment and possibly Alzheimer’s disease (Sachdev, 2004), diabetic nephropathy (House et al., 2010), stroke and vascular-heart disease (Elias and Eng, 2005, Smulders and Blom, 2011). Hcy has been considered to play an important role in vascular injury, resulting in the development of peripheral and coronary arterial disease (Elias and Eng, 2005). Mild homocysteinemia is an independent risk factor for atherosclerosis, atherothrombosis and may even increase the risk of CVD in people with T2D (Smulders and Blom, 2011, Zeman et al., 2006). On the other hand, in patients with T2D, serum Hcy levels are higher than normal, which is associated with endothelial dysfunction, insulin resistance, diabetic nephropathy and prothrombosis (Huang et al., 2012). High Hcy levels may independently play a direct causative role in the pathogenesis of T2D obesity and metabolic syndrome by promoting oxidative stress, systematic inflammation and endothelial dysfunction (Hofmann et al., 2001, Stamler et al., 1993, Weiss et al., 2003). In human studies, plasma Hcy levels were strongly associated with insulin concentration. As such, this association may also help to explain the discrepancy between plasma Hcy levels in diabetic patients and in healthy ones (Elias and Eng, 2005).

N-3 polyunsaturated fatty acids (n-3 PUFAs), particularly eicosapentaenoic acid (EPA, C 20:5 n-3) and docosahexaenoic acid (DHA, C 20:6 n-3), found in fish oil are known to have potential anti-atherosclerotic effects and anti-inflammatory properties and also reduce deaths due to CVD (D’Alessandro et al., 2002, Grundt et al., 2003). In addition, a high intake of marine n-3 PUFAs is associated with lower risk of CVD (Yokoyama et al., 2007). In non-diabetic people, supplementation with n-3 PUFAs have potential protective effects on cardiovascular system, such as anti-inflammatory effects, fixing atherosclerotic plaques, increasing fibrinolysis, anti-thrombotic effects and lowering of blood pressure (Hartweg et al., 2007). There is a low prevalence of diabetes in Greenland and Alaskan Eskimos; populations known for a very high intake of n-3 PUFAs (De Caterina et al., 2007). Based on previous researches, supplementation with n-3 PUFAs have been suggested as one of the methods of reducing Hcy levels (Grundt et al., 2003, Huang et al., 2012). In recent years, several studies regarding n-3 fatty acids have been conducted, but none of them have been able to completely show its effects on diabetes patients. However, the results of n-3 PUFAs effects on Hcy are still contradictory (De Caterina et al., 2007, Huang et al., 2012). Several clinical trials with small sample size and short duration of the effect of n-3 PUFAs on plasma Hcy have been performed (De Caterina et al., 2007, Selhub, 1999, Zeman et al., 2006). While few studies have been conducted on T2D (De Caterina et al., 2007).

The mechanism of action of n-3 PUFAs on blood Hcy is yet to be well understood. N-3 PUFAs effects on vascular endothelial function can neutralize the effect of Hcy (Grundt et al., 2003). But on the other hand, some studies asserted that supplementation with n-3 PUFAs may lead to increase in oxidative stress (Saedismeolia et al., 2009). Therefore, the present study was carried out to determine the effect of n-3 PUFAs supplementation on Hcy, lipid and glycemic profile in T2D patients.

**Materials and Methods**

**Patients and study design:** This study is a double-blind controlled trial involving 70 patients with T2D selected from Yazd Diabetes Research Center in 2013. Inclusion criteria included: (1) less than 60 years, (2) diagnosed diabetes, (3) a
minimum of 5 years’ experience in diabetes, (4) without any kidney, liver, heart, thyroid or bleeding disorders and malignancies, (5) not taking omega-3 supplementation during recent month, (6) without insulin therapy and (7) not pregnant or lactating. Exclusion criteria included: (1) taking less than 80% of the capsules, (2) changing the type and dose of routine medicines and (3) consumption of B vitamins supplementations during the study. Patients were randomly assigned into 2 groups, and they received either omega-3 soft gels (OG) or placebo (PG).

In previous studies, supplementation dose ranged from 200 to 6 g/day (Huang et al., 2011), but based on similar studies, an effective dose at 2 g/day was intended for this study. The OG received was 2 g/day omega-3 soft gels (Zahravi Pharmaceutical Co, Tabriz, Iran, consists of 240 mg of DHA, 360 mg EPA) and the PG received was 2 g/day placebo (Zahravi Pharmaceutical Co, Tabriz, Iran). The duration of our intervention was 6 weeks. Participants were asked not to change their lifestyle, diet, dietary patterns, physical activity and medication within the intervention. During this study, each person compulsorily took 84 capsules. At the beginning of the study, half of the capsules (42 capsules) were given to participants. After 3 weeks, they were invited to receive the second package of soft gels (another 42 soft gels). During the second session, residual capsules were counted. After completion of intervention, the remaining capsules were counted again.

Measurements: General information questionnaire including age, height, weight, sex, occupation, duration of disease, blood pressure, type and dose of medication etc., was completed. To evaluate anthropometric indices, weight was measured using digital scale (Seca, Germany) with minimal clothing and accuracy of 100 g, and height was measured with a stadiometer with an accuracy of 0.5 cm without shoes. At the beginning and end of the study, 24-h dietary recall questionnaire was used to estimate the intake of energy, macro and micronutrients, and also to check whether the person’s eating habits have changed during the study or not.

Biochemical measurements recorded were plasma fasting glucose (FBG), plasma fasting insulin, lipid profile (triglycerides (TG), HDL-c, LDL-c and total cholesterol (TC)) and serum Hcy. At the baseline and after 6-week, 10 ml of blood samples were taken after 12 h of fasting. For serum separation, samples were centrifuged at 3000 rpm for 10 min at room temperature.

Serum Hcy was measured using enzymatic cycling method (REAGENT kit by Axis-shield of England) and Alfa classic autoanalyzer (Iran). The normal range of Hcy based on the kits is 5-15 µm/L. Serum insulin was measured by ELISA method using monobind kits (made by USA) and with autoanalyzer having a sensitivity of 2 µIU per ml. Serum glucose, TG, TC and HDLc were assessed using enzymatic-colorimetric method with autoanalyzer, and the serum LDLc was calculated with FriedWald formula. In order to calculate the insulin resistance (IR), insulin sensitivity (IS) and β-cell function (B%), HOMA Calculator Software (version 2.2.2, Diabetes trials Unit University of oxford) was used.

Data analysis: In this study, to analyze the 24-h dietary recall data, Nutritionist4 was used. Data were analyzed using SPSS software v.16. Descriptive statistics were used to explain the general characteristics of the participants. Student’s t-test was used to compare the mean of variables before and after the intervention between the groups and paired t-test for within group caparison. A P-value < 0.05 was considered to be statistically significant.

Ethical considerations: Written consent was obtained from the participants before beginning the study. Entering and leaving of the study was completely voluntary, and all experiments were performed free of charge. This study was approved by the Shahid Sadoughi University of Medical Sciences Research Ethic Committee. In addition, it was registered with the Iranian Clinical Trial Registration Center (www.irct.ir) under the code of IRCT2013011312122N1.
Results

Sixty five out of 70 participants completed the study and 5 patients were excluded (Figure 1). From the 65 participants, 21 were men (52% in OG and 48% in the PG) and 44 were female (54.5% in the OG and 45.5% in the PG). Before the intervention, 71.4% and 73.3% of subjects received OG and PG, respectively and had normal serum Hcy concentration (> 15 µm/L). But at the end of the study, it was 41.2% and 57.1% that received OG and PG, respectively.

The baseline characteristics, such as age, height, weight, body mass index (BMI), and sex at the beginning of the study are shown in Table 1. There were no significant differences in baseline variables between the two groups. The dietary energy and the other nutrients intakes are shown in Table 2. No significant differences were observed in dietary intakes between the two groups.

The mean Hcy level was compared in between and within groups in Table 3. Omega-3 supplementation caused a significant increase in Hcy level as compared with placebo ($P = 0.007$). But there was not any significant difference in mean change between the groups. Table 4 shows the mean of TC, TG, LDLc, HDLc concentration and HDLc/LDLc. According to these findings, we observed no significant differences in TG and TC before and after the intervention between groups. But LDLc and HDLc/LDLc ratio were significantly increased, while HDLc concentration was significantly decreased in OG ($P < 0.05$). No significant differences were observed in mean changes of TG, TC, LDLc, HDLc concentration and HDLc/LDLc between groups.

Table 5 shows the changes of insulin, IR, IS, β-cell function and FBG before and after the study. Insulin and IR significantly increased, IS significantly decreased in both groups and β-cell function was significantly increased only in OG, while there were no differences in mean changes of fasting insulin, IR, IS, β-cell function.

![Figure 1. The Intervention framework](image-url)
Effect of omega-3 on homocysteine, insulin resistance and blood lipids in type 2 diabetes patients

Table 1. Baseline characteristics of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Omega-3 Group (N = 35)</th>
<th>Placebo Group (N = 30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>48.51 ± 6.80</td>
<td>50.66 ± 6.62</td>
<td>0.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.12 ± 7.75</td>
<td>161.97 ± 8.83</td>
<td>0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.97 ± 13.46</td>
<td>72.27 ± 12.31</td>
<td>0.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.16 ± 4.96</td>
<td>27.50 ± 4.63</td>
<td>0.8</td>
</tr>
<tr>
<td>Sex</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11 (31.4)</td>
<td>10 (33.3)</td>
<td>0.6</td>
</tr>
<tr>
<td>Female</td>
<td>24 (68.6)</td>
<td>20 (66.7)</td>
<td></td>
</tr>
</tbody>
</table>

*: Mean±SD, b: Student t-test, c: Chi square test

Table 2. Daily dietary energy and nutrients intake

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before</th>
<th>After</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy(Kcal)</td>
<td>Omega-3 Group 1450.44 ± 400.45</td>
<td>Placebo Group 1532.65 ± 634.12</td>
<td>0.43</td>
</tr>
<tr>
<td>P-value b</td>
<td>0.70</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate(g/day)</td>
<td>Omega-3 Group 143.43 ± 48.65</td>
<td>Placebo Group 165.48 ± 85.34</td>
<td>0.24</td>
</tr>
<tr>
<td>P-value</td>
<td>0.45</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>Omega-3 Group 53.21 ± 27.43</td>
<td>Placebo Group 53.23 ± 54.23</td>
<td>0.33</td>
</tr>
<tr>
<td>P-value</td>
<td>0.45</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>Omega-3 Group 60.42 ± 22.14</td>
<td>Placebo Group 65.15 ± 34.55</td>
<td>0.94</td>
</tr>
<tr>
<td>P-value</td>
<td>0.28</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Omega-3 (g/day)</td>
<td>Omega-3 Group 6.43 ± 6.51</td>
<td>Placebo Group 5.98 ± 5.83</td>
<td>0.23</td>
</tr>
<tr>
<td>P-value</td>
<td>0.64</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>B6 (mg/day)</td>
<td>Omega-3 Group 1.21 ± 0.57</td>
<td>Placebo Group 1.36 ± 0.37</td>
<td>0.54</td>
</tr>
<tr>
<td>P-value</td>
<td>0.12</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>B12 (µg /day)</td>
<td>Omega-3 Group 2.23 ± 0.98</td>
<td>Placebo Group 3.02 ± 1.45</td>
<td>0.54</td>
</tr>
<tr>
<td>P-value</td>
<td>0.12</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Folate (µg/day)</td>
<td>Omega-3 Group 210.34 ± 54.12</td>
<td>Placebo Group 189.12 ± 43.23</td>
<td>0.94</td>
</tr>
<tr>
<td>P-value</td>
<td>0.54</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

*: Mean±SD, b: Student t-test, c: Paired t-test
Table 3. Means of Homocysteine concentration (µm/L) before and after the study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
<th>P-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega-3 Group</td>
<td>13.21 ± 4.97</td>
<td>15.24 ± 5.53</td>
<td>2.02 ± 3.80</td>
<td>0.007</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>13.19 ± 4.89</td>
<td>14.40 ± 5.68</td>
<td>1.20 ± 4.82</td>
<td>0.21</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.98</td>
<td>0.57</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): Paired t-test, \(^b\): Student t-test

Table 4. Means of total cholesterol, TG, LDLc, HDLc, HDLc/LDLc concentration before and after the study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before</th>
<th>After</th>
<th>Change</th>
<th>P-value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Group</td>
<td>163.8 ± 29.77</td>
<td>163.88 ± 35.45</td>
<td>0.07 ± 31.68</td>
<td>0.98</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>184.07 ± 46.06</td>
<td>177.14 ± 45.29</td>
<td>- 6.92 ± 46.43</td>
<td>0.43</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.10</td>
<td>0.20</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Group</td>
<td>168.58 ± 105.37</td>
<td>156.51 ± 105.25</td>
<td>12.07 ± 72</td>
<td>0.33</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>155.16 ± 60.90</td>
<td>165.22 ± 97.66</td>
<td>10.05 ± 70.03</td>
<td>0.46</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.36</td>
<td>0.75</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>LDLc (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Group</td>
<td>84.26 ± 22.93</td>
<td>96.40 ± 32.36</td>
<td>12.14 ± 28.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>105.27 ± 44.78</td>
<td>96.24 ± 37.39</td>
<td>- 9.02 ± 36.71</td>
<td>0.21</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.07</td>
<td>0.97</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>HDLc (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Group</td>
<td>47.63 ± 11.63</td>
<td>40.50 ± 11.45</td>
<td>7.13 ± 10.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>47.67 ± 10.49</td>
<td>44.55 ± 8.61</td>
<td>3.12 ± 10.31</td>
<td>0.12</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.89</td>
<td>0.12</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>HDLc/LDLc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omega-3 Group</td>
<td>0.59 ± 0.18</td>
<td>0.48 ± 0.26</td>
<td>- 0.11 ± 0.21</td>
<td>0.006</td>
</tr>
<tr>
<td>Placebo Group</td>
<td>0.54 ± 0.27</td>
<td>0.51 ± 0.18</td>
<td>- 0.02 ± 0.25</td>
<td>0.59</td>
</tr>
<tr>
<td>P-value(^b)</td>
<td>0.36</td>
<td>0.49</td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): Paired t-test, \(^b\): Student t-test

Discussion

In this study, which involved 65 T2D patients, after 6 weeks of n-3 PUFAs supplementation, the overall results showed that a daily intake of 2 g n-3 PUFAs capsules caused a significant decrease in HDLc, HDLc/LDLc and IS; a significant increase in LDLc, Hcy, fasting insulin, IR and β-cell function; a reduction in TG; and an increase in FBG level. But based on mean changes, n-3 PUFAs supplementation had no positive effects on Hcy, lipid and insulin profile.

The results of the present study on Hcy are in line with some previous studies (Piolot et al., 2003) and inconsistent with some others (Grundt et al., 2003, Zeman et al., 2006). Zeman et al. (Zeman et al., 2006) recommended that 3.6 g PUFA n-3 supplementation for 3 months decreased Hcy levels in diabetic dyslipidemia. But in the present study, omega-3 supplementation was accompanied by statin-fibrate treatment. Several studies have shown that supplementation with omega-3 can reduce Hcy concentration in T2D (Benito et al., 2006, Tayebi-Khosroshahi et al., 2013). Fiedler et al.
Effect of omega-3 on homocysteine, insulin resistance and blood lipids in type 2 diabetes patients

Table 5. Means of insulin, insulin resistance, insulin sensitivity, beta cell function and fasting glucose before and after the study.

| Variables                        | Before          | After           | Change          | P-value*
|----------------------------------|-----------------|-----------------|-----------------|---------
| Insulin (mU/L)                   |                 |                 |                 |         
| Omega-3 Group                    | 7.04 ± 4.72     | 10.04 ± 4.53    | 2.99 ± 3.66     | <0.001  
| Placebo Group                    | 7.73 ± 4.98     | 11.07 ± 7.49    | 3.34 ± 4.56     | 0.001   
| P-value                          | 0.73            | 0.50            | 0.74            |         
| Fasting blood glucose (mg/dL)    |                 |                 |                 |         
| Omega-3 Group                    | 137.01 ± 40.48  | 142.53 ± 48.34  | 5.07 ± 27.19    | 0.28    
| Placebo Group                    | 150.98 ± 48.57  | 159.11 ± 48.08  | 5.09 ± 26.15    | 0.25    
| P-value                          | 0.21            | 0.18            | 0.9             |         
| Insulin resistance               |                 |                 |                 |         
| Omega-3 Group                    | 1.06 ± 0.66     | 1.52 ± 0.67     | 0.45 ± 0.49     | <0.001  
| Placebo Group                    | 1.21 ± 0.66     | 1.74 ± 0.98     | 0.52 ± 0.62     | <0.001  
| P-value                          | 0.76            | 0.28            | 0.64            |         
| Insulin sensitivity (%)          |                 |                 |                 |         
| Omega-3 Group                    | 124.03 ± 63.47  | 78.86 ± 37.42   | -45.16 ± 57.59  | <0.001  
| Placebo Group                    | 110.66 ± 73.72  | 84.05 ± 60.14   | -26.60 ± 56.13  | 0.02    
| P-value                          | 0.99            | 0.66            | 0.23            |         
| Beta cell function (%)           |                 |                 |                 |         
| Omega-3 Group                    | 46.89 ± 23.13   | 62.07 ± 34.67   | 15.18 ± 28.40   | 0.005   
| Placebo Group                    | 46.01 ± 31.38   | 51.84 ± 34.52   | 15.83 ± 25.92   | 0.29    
| P-value                          | 0.86            | 0.32            | 0.21            |         

*: Paired t-test, #: Student t-test

(Fiedler et al., 2005) administered 1.2 g of omega-3 with 11.2 g of pectin for 12 weeks to 11 hemodialysis patients. They conducted a short-term intervention and found no reduction in Hcy level. Piolot et al. (Piolot et al., 2003) observed a progressive and significant increase in Hcy concentration in 16 normolipidemic subjects, which is in line with our study. However, certain effect of omega-3 fatty acids on Hcy is equivocal. This inconsistency may be due to different target groups, supplementation doses, intervention duration, sample size, type of medications and disease progression in different studies. Fiedler (2005) suggested that high doses of omega-3 for a long time may have anti-inflammatory effects (Fiedler et al., 2005). In addition, the initial Hcy concentration in subjects may also be effective in results.

Some proposed mechanisms of the association between omega-3 and Hcy: Omega-3 PUFAs in cell membranes can cause modulation of the expression of enzymes involved in Hcy metabolism (Li et al., 2007), such as Cystathionine beta synthase. Dietary fatty acids may interact with methylene tetrahydrofolate reductase (MTHFR) and methionine adenosyl transferase I, alpha (MAT1A) genetic variants in determining the Hcy level (Huang et al., 2012). Omega-3 fatty acids may counteract the adverse effects of Hcy on endothelial function (Grundt et al., 2003), they may increase nitric oxide (NO) production and subsequently inhibit methionine synthase (Haglund et al., 1993, Zeman et al., 2006). The increased Hcy concentrations during supplementation with n-3 fatty acids may be explained by the last mechanism, but other studies are needed to identify more precise mechanisms.

According to results of the present study, there was unfavorable effect on LDLc and HDLc. Thus
there was no change in total cholesterol, and non-
significant reduction in TG concentration in OG
was observed. As such, n-3 PUFAs did not
ameliorate lipid profiles in diabetic patients. N-3
PUFAs have been shown to improve plasma lipid
profile in normal and hypertriglyceridemic
subjects in some studies (Friday et al., 1989,
Haglund et al., 1993) but in contrast, some
others did not show the same (García-Alonso
et al., 2012). The result obtained in a study by
West et al. (West et al., 2005) on diabetic
patients is in line with the finding of the present
study in terms of LDLc. Piolot (Piolot et al.,
2003), Schiano (Schiano et al., 2008) and Garci
(García-Alonso et al., 2012) in different studies
showed that supplementation with n-3 PUFAs
had not significant effects on HDLc and total
cholesterol. But another study on patients with
hyperlipidemia proved increasing HDLc
(Haglund et al., 1993). Crochemore suggested
that the reduction effects of omega-3 PUFAs on
total cholesterol and TG are dose dependent
(Crochemore et al., 2012).

The hypotriglyceridemic effect of n-3 PUFAs
have been proven, but this effect is dose
dependent (Kris-Etherton et al., 2003). Based on
previous studies, n-3 PUFAs reduced TG, mainly
by declining the production and secretion of
hepatic VLDL and also increasing the
catabolism of VLDL (Grimsgaard et al., 1997).
After n-3 PUFA supplementation, PPARα
increased which resulted in an increase in
lipoprotein lipase (LPL), thereby reducing
VLDL production (Schmidt et al., 2012). In
addition, it has been observed that the lowering
effects of omega-3 fatty acids consumption on
postprandial TG (PPTG) is significant (Kris-
Etherton et al., 2003). That ≤ 2 g of omega-3
consumption can significantly reduce
PPTG(Roche and Gibney, 1996).

In the present study, fasting insulin level and
IR were significantly increased in both groups
after the intervention, while IS was significantly
reduced, β -cell function and fasting glucose level
had increasing trend in both groups, which was
statistically significant in the intervention group.
Several studies have shown the useful effects of
omega-3 fatty acids on insulin function in animals
(Chicco et al., 1996, D’Alessandro et al., 2002,
Vessby, 2000). However, limited and conflicting
results on humans, especially on diabetic patients
are available. It has been shown that Greenland
Eskimos, with diets rich in fish, have a low
prevalence of diabetes. Giacco et al. (Giacco
et al., 2007) observed in their study that 3.6 g/day
omega-3 PUFAs supplementation on 162
healthy subjects had no effect on IS, insulin
secretion, beta-cell function and glucose
tolerance. In another study, omega-3 fatty acids
supplementation did not cause detrimental
glycemic effects (Holness MJ, 2003).

Similar to our study, In overall, n-3 PUFAs
may lead to higher glucose concentrations.
Possible mechanism may: (1) lower glucose
utilization and increase glucagon-stimulated C-
peptide (2) increase gluconeogenesis in liver, and
(3) increase glucose circulation (Kaushik et al.,
2009). Several mechanisms have been suggested
for n-3 PUFAs mediated effects on insulin
action: (1) the prevention of decrease by
phosphatidylinositol 3’ kinase (PI3 kinase)
activity; (2) prevention of the depletion of
glucose transporter protein GLUT4 in muscles;
(3) the prevention of decrease in expression of
GLUT4 in adipose tissue; and (4) inhibition of
both the activity and expression of liver glucose-
6-phosphatase (Delarue et al., 2004, Taouis et
al., 2002). With regard to the adverse effect of
omega-3 supplementation on insulin function,
some studies suggested that dioxins or methyl
mercury can disrupt insulin signaling pathways
(Kaushik et al., 2009, Lee et al., 2006).

Omega-3 fatty acids can cause oxidative stress by
increasing the production of reactive oxygen species
and intracellular antioxidant defense reduction.
Experimental studies have shown that oxidative
stress can disrupt insulin signaling and insulin
secretion. Endothelial dysfunction, due to Omega-3
fatty acids, lowers insulin delivery to insulin
sensitive tissues, which in turn impairs the insulin-
dependent glucose metabolism, leading to insulin
resistance. Dietary intake of n-3 PUFAs s may have
favorable effects on T2D prevention, but supplementation in people diagnosed with T2D seems not to be suitable.

The limitations of our study were short duration of intervention and low DHA concentration in omega-3 soft gels. To determine the pure effect and mechanisms of omega-3 fatty acids in T2D, future studies with longer periods are needed.

Conclusions
The present study found no beneficial effects of 2 g/day omega-3 supplement for 6 weeks on biomarkers of Hcy, FBG, insulin and lipid profile in patients with T2D.

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Authors’ contribution
Mozaffari-Khosravi H participated to conception and design of study, managing the project and drafting the manuscript. Naghdipour Biregani A and Poursoleiman F participated to acquisition of data, data analysis and drafting the manuscript. All authors read manuscript and they finally verified it.

Conflict of interest
The authors declare that there is no any conflict of interests.

References


Effect of omega-3 on homocysteine, insulin resistance and blood lipids in type 2 diabetes patients


