Diabetic cardiac autonomic neuropathy: insulin resistance, lipid profile, and omega-3 polyunsaturated fatty acids

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Abstract.
The significance of cardiac autonomic neuropathy (CAN) in patients with type 2 diabetes mellitus (T2DM) has been not fully appreciated and there is no unified treatment algorithm. Aim: The aim of study was to investigate the effects of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) on blood lipid profile and insulin resistance (IR) parameters in patients with T2DM and definite CAN. The study involved 33 patients with T2DM and definite CAN. Patients were allocated into two treatment groups: 1st group - 15 patients received standard hypoglycaemic therapy - control (n = 15); 2nd group (n = 18) - standard hypoglycaemic therapy and 1 capsule/day of the ω-3 PUFAs (1 g, including ~90 % ω-3 PUFAs) for three months. The concentrations of glucose, glycated haemoglobin A1c, immunoreactive insulin in the blood were determined. Lipid metabolism was assessed by the concentration of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG) measurements. The insulin resistance Homeostasis model assessment, atherogenic coefficient (AC), TG/LDL-C, TG/TC, TG/LDL-C and TG glucose (TyG) index were calculated. Obtained results of our study could witness that the prescription of ω-3 PUFAs was accompanied by a statistically significant decrease in TG concentration; AC, TG/LDL-C, TG/TC, TG/LDL-C and TG glucose (TyG) index parameters and increase in HDL-C levels (compared to control). Conclusions. Obtained results justify the appropriateness of ω-3 PUFAs prescriptions to patients with T2DM and definite CAN.

Keywords:
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**Introduction**

It was estimated that there were 415 million people with diabetes mellitus (DM) aged 20-79 years in 2015, and the number was predicted to rise to 642 million by 2040 [1]. The development of cardiac autonomic neuropathy (CAN) is associated with the lesion of the autonomic nervous system, and maybe accompanied by coronary vessels ischemia, arrhythmias, “silent” myocardial infarction, severe orthostatic hypotension and sudden death syndrome [2-6]. Therefore, the problem of effective treatment of CAN is particularly relevant. Pathogenetic treatment of CAN includes: balanced diet and physical activity; reducing insulin resistance; optimization of glycaemic control; treatment of dyslipoproteinemia (DLP); correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors; γ-linolenic acid, acetyl-L-carnitine, antioxidants, use of ω-3 polyunsaturated fatty acids (ω-3 PUFAs), vasodilators, fat-soluble vitamin B1, aminoguanidine; symptomatic treatment of concomitant diseases and syndromes [hypertension, coronary heart disease (CHD), heart failure and arrhythmias] and others [7-10]. Numerous studies report salutary effects of ω-PUFAs, i.e. eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) on cardiovascular diseases (CVD) risk factors. These effects include lowering of serum triglyceride (TG) by reducing of hepatic TG production; lowering of blood pressure (BP) by improving of endothelial cell function; decreasing of platelet aggregation by reducing of prothrombotic prostanoids; decreasing inflammation via reduction in 4-series leukotrienes production; protection from arrhythmias by modulation of electrophysiological properties of cardiomyocytes. Systematic meta analysis suggests that high doses of ω-3 PUFAs (~3 g/day) produce a small, but significant decrease in systolic BP in older and hypertensive subjects [11-12]. The aim of this study was to investigate the effects of omega-3 polyunsaturated fatty acids on blood lipid profile, triglyceride-glucose index and insulin resistance parameters in patients with type 2 diabetes mellitus and definite cardiac autonomic neuropathy.

**Materials and methods**

The study involved 33 patients with type 2 DM (T2DM) and
definite CAN. Median age of patients was 55.1 ± 0.63 yrs, disease duration - 3.52 ± 0.29 yrs and median glycated haemoglobin (HbA1c) - 7.09% ± 0.12%. CAN was diagnosed according to previously proposed criteria [2]. The work was done according to the principles of the Helsinki Declaration II and was approved by the Medical Ethics Committee of Danylo Halytsky Lviv National Medical University. All participants signed informed consent prior to their inclusion into the study. First group received traditional antihyperglycaemic therapy (n = 15, control group) for three months; patients in group 2 (n = 18), received in addition to standard treatment 1 capsule/day of the ω-3 PUFAs for three months. The capsule contains 1 g, including ~90 % ω-3 PUFAs, mainly EPA and DHA and 4 mg of α-tocopherol acetate. The duration of the treatment was three months.

The concentration of glucose in the blood was determined by the glucose oxidase method while HbA1c level was assessed by using a highly sensitive method of ion-exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States). Determination of immunoreactive insulin (IRI) was performed using commercial kits from Immunotech insulin immunoradiometric assay reagents (Czech Republic). Lipid metabolism was assessed by the concentration of total cholesterol (TC), TG, low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C); atherogenic coefficient (AC), TG/LDL-C, TC/TG, TG/HDL-C parameters. The TG glucose (TyG) index was calculated by the Ln [fasting TG (mg/dL) x fasting glucose (mg/dL)/2] [13]. TyG index, the product of fasting glucose and TG in the blood, has been proposed as a simple method for determining insulin resistance (IR) in healthy subjects [14-15]. The use of the homeostasis model assessment (HOMA) IR (HOMAIR), [16,17] the insulin suppression test, [14] and the hyperinsulinemic-euglycemic clamp [16,17] suggested that the TyG index correlates with IR [18]. TyG indexes are reported to be elevated in T2DM patients compared to parameters in patients with prediabetes [18-20]. The lipid fractions were determined by using HUMAN reagents (Germany) for the analyzer Humanlayzer 2000. HOMA-IR index was calculated according to the formula: fasting IRI (mIU/mL) x fasting glucose (mmol/L)/22.5 [21]. Resting 12-lead surface
electrocardiography (ECG) with a paper speed of 25 mm/s and a signal size of 10 mm/mV was recorded in the morning period. We performed resting ECG analysis included measurement of the following parameters: heart rhythm, heart rate, conduction intervals, and Holter-ECG [(ECG “EC-3H” (“Labtech,” Hungary)] analysis included measurement of 24 hours ECG, circadian indexes and heart rate variability parameters. Statistical analysis Statistical analysis was based on the variational method using a statistical parametric t-test, nonparametric Wilcoxon t-test, and Fisher's Pearson correlation coefficient. Data are presented as mean ± standard error of the mean (SEM). All tests were performed using the ANOVA (MicroCal Origin v. 8.0) software. Statistical significance was set at p < 0.05.

Results

We found out that the HbA1c of patients with T2DM and definite CAN was not statistically significantly influenced by the treatment (p > 0.05).

As a result of our study, we found out that the use of ω-3 PUFAs do not affect the concentration of IRI and HOMA-IR parameters. As we have previously reported, the definite CAN in patients with T2DM is characterized by an increase in IRI concentration (+136.04%) compared to healthy volunteers (p < 0.001); compared to patients without CAN (p < 0.001); compared to patients with subclinical CAN (p < 0.001), and HOMA-IR (+240.82% compared to healthy volunteers (p < 0.001); compared to patients without CAN (p < 0.01). Therefore, the most statistically significant hyperinsulinaemia (determined according to the IRI concentration) as well as IR (HOMA-IR) were verified in patients with T2DM and definite CAN[5]. The definite CAN is characterized by a significant increase in the content of TC, TG; LDL-C, and a significant decrease in the concentration of HDL-C [5].

Treatment with the ω-3 PUFAs among patients with T2DM and definite CAN lead to a significant increase of the HDL-C level [+9.73 ± 2.57% (p < 0.05)] and decrease TG concentration [-33.35 ± 2.73%, p < 0.001]. Obtained results of this study could prove that prescription of ω-3 PUFAs is accompanied by hypolipidaemic effect without influence on glucose metabolism.
As a result of our study, we found out that the use of ω-3 PUFAs was accompanied by a statistically significant decrease in the AC, TG/LDL-C, TG/TC, TG/HDL-C, and TyG index parameters (compared with the control group).

**Discussion**

Several experimental studies have shown that long-chain ω-PUFAs inhibit the absorption of cholesterol in the intestine and its synthesis in the liver, lead to increased clearance of lipoproteins in the blood, prevent the development of IR in experimental diabetes, increase the level of glucose transporter 4 in skeletal muscles, have a positive effect on age related decrease of blood flow in the brain and improve glucose utilization under stress; there isn't any influence on the development of hypertension and metabolic syndrome. Ω-3 PUFAs decrease level of BP, dose-dependent prevent the development of T2DM, IR, contribute to positive changes of blood coagulation parameters; enhance endothelial cell migration and inhibits the proliferation of smooth muscle cells. [22] A meta-analysis of 18 studies found a significant effect of fish oil to lower TG concentrations and increase HDL-C in the blood; while there were no statistically significant changes in preprandial glucose, HbA1c, TC, LDH-C levels. Ω-3 PUFAs may affect the IR and glucose homeostasis by inhibition of IR in the muscle tissue > adipose tissue >> liver, inhibition of insulin secretion, which defer the development of T2DM; and on the state of lipid metabolism (in particular, reduce the concentration of TG, very low density-lipoprotein cholesterol (VLDL-C), increase of HDLC, improve lipid profile by mixed hyperlipidaemia, slightly decrease BP, improve endothelial function, have an positive impact on the antioxidant status and inflammatory reactions[23] . Ω-3 PUFAs decrease VLDL assembly and secretion, resulting in diminished TG production, through a decreased sterol receptor element binding protein-1c activity. [24,23] Clinical trials clearly suggest beneficial effects of ω-3 PUFAs consumption on plasma TG levels. A review of placebo-controlled human studies concludes that an average intake of 3-4 g·day⁻¹ of ω-3 PUFAs decreases serum TG concentrations by 25-30% in a dose-dependent manner. The same intake does not affect TC but
MEDICINE AND PHARMACY

increases LDL levels by 5-10% and HDL by 1-3%. [23] The increase in LDL is mainly through a rise in amounts of the larger, more buoyant and potentially less atherogenic LDL particles, whereas the smaller, denser and potentially more atherogenic LDL particles decrease. [22,23,25] Some studies reported that prescription of EPA and DHA to patients with severe lipid metabolism disorders (in particular with hypertriglyceridemia, primary hypercholesterolaemia, combined DLP, family combined DLP, CHD, and persistent hypertriglyceridemia) decreased TC levels or did not affect it and increased LDL-C, HDL-2 and apolipoprotein A concentrations. [26] Results of several studies, including GISSI and JELIS, showed only insignificant changes of TG levels. [22,23] Prescription of ω-3 PUFAs resulted in positive changes in lipid metabolism profile in a patient with T2DM and DLP, which was characterized by increased TG and reduced HDL-C levels. However, studies of ω-3 PUFAs prescription to patients with DM without diagnosed CHD (despite accumulated evidence that T2DM showing as the equivalent of CHD [27-29] are few and obtained results do not suggest their effectiveness. The highly concentrated pharmaceutical preparation Omacor™ (Pronova Biocare, Lysaker, Norway), known as Lovaza™ (GlaxoSmithKline, St Petersberg, FL, US) in North America is approved by the FDA as an adjunct to diet to reduce very high TG levels (≥ 500 mg·dL⁻¹ ) in adults. Each 1-g capsule of ω-3-acid ethyl esters contains ethyl esters of EPA (0.465 g) and DHA (0.375 g). Patients take a q.d. dose of 4-g or two 2-g doses (two capsules b.i.d.) [30] . Clinical trials have shown that administration of 4 g·day⁻¹ of Lovaza™ results in a decrease in TG levels of 30- 50%; does not affect the efficacy of statins. [31] In patients with combined DLP, coadministration of Lovaza™ with statins was a safe and effective means of lowering serum TG, despite the persistent high TG levels when the patients received statins alone. [32] The relative risk of CHD in patients who consumed fish less than one time per month was 0.70, 1- 3 times a month - 0.60, once a week - 0.64, 5 times a week - 0.36. Thus, a higher consumption of fish (including ω-3 PUFAs) contributed to the reduction of CHD incidence and
reduced total mortality rate significantly. However, effects of $\omega$-3 PUFAs on the development/progression of CHD and mortality in patients with DM are not entirely understood. The question of the feasibility and additional benefits of $\omega$-3 PUFAs administration in combination with statins to avoid polypragmasia in the treatment of diabetic vascular disorders is open. [26,23] Therefore, dietary consumption of $\omega$-3 PUFAs is recommended in international guidelines for the general population to prevent the occurrence of CHD. However, the precise mechanisms underlying the cardioprotective effects of $\omega$-3 PUFAs are not fully understood. $\Omega$-3 PUFAs can be incorporated into the phospholipid bilayer of cell membranes and can affect membrane fluidity, lipid microdomain formation, and signaling across membranes. $\Omega$-3 PUFAs also modulate the function of membrane ion channels, such as Na+ and L-type Ca2+ channels, to prevent lethal arrhythmias. Moreover, $\omega$-3 PUFAs also prevent the conversion of arachidonic acid into pro-inflammatory eicosanoids by serving as an alternative substrate for cyclooxygenase or lipoxygenase pathways, resulting in the production of less potent products. In addition, a number of enzymatically oxygenated metabolites derived from $\omega$-3 PUFAs were recently identified as anti-inflammatory mediators. These $\omega$-3 metabolites may contribute to the beneficial effects against CHD that are attributed to $\omega$-3 PUFAs. [33,34].

Conclusion

Development of hyperinsulinemia and IR among patients with T2DM and CAN are accompanied by atherogenic changes in lipid profile namely by an increase in TC, TG, LDL-C, AC, TyG index and decrease in HDL-C. The most pronounced atherogenic changes observed among patients with definite CAN. Obtained results could witnes about the essential importance of hyperinsulinaemia, IR and DLP in the pathogenesis of the CAN. Prescription of $\omega$-3 PUFAs contributed to a statistically significant decrease in the concentration of TC, LDL-C, TG, AC, TG/LDL-C, TG/TC, TG/HDL-C, TyG index parameters, and an increase in the content of HDL-C (compared to the control). Our results suggest that the efficacy of $\omega$-3 PUFAs is not associated with improved glycemic control of T2DM in patients.
with definite stage of CAN, but is rather the result of a direct effect of the pharmacological agent on the investigated metabolic indexes. Therefore, the appointment of \( \omega-3 \) PUFAs is necessary in the treatment of DLP in patients with T2DM with definite CAN. However, existing data are not consistent perhaps due to a significant heterogeneity (variable doses of \( \omega-3 \) PUFAs, different duration of intake, different populations, and end-points) in the interventional studies. For prevention/treatment of CAN events supplementation with \( \omega-3 \) PUFAs should be integrated into a more global strategy that includes focusing on other components of a healthy lifestyle (diet, weight control, physical activity, smoking cessation) and on tight control of glucose and lipid profile when indicated. Thus, further research to understand the mechanism of action and confirm the beneficial effect of \( \omega-3 \) PUFAs on BP profile, artery stiffness, and heart rate variability parameters is needed.

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**References:**


