The Efficacy of Eicosapentaenoic acid Supplementation on the Serum Levels of Homocysteine and Malondialdehyde in the Patients with Type 2 Diabetes Mellitus

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Abstract: Background: The major cause of mortality among diabetic patients is cardiovascular diseases. The high plasma or serum levels of homocysteine and malondialdehyde are known as modifiable risk factors for CVDs. EPA has the antioxidant, antiinflammatory, antithrombogenic, and antiarteriosclerotic properties. Therefore, we investigated the effect of EPA supplementation on the serum levels of Hcy and MDA in the diabetic patients. **Methods:** This study was designed as a randomized, double-blind, and placebo-controlled clinical trial. Thirty six patients with type 2 diabetes were given written; informed consent, randomly were classified into 2 groups. They were supplemented with 2 g/day of the capsules of EPA or placebo. Blood sample for measurement of the serum levels of Hcy, MDA, and lipids, as well as FBS and HbA1c were given. **Results:** The patients supplemented with EPA showed a significant decrease in the serum levels of Hcy and MDA. There were no significant differences between the two groups regarding any demographic, clinical or biochemical data, total energy intake, and macronutrient intake at the baseline, and during the intervention, except for a significant increase of protein intake and the levels of HbA1c in the placebo group, and a significant increase of HDL-c, as well as a slight reduce of total cholesterol, LDL-c, TG and FBS in the supplement group. **Conclusions:** EPA is atheroprotective via decrease in the serum levels of Hcy and MDA, as well as change in the serum levels of lipids, FBS and HbA1c.
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Introduction:

Type 2 diabetes is one of the most common endocrine disorders resulting from defect in the insulin secretion, or the insulin resistance in peripheral tissues such as the liver, skeletal muscle and adipose tissue[1].

This disease is recognized as a major public health problem all over the world [2]. Over the last two decades, the prevalence of diabetes, in particular type 2 diabetes, has increased rapidly [3]. This could be because of ageing, population growth, urbanization, changes in dietary habits, obesity and sedentary lifestyle[4]. According to the estimation of International Diabetes Federation (IDF), every ten seconds two people become affected diabetes worldwide, and one person dies from complications of diabetes [5].

Homocysteine (Hcy) is a nonessential amino acid containing sulfur and a byproduct generated from the amino acid methionine; which serves as a precursor; via the biologic transmethylation reactions, and removes from the body [6]. In the normal conditions, total homocysteine (tHcy) is used to the production of RNA and DNA for making and maintenance of tissue[7], while findings of several studies among epidemiological studies and clinical trials have shown that elevated the plasma or serum levels of Hcy is well-known as an modifiable and independent risk factor for atherosclerosis in peripheral, coronary, and cerebral arterial diseases such as CVD, and in venous thrombosis [8]. Diabetic patients with the high serum levels of Hcy have a higher intima-media thickness, and are more susceptible to the harmful effects of the high serum levels of Hcy than nondiabetic individuals [9]. Therefore, research for this modifiable risk factor in the patients with type 2 diabetes mellitus is important.

Malondialdehyde (MDA) is one of highly toxic products of the peroxidation of polyunsaturated fatty acids (PUFAs) of membrane phospholipids due to exposure to reactive oxygen species (ROS) and free radicals, and it may be used as an index of the

oxidative damage of membrane, which is usually measured as thiobarbituric acid reactive substances (TBARS) reaction [10].

Eicosapentaenoic acid (EPA) is one of ω-3 PUFAs which are present at the great amounts in the fish oil [11]. The findings of several studies have shown that EPA has the antioxidant [12], antiinflammatory [13], antithrombogenic [14], and antiarteriosclerotic [15]properties. The aim of our study was to determine and compare the effects of Eicosapentaenoic acid supplementation on homocysteine and malondialdehyde in the patients with type 2 diabetes mellitus.

Hyperhomocysteinemia:

Although severe and mild hyperhomocysteinemia are respectively rare and very prevalent in the general population [16], but there is a moderate increase in the plasma concentration of Hcy in up to 8% of the general and apparently healthy population, and this extent of increase in the plasma levels of Hcy is existent in 20-40% of patients with coronary and peripheral vascular diseases [17].

The increment in plasma levels of Hcy above 9–10 μmol/L is indicating a graded independent risk factor for arteriosclerotic vascular diseases. It is believed to a 5μmol/L increment in the plasma levels of tHcy increases the risk of coronary artery disease (CAD) by as much as would cause due to an increase in the cholesterol levels of 0.5 mmol/L (20 mg/dL) [18].

Major Factors Increasing the Serum or Plasma Levels of Homocysteine:

Delays in processing blood specimens, nonfasting specimens following meals rich in protein, low intake of dietary folate, vegetarian diet (low intake of vitamin B12), impaired the absorption of folate or vitamin B12 and moderate or severe deficiency of both them, impaired renal function, increment of age, drugs affecting the Hcy metabolism, several diseases, coffee and chronic consumption of alcohol, smoking and physical inactivity and Homocystinuria (genetic defects in enzymes metabolizing Hcy such as cystathione β-synthase) [19].

Cellular Mechanisms of Homocysteine:

Possible cellular mechanisms by which Hcy may induce the endothelial dysfunction and modify the adhesive properties of endothelium; which thereby contribute to CVD; be multifactorial, including the unfolded protein response, oxidative stress and altering the balance between the production of ROS and their neutralization, inactivate nitric oxide (NO) via the formation of S-nitrosation of Hcy and decrease its bioavailability [20], the induction of proinflammatory factors [21], damaging cell survival and proliferation, increase apoptosis, the lipid metabolism and peroxidation [22], N-homocysteinylation of fibrinogen, enhanced in the platelet aggregation and activation of coagulation system [23], inhibiting anticoagulants[7], impaired vasodilatory capacity of vascular[24], and decrease in the tissue levels of ω-3 PUFAs [25].

The conversion of nonprotein amino acid Hcy to HCTL as its metabolite takes place in the cells of all tissues [26] in an error editing process during the synthesis of protein[27] by methionyl-tRNA synthetase [26], and several studies have reported that HCTL is potentially harmful on the vascular cells and may be the molecular basis of vascular damage induced by Hcy[28]. Furthermore, there is an inverse relationship between Hcy, and the hepatic expression and synthesis of HDL-c and proteins associated with HDL-c, such as lecithin-cholesterol acyltransferase (LCAT) and apolipoprotein A-1 (apo A-1), as well as Hyperhomocysteinemia results in the intensification of HDL-c catabolism and decrease in the levels and activity of HDL-c[29].

Malondialdehyde and Diabetes:

Many studies have shown that the production of MDA increases significantly in the erythrocyte membranes of diabetic patients [30], and leads to damaging in erythrocyte cells membrane, thereby, it may be has an important role not only in the progress of diabetes but also for the development of diabetes complications [31].

Material and Methods

1. Patients and Study Design:

1. 1. Patients:

The study subjects were 36 patients with type 2 diabetes mellitus who were selected from Iran Diabetes Association (Tehran, Iran). Only patients with a previous clinical diagnosis of type 2 diabetes mellitus according to the criteria for the diagnosis of diabetes as recommended by American Diabetes Association[32] were recruited.

1.1.1. Inclusion/Exclusion Criteria:

Inclusion criteria for the participation in the study were, willingness to collaborate in the study, aged 35-50 years, having a history of at least 1 year of the diagnosis of type 2 diabetes mellitus before the participation in the study based on FBS ≥126 mg/dl or 2hPG ≥200 mg/dl (2-hour plasma glucose), 25≤BMI<30 kg/m^2, identified and maintaining of the antidiabetic's drug (s) dose from 3 months ago.

Participants were excluded from the study if they had, unwillingness to continue the cooperation in the study, need to take insulin, change in the dose (s) and type of medication to the treatment of diabetes, change in the levels of physical activity, do not use (noncompliance) supplements (<10%), affected to the acute inflammatory diseases; according to the consultant physician endocrinologist.

1.2. Study Design:

The study protocol was designed as a randomized, double-blind, and placebo-controlled clinical trial. At the first, the study protocol was approved by the ethics committee of Tehran University of Medical Sciences, and all participants gave written, informed consent before the participation in the study.

The patients were randomly classified into 2 groups to the supplementation with 2 g/day of the softgels of EPA or placebo (supplied as 1-g softgels), the two groups were randomly allocated to the supplement and placebo groups by balanced permuted block on the sex. The softgels containing Eicosapentaenoic acid ethyl ester (75%) [EPA, Mino Pharmaceutical Co. Iran], or edible paraffin were provided by Mino Pharmaceutical Co., Iran. They were strictly advised to maintain their usual diets and nutritional habits, level of physical activity, and not to change their medication dose (s) during the study, as well as were asked to record and report any side effect of taking capsules gave to them.

Compliance with the supplementation was assessed by counting the number of softgels had used and the number of softgels returned to the study centerat the time of specified visits. The patients were followed up by telephone each week.

1.2.1. Nutritional Assessment:

At the beginning and at the end of the intervention, nutrients intakes were estimated using a 24-hour diet recall questionnaire for 3 days.

1.2.2. Questionnaires, Anthropometric and Biometric Measurements:

At the start and at the end of the study, each participant was evaluated with the physical examination and a general questionnaire containing questions regarding demographic variables (age, sex), anthropometric data (weight, height, waist and hip circumference, heart rate, and measurements of systolic, diastolic and mean blood pressure (SBP, DBP and MBP), and pulse pressure (PP)), family history of diseases (diabetes, hyperlipidemia and hypertension, cardiovascular, etc), age at the diagnosis of type 2 diabetes, type of the treatment and medication used, and lifestyle habits (including the history of smoking, alcohol consumption). The average of type and duration of all physical activities were measured using the International Physical Activity Questionnaire (IPAQ), at the beginning and at the end of the intervention.

Anthropometric measurements, including weight, height, as well as waist and hip circumference, and blood pressure were measured at the start and at the end of the study. Weight, changes in the level of physical activity, and any disease were recorded at the baseline and during weeks 2, 4, 6, and 8 of the intervention.

Subjects were weighed without shoes, in light indoor clothes by a Seca scale with an accuracy of ±100 g. Standing height was measured without shoes to the nearest 0.5 cm using a commercial stadiometer. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). According to the recommendation of International Diabetes Federation, hypertension was defined as blood pressure ≥130/85 mmHg [33].

Each participant gave a blood sample in the early morning after an overnight fast for 10–12 hours and before taking any oral hypoglycemic agent (s) at the beginning and at the end of intervention (8th week). Samples were drawn from the antecubital vein, and were collected into blood tubes containing EDTA or heparin. After at least 30 minutes, plasma and serum were separated by centrifugation at 3000 ×g for 10 minutes at 4 °C. Serum and plasma aliquots of each sample stored at −80 °C, for analysis of biochemical parameters [Serum levels of Hcy and MDA, FBS (fasting blood sugar), HbA1c, the serum total cholesterol (TC), triglyceride (TG), LDL-c and HDL-c]. The blood samples were collected only for this study.

1.2.3. Measurement of the Serum Levels of Homocysteine and Malondialdehyde:

The serum levels of MDA was measured colorimetrically using thiobarbiruric acid (TBA) reagent (Daiichi Pure Chemical Co. Ltd, Tokyo) dissolved in 2M sodium sulfate by heating with 1,1,3,3-tetraethoxy-propane (Tokyo Kasei Co. Ltd, Tokyo) as a standard solution [34].

1.2.4. Other Laboratory Analyses:

Serum was used for the determination of lipids and glucose. Glucose and HbA1c were measured by enzymatic methods. Serum lipid (serum total cholesterol, HDL-cholesterol, triglyceride and LDL-cholesterol) analyses were performed by spectrophotometric method (Pars azmoon, Iran).

1.2.5. Statistical Analyses:

The data were analysed using SPSS software (version 16.0 for Windows; SPSS Inc., Chicago, IL, USA), and the results are expressed as mean ± SD. The Independent t-test was used for the comparison of variables between two groups. The Paired t-test and Levene's test were also used for data analysis. 24-hour diet recalls analysed using Food processor II software [35], and the comparison of means in different intervals of 24-hour diet recalls was performed using Independent t-test. Values of $p < 0.05$ were considered statistically significant.

Results

1. Patient Characteristics:

The baseline characteristics of the two groups of patients are shown in Table 1. There were no significant differences in age, sex, duration of diabetes, weight, height, body mass index (BMI), waist circumference, hip circumference, waist/hip ratio, measurements of systolic, diastolic and mean blood pressure (SBP, DBP and MBP), pulse pressure, heart rate and biochemical data between the two groups at the baseline.

2. Dietary Intake and Lifestyle:

There were no significant differences in total energy intake, macronutrient intake, and body weight between the two groups of patients at the baseline (Table 1), and no significant changes observed during the intervention (data not shown). Medication dose (s), and the levels of physical activity from both groups had no significant difference at the baseline, and remained constant during the intervention period (data not shown).

3. Compliance and Side Effect:

All patients were fulfilled the intervention program, and were well tolerated intervention with study capsules for 8 weeks. Also, they were reported no side effects throughout the study.

4. The Serum Levels of Homocysteine and Malondialdehyde:

There were no significant differences in the serum levels of Hcy between the two groups of patients at the baseline (Table 2), whereas as shown in Table 2, the serum levels of Hcy decreased significantly ($p < 0.05$) in the EPA receiving patients compared with the placebo receiving patients.

As shown in Table 2, no statistically significant differences were observed between the two groups of patients at the baseline with regard to the serum levels of MDA, whereas the serum levels of MDA in the EPA receiving patients compared with the placebo receiving patients decreased significantly ($p < 0.001$).

5. The SerumLevels of Lipids:

The serum total cholesterol was 226.27 ± 38.73 mmol/L after receiving placebo and 207.16 ± 39.69 mmol/L after the supplementation with EPA. The serum LDL-cholesterol was 95.73 ± 29.86 mmol/L after receiving placebo and 81.4 ± 32.63 mmol/L after the supplementation with EPA. The serum HDL-cholesterol was 76.50 ± 20.81 mmol/L after receiving placebo and 101.61 ± 16.37 mmol/L after the supplementation with EPA. The serum triglycerides was 162.8 ± 158.81 mmol/L after receiving placebo and 176.48 ± 133.75 mmol/L after the supplementation with EPA (Table 3).

Discussion:

1. Functions and Molecular Mechanisms of Action of EPA:

Several studies have shown that EPA has various effects, including preventing of the insulin resistance [36], increasing the insulin secretion[37], enhancing the size of LDL-c particle[38], reducing the serum levels of triglyceride, lowering the blood viscosity, increasing the production of NO, having the antiinflammatory and antithrombotic properties[39, 40], and decreasing the blood pressure [41].

It has been demonstrated that EPA is more effective than docosahexaenoic acid (DHA) in the suppression of inflammatory response [42]. EPA plays as a substrate that decreases the production of inflammatory eicosanoids from arachidonic acid, via competing for the cyclooxygenase-2 and lipooxygenase (COX-2/LOX) enzymes. These alternative eicosanoids, which are termed E-series resolvins, have identified as a group of mediators to exert the antiinflammatory functions. Moreover, both DHA and EPA reduce the release of arachidonic acid via the inhibition of Phospholipase A2 (PLA2) [43, 44].

Also, EPA has an inhibitory role on the endotoxin-induced expression of adhesion molecules upon the endothelial cells (ECs) of human vein, and results in the excessive reduction of monocytes attached to the arterial endothelium [45].

The findings of an epidemiological study of Greenland Eskimos suggested that EPA could be has the antithrombogenic and antiarteriosclerotic properties [15]. It has been postulated that the mechanisms of these actions are including the suppression of platelet aggregation and the improvement of blood rheologic properties [46].

It has also been reported that EPA has beneficial effects on the serum levels of lipids to is suggesting that EPA may be useful as a supplement for the prevention and treatment of arteriosclerotic disease [47]. These results suggest that the administration of EPA to the patients with type 2 diabetes may prevent of the development of cardiovascular complications caused by some different risk factors. It seems that a combination of these actions and mechanisms explained above are responsible for the antiinflammatory, antiatherosclerotic, and antithrombotic effects caused by EPA.

2. Basis Evidence of Role of Homocysteine in atherothrombosis:

The strongest evidence in regard to causal role of Hcy in atherothrombosis comes from studies of severe genetic hyperhomocysteinemia in the atherothrombosis of humans[48], as well as genetic and nutritional hyperhomocysteinemia in animals [49].

3. Possible Mechanisms of Action of ω-3 PUFAs on Total Homocysteine:

Although as yet, the exact mechanism decrease in the levels of tHcy by ω-3 PUFAs is unknown, however, possible mechanisms described in the previous studies are including, the induction of oxidative stress and stimulation in the oxidative catabolism of tHcy, the alteration of cysteine/tHcy ratio via the transsulphuration pathway [7], the inhibition of methionine synthase activity and a physiologic regulation of Hcy metabolism by way increase in the production of nitrous oxide [50], and the modulation of gene expression of enzyme(s) which are involved in the formation of Hcy[51].

4. ω-3 PUFAs and Homocysteine:

The effects of ω-3 PUFAs on Hcy are contradictory, and these inconsistencies could be related to the same reasons that explained to MDA (see below), and in study performed by Grundt et al. is also mentioned [52]. Only several studies have shown that ω-3 PUFAs decrease the production of Hcy and its serum levels in humans [52]. Furthermore, in a animal study performed in this regard, Baydas et al. observed that the fish oil can decrease the plasma levels of Hcy in rats [53].

As yet, the effect of EPA on the serum levels of Hcy in vitro and in vivo was not studied, and this is the first time that has been demonstrated EPA can decrease the serum levels of Hcy in vivo. Our present study clearly shows that the supplementation of EPA for 8 weeks in the patients with type 2 diabetes mellitus leads to a significant reduction in the serum levels of Hcy than the placebo group (Table 2).

5. Mechanisms of Action of ω-3 PUFAs on malondialdehyde:

Potential mechanisms for reducing in the levels of MDA by ω-3 PUFAs may be associated with the assembly of ω-3 PUFAs in the membrane lipids and lipoproteins, which this results in the less available of double bonds for free radical attack, inhibition of the PLA2 prooxidant enzyme, up regulation of the gene expression and stimulation in the activity of antioxidant enzymes, as well as down regulation of the genes related to the production of ROS [34, 54].

6. ω-3 PUFAs and Malondialdehyde:

The data relating to the effects of ω-3 PUFAs on MDA in vivo are contradictory[55], and these inconsistencies can be attributed to several factors, such as discrepancies in the population studied, the duration of study, the content of ω-3 PUFA in the supplement or the history of diet. However, the most acceptable explanation is differences in the methodologies used to assessment of lipid peroxidation [56]. Only in a small number of studies have reported that ω-3 PUFAs decrease lipid peroxidation and levels of TBARS [34, 57]. In addition, in an animal study on KKAy/Tamice was observed that EPA can decrease the serum levels of MDA [58].

Our findings clearly show that the supplementation of EPA for 8 weeks in the patients with type 2 diabetes mellitus significantly reduces the serum levels of MDA (Table 2). This is the first time that has been demonstrated EPA can decrease the serum levels of MDA in vivo, and this finding is in accordance with that of animal model studied in this regard.

Thus, it is significant to point out that our data provide evidence compatible with the hypothesis that EPA influences the serum levels of Hcy and MDA in the patients with type 2 diabetes mellitus.

7. ω-3 PUFAs and the lipid profile

Meanwhile, several studies have shown that the ω-3 PUFAs have various effects on the lipid profile in type 2 diabetic patients, including enhancing the size of LDL-c particle [59], reducing the serum levels of TG [60], increasing the plasma levels of HDL-c and HDL2-c [60, 61], and decreasing the plasma levels of HDL3-c [60]. This study demonstrated that EPA can significantly increase the serum levels of HDL-c which is compatible with the results in the other studies with ω-3 PUFAs [60, 61], but did not significantly affect the other serum levels of lipids.

8. The study limitations:

There were several limitations for our study. First, a relatively small sample size of patients, therefore, it should point out that the results of our study are preliminary and need to be confirmed in a larger sample size of patients. Second, the exact mechanism by which EPA decrease the serum levels of Hcy and MDA has not been clarified, and further work is necessary to delineate the molecular mechanism of action of EPA on the regulation of serum levels of Hcy and MDA. Third, it is better and important that the serum levels of AdoMet, AdoHcy, AdoMet/AdoHcy ratio, CPR, and inflammatory cytokines, as well as the percentage of EPA in the membrane of RBC measure in the further studies. For these reasons, additional studies will be necessary to determine the general applicability of our study results.

Conclusions:

From these findings it can be concluded that the supplementation of EPA is very effective in decrease of the oxidative stress through an improvement in the serum levels of Hcy and MDA, which may contribute in the prevention of vascular complications in the patients with type 2 diabetes mellitus.

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References

1. Nyenwe, E.A., et al., *Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes.* Metabolism, 2011. 60(1): p. 1-23.
2. Blonde, L., *State of diabetes care in the United States.* Am J Manag Care, 2007. 13 Suppl 2: p. S36-40.
3. Shaw, J.E., R.A. Sicree, and P.Z. Zimmet, *Global estimates of the prevalence of diabetes for 2010 and 2030.* Diabetes Res Clin Pract, 2010. 87(1): p. 4-14.
4. Hu, F.B., et al., *Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women.* JAMA, 2003. 289(14): p. 1785-91.
5. Federation, I.D., *Diabetes Atlas*. 3rd edn ed. 2006: Brussels: International Diabetes Federation.
6. Refsum, H., et al., *Facts and recommendations about total homocysteine determinations: an expert opinion.* Clin Chem, 2004. 50(1): p. 3-32.
7. Beavers, K.M., et al., *Omega-3 fatty acid supplementation and total homocysteine levels in end-stage renal disease patients.* Nephrology (Carlton), 2008. 13(4): p. 284-8.
8. Antoniades, C., et al., *Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials.* Eur Heart J, 2009. 30(1): p. 6-15.
9. Becker, A., et al., *Plasma homocysteine and S-adenosylmethionine in erythrocytes as determinants of carotid intima-media thickness: different effects in diabetic and non-diabetic individuals. The Hoorn Study.* Atherosclerosis, 2003. 169(2): p. 323-30.
10. Karatas, F., M. Karatepe, and A. Baysar, *Determination of free malondialdehyde in human serum by high-performance liquid chromatography.* Anal Biochem, 2002. 311(1): p. 76-9.
11. Hagiwara, S., et al., *Eicosapentaenoic acid ameliorates diabetic nephropathy of type 2 diabetic KKAy/Ta mice: involvement of MCP-1 suppression and decreased ERK1/2 and p38 phosphorylation.* Nephrol Dial Transplant, 2006. 21(3): p. 605-15.
12. Demoz, A., N. Willumsen, and R.K. Berge, *Eicosapentaenoic acid at hypotriglyceridemic dose enhances the hepatic antioxidant defense in mice.* Lipids, 1992. 27(12): p. 968-71.
13. Figueras, M., et al., *Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status.* Obesity (Silver Spring), 2011. 19(2): p. 362-9.
14. Nomura, S., S. Kanazawa, and S. Fukuhara, *Effects of eicosapentaenoic acid on platelet activation markers and cell adhesion molecules in hyperlipidemic patients with Type 2 diabetes mellitus.* J Diabetes Complications, 2003. 17(3): p. 153-9.
15. Dyerberg, J., et al., *Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis?* Lancet, 1978. 2(8081): p. 117-9.
16. Anderson, J.L., et al., *Plasma homocysteine predicts mortality independently of traditional risk factors and C-reactive protein in patients with angiographically defined coronary artery disease.* Circulation, 2000. 102(11): p. 1227-32.
17. Vollset, S.E., et al., *Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study.* Am J Clin Nutr, 2001. 74(1): p. 130-6.
18. Hankey, G.J. and J.W. Eikelboom, *Homocysteine and vascular disease.* Lancet, 1999. 354(9176): p. 407-13.
19. De Bree, A., et al., *Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease.* Pharmacol Rev, 2002. 54(4): p. 599-618.
20. Upchurch, G.R., Jr., et al., *Homocyst(e)ine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase.* J Biol Chem, 1997. 272(27): p. 17012-7.
21. Garaliene, V., *[The main determinants of endothelial dysfunction]*. Medicina (Kaunas), 2006. 42(5): p. 362-9.
22. Jakubowski, H., *The pathophysiological hypothesis of homocysteine thiolactone-mediated vascular disease.* J Physiol Pharmacol, 2008. 59 Suppl 9: p. 155-67.
23. Undas, A., et al., *Plasma homocysteine affects fibrin clot permeability and resistance to lysis in human subjects.* Arterioscler Thromb Vasc Biol, 2006. 26(6): p. 1397-404.
24. Dayal, S. and S.R. Lentz, *ADMA and hyperhomocysteinemia.* Vasc Med, 2005. 10 Suppl 1: p. S27-33.
25. Severus, W.E., A.B. Littman, and A.L. Stoll, *Omega-3 fatty acids, homocysteine, and the*

increased risk of cardiovascular mortality in major depressive disorder. Harv Rev Psychiatry, 2001. 9(6): p. 280-93.
26. Billecke, S., et al., *Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters.* Drug Metab Dispos, 2000. 28(11): p. 1335-42.
27. Jakubowski, H., *Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation.* J Biol Chem, 2000. 275(6): p. 3957-62.
28. Jakubowski, H., *The molecular basis of homocysteine thiolactone-mediated vascular disease.* Clin Chem Lab Med, 2007. 45(12): p. 1704-16.
29. Liao, D., et al., *Hyperhomocysteinemia decreases circulating high-density lipoprotein by inhibiting apolipoprotein A-I Protein synthesis and enhancing HDL cholesterol clearance.* Circ Res, 2006. 99(6): p. 598-606.
30. Kesavulu, M.M., et al., *Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease.* Diabetes Res Clin Pract, 2001. 53(1): p. 33-9.
31. Devaraj, S. and I. Jialal, *Low-density lipoprotein postsecretory modification, monocyte function, and circulating adhesion molecules in type 2 diabetic patients with and without macrovascular complications: the effect of alpha-tocopherol supplementation.* Circulation, 2000. 102(2): p. 191-6.
32. Association, A.D., *Clinical practice recommendations.* Diabetes Care 2010. 33: p. S1-S100.
33. Alberti, K.G., P. Zimmet, and J. Shaw, *International Diabetes Federation: a consensus on Type 2 diabetes prevention.* Diabet Med, 2007. 24(5): p. 451-63.
34. Kesavulu, M.M., et al., *Effect of omega-3 fatty acids on lipid peroxidation and antioxidant enzyme status in type 2 diabetic patients.* Diabetes Metab, 2002. 28(1): p. 20-6.
35. Stark, K.D., et al., *Effect of a fish-oil concentrate on serum lipids in postmenopausal women receiving and not receiving hormone replacement therapy in a placebo-controlled, double-blind trial.* Am J Clin Nutr, 2000. 72(2): p. 389-94.
36. Fedor, D. and D.S. Kelley, *Prevention of insulin resistance by n-3 polyunsaturated fatty acids.* Curr Opin Clin Nutr Metab Care, 2009. 12(2): p. 138-46.
37. Mustad, V.A., et al., *Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse.* Metabolism, 2006. 55(10): p. 1365-74.
38. Suzukawa, M., et al., *Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages.* J Lipid Res, 1995. 36(3): p. 473-84.
39. Okuda, Y., et al., *Eicosapentaenoic acid enhances nitric oxide production by cultured human endothelial cells.* Biochem Biophys Res Commun, 1997. 232(2): p. 487-91.
40. Kawano, H., et al., *Changes in aspects such as the collagenous fiber density and foam cell size of atherosclerotic lesions composed of foam cells, smooth muscle cells and fibrous components in rabbits caused by all-cis-5, 8, 11, 14, 17-icosapentaenoic acid.* J Atheroscler Thromb, 2002. 9(4): p. 170-7.
41. Miyajima, T., et al., *Effects of eicosapentaenoic acid on blood pressure, cell membrane fatty acids, and intracellular sodium concentration in essential hypertension.* Hypertens Res, 2001. 24(5): p. 537-42.
42. Verlengia, R., et al., *Comparative effects of eicosapentaenoic acid and docosahexaenoic acid on proliferation, cytokine production, and pleiotropic gene expression in Jurkat cells.* J Nutr Biochem, 2004. 15(11): p. 657-65.
43. Serhan, C.N., et al., *Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions.* J Physiol Pharmacol, 2000. 51(4 Pt 1): p. 643-54.
44. Serhan, C.N., et al., *Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals.* J Exp Med, 2002. 196(8): p. 1025-37.
45. Kim, D.N., J. Schmee, and W.A. Thomas, *Dietary fish oil added to a hyperlipidemic diet for swine results in reduction in the excessive number of monocytes attached to arterial endothelium.* Atherosclerosis, 1990. 81(3): p. 209-16.
46. Terano, T., et al., *Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects.* Atherosclerosis, 1983. 46(3): p. 321-31.
47. Singer, P., et al., *Lipid and blood pressure-lowering effect of mackerel diet in man.* Atherosclerosis, 1983. 49(1): p. 99-108.
48. Kluijtmans, L.A., et al., *The molecular basis of cystathionine beta-synthase deficiency in Dutch patients with homocystinuria: effect of CBS genotype on biochemical and clinical phenotype*

and on response to treatment. Am J Hum Genet, 1999. 65(1): p. 59-67.
49. Lentz, S.R., *Mechanisms of homocysteine-induced atherothrombosis.* J Thromb Haemost, 2005. 3(8): p. 1646-54.
50. Zeman, M., et al., *N-3 fatty acid supplementation decreases plasma homocysteine in diabetic dyslipidemia treated with statin-fibrate combination.* J Nutr Biochem, 2006. 17(6): p. 379-84.
51. Li, D., et al., *Platelet phospholipid n-3 PUFA negatively associated with plasma homocysteine in middle-aged and geriatric hyperlipaemia patients.* Prostaglandins Leukot Essent Fatty Acids, 2007. 76(5): p. 293-7.
52. Grundt, H., et al., *Reduction in homocysteine by n-3 polyunsaturated fatty acids after 1 year in a randomised double-blind study following an acute myocardial infarction: no effect on endothelial adhesion properties.* Pathophysiol Haemost Thromb, 2003. 33(2): p. 88-95.
53. Baydas, G., et al., *Effects of certain micronutrients and melatonin on plasma lipid, lipid peroxidation, and homocysteine levels in rats.* Arch Med Res, 2002. 33(6): p. 515-9.
54. Takahashi, M., et al., *Fish oil feeding alters liver gene expressions to defend against PPARalpha activation and ROS production.* Am J Physiol Gastrointest Liver Physiol, 2002. 282(2): p. G338-48.
55. Nenseter, M.S. and C.A. Drevon, *Dietary polyunsaturates and peroxidation of low density lipoprotein.* Curr Opin Lipidol, 1996. 7(1): p. 8-13.
56. Mori, T.A., et al., *Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects.* Free Radic Biol Med, 2003. 35(7): p. 772-81.
57. Shidfar, F., et al., *Effects of omega-3 fatty acid supplements on serum lipids, apolipoproteins and malondialdehyde in type 2 diabetes patients.* East Mediterr Health J, 2008. 14(2): p. 305-13.
58. Zhang, M., et al., *Effects of eicosapentaenoic acid on the early stage of type 2 diabetic nephropathy in KKA(y)/Ta mice: involvement of anti-inflammation and antioxidative stress.* Metabolism, 2006. 55(12): p. 1590-8.
59. Patti L, M.A., Iovine C, et al, *Long term effects of fish oil on lipoprotein subfractions and low density lipoprotein size in non-insulin-dependent diabetic patients with hypertriglyceridemia.* Atherosclerosis, 1999. 146: p. 361-367.
60. Woodman, R.J.M., T. A. Burke, V. Puddey, I. B. Watts, G. F. Beilin, L. J., *Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension.* Am J Clin Nutr, 2002. 76(5): p. 1007-15.
61. Luo, J.R., S. W. Vidal, H. Oppert, J. M. Colas, C. Boussairi, A. Guerre-Millo, M. Chapuis, A. S. Chevalier, A. Durand, G. Slama, G., *Moderate intake of n-3 fatty acids for 2 months has no detrimental effect on glucose metabolism and could ameliorate the lipid profile in type 2 diabetic men. Results of a controlled study.* Diabetes Care, 1998. 21(5): p. 717-24.

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