EFFECTS OF OMEGA-3 POLYUNSATURATED FATTY ACIDS ON THE CIRCADIAN RHYTHM OF HEART RATE VARIABILITY PARAMETERS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CARDIOVASCULAR AUTONOMIC NEUROPATHY

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Aim. The aim of the study was to analyze the effect of ω-3 polyunsaturated fatty acids (ω-3 PUFAs) on the heart rate variability (HRV) parameters in patients with type 2 diabetes mellitus (T2DM) and advanced stage of cardiovascular autonomic neuropathy (CAN).

Material and methods. We have examined 36 patients with T2DM and advanced stage of CAN, aged between 50-59 years with disease duration 1-6 years and median glycated hemoglobin A1c 7,1%±0,12%. Patients with T2DM and advanced stage of CAN were divided into 2 groups. The first group received traditional antihyperglycemic therapy (n=15, control) for three mo; patients in group 2 (n=21) received in addition 1 g/day of the ω-3 PUFAs for three months.

Results. Prescription of the ω-3 PUFAs to the patients with T2DM and advanced stage of CAN was accompanied by a statistically significant increase of the time-domain HRV parameters; the spectral HRV parameters during the active and passive periods compared to the control group.

Conclusion. Obtained results suggest that the efficacy of ω-3 PUFAs is the result of a direct effect of the ω-3 PUFAs on the investigated indexes.

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ВЛИЯНИЕ ОМЕГА-3 ПОЛИНЕНАСЫЩЕННЫХ ЖИРНЫХ КИСЛОТ НА ЦИРКАДНЫЕ РИТМЫ ВАРИАБЕЛЬНОСТИ РИТМА СЕРДЦА ПРИ САХАРНОМ ДИАБЕТЕ 2 ТИПА И СЕРДЕЧНОЙ АВТОНОМНОЙ НЕЙРОПАТИИ

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Цель. Проанализировать влияние ω-3 полиненасыщенных жирных кислот (ω-3 ПНЖК) на параметры вариабельности ритма сердца (ВРС) у пациентов с диабетом 2 типа (СД2) и развёрнутой стадией сердечно-сосудистой автономной полинейропатии (САП).

Материал и методы. Мы изучили данные 36 пациентов с СД2 и развёрнутой стадией САП, возраст 50-59 лет, с длительностью заболевания 1-6 лет с медианой уровня гликированного гемоглобина 7,1%±0,12%. Пациенты были разделены на 2 группы. Первая группа получала стандартную гипогликемическую терапию (n=15, контроль); пациенты из группы 2 (n=21) получали дополнительно 1 г в день ω-3 ПНЖК в течение 3 месяцев.

Результаты. Назначение ω-3 ПНЖК пациентам с СД2 и развёрнутой стадией САП сопровождалось статистически значимым увеличением временных параметров ВРС; спектральные параметры ВРС в активный и пассивный периоды были сравнимы с таковыми у контроля.

Заключение. Полученные результаты предполагают, что эффективность ω-3 ПНЖК является результатом прямого влияния ω-3 ПНЖК на изученные показатели.

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Diabetes mellitus (DM) is a global epidemic affecting at least 8,3% of the population and 371 million people worldwide with a significant proportion (~50%) remaining undiagnosed. Approximately one in six people are currently at risk of developing diabetes-related complications [1].

The majority of patients with chronic DM [mainly type 2 diabetes mellitus (T2DM)] are diagnosed with coronary heart disease (CHD) due to coronary artery atherosclerosis. Often the course of CHD is complicated by a combination of hypertension, specific kidney arterial involvement, eyes and lower limbs affection. Metabolic alterations in the myocardium are combined with early coronary atherosclerosis. All these changes in the heart occur over a prolonged duration of DM among middle age, and elderly patients

[coronary vessels affection, myocardium changes, diabetic cardiovascular autonomic neuropathy (CAN) and sclerotic arterial disease] are associated with the term "diabetic heart" or "diabetic cardiomyopathy" [2].

Based on the CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy [3], CAN is defined as the impairment of cardiovascular autonomic control among patients with established DM following the exclusion of other causes. Cardiac autonomic neuropathy among T2DM patients, is characterized by lesion of nerve fibers in the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS), is diagnosed unsatisfactorily and may be accompanied by severe postural hypotension, decreased tolerance to physical load, and cause of the cardiac arrhythmias, ischemia of coronary vessels, "silent" myocardial infarction (MI), sudden death syndrome [3-5]. Type 2 DM individuals present reduced autonomic function in cardiovascular system evidenced by decreased heart rate variability (HRV), which results in CAN and increased risk for sudden cardiac death [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 glycemic control; treatment of dyslipoproteinemia; 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 B_1 (benfotiamine), aminoguanidine; symptomatic treatment of concomitant diseases and syndromes (hypertension, CHD, heart failure and arrhythmias) and others [2, 7, 8].

Thus, we aimed to evaluate the effects of ω-3 PUFAs on the circadian rhythm of HRV parameters in patients with T2DM and advanced stage of CAN.

Material and methods

To explore the effectiveness of some abovementioned compounds we examined 36 patients with T2DM and advanced stage of CAN, patients were aged between 50-59 years with disease duration 1-6 years and median glycated hemoglobin A1c (HbA$_{1c}$) 7,1%±0,12%.

T2DM was diagnosed with revised criteria provided by American Diabetes Association when source documents were reviewed [1]. When one or more of the following components were found (HbA$_{1c}$ ≥6,5%; fasting plasma glucose ≥7 mmol/L; 2-h plasma glucose ≥11,1 mmol/L during an oral glucose tolerance test; a random plasma glucose ≥11,1 mmol/L; exposure of insulin or oral antidiabetic drugs; a previous diagnosis of T2DM was determined. CAN was diagnosed according to previously proposed criteria [3].

Patients with T2DM and CAN were divided into 2 groups. First group received traditional antihyperglycemic therapy (n=15, control group) for three months; patients in group 2 (n=21), received in addition to standard treatment 1 capsule/day of the ω-3 PUFA for three months. The capsule contains 1 g, including ~90% ω-3 PUFAs, mainly eicosapentaenoic (EPA) and docosahexaenoic acids (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 HbA$_{1c}$ level was assessed by using a highly sensitive method of ion exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States).

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 following parameters: heart rhythm, heart rate (HR), conduction intervals and Holter-ECG [(ECG "EC-3H" ("Labtech," Hungary)] analysis included measurement of 24 hours ECG, circadian indexes and following HRV parameters [9]: standard deviation of all NN intervals (SDNN), standard deviation of the means of all NN intervals for all 5-mins segments of the entire recording (SDANNi), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (pNN50), the very low frequency component of HRV (VLF), the high-frequency component of HRV (HF), the low-frequency component of HRV (LF), ratio of low to high frequency power components [sympathetic/parasympathetic ratio (LF/HF)].

The study was performed according to the Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol was approved by the Ethics Committees of all the study centres involved. Written informed consent was obtained from all participants prior to their inclusion in the study.

Statistical analysis was based on the variational method using 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 HbA$_{1c}$ of patients with T2DM and advanced stage of CAN was not statistically significant influenced by the treatment (p>0,05).

The features of the time-domain HRV parameters in patients with T2DM and advanced stage of CAN after treatment with ω-3 PUFAs are given in table 1.

As can be seen from table 1 prescription of ω-3 PUFAs to patients with T2DM and advanced stage of CAN pro-

Table 1

Time-domain heart rate variability parameters after 3-months of ω-3 PUFAs therapy (Δ%, Mean±SEM)

Parameter	Patients with T2DM and advanced stage of CAN (n=36)				
	Groups	Baseline	After treatment	% change from baseline	p
SDNN (ms)	Control (n=15)	94,7±4,84	91,3±3,33	-0,82%±2,73%	>0,05
	Treatment (n=21)	93,57±2,89	104,29±2,97	+12,2%±2,64%	<0,05
SDANNi (ms)	Control (n=15)	72,3±4,08	74,7±3,33	+4,72%±2,67%	>0,05
	Treatment (n=21)	81,19±3,23	93,57±3,72	+16,13%±2,82%	<0,05
RMSSD (ms)	Control (n=15)	19,1±1,36	18,7±0,77	+0,96%±3,67%	>0,05
	Treatment (n=21)	18,14±1,03	21,9±1,08	+23,0%±3,69%	<0,05
pNN50 (%)	Control (n=15)	3,9±0,42	3,7±0,23	+7,1%±9,59%	>0,05
	Treatment (n=21)	4,0±0,35	5,05±0,37	+31,9%±4,98%	<0,05

Note: the results are presented as absolute values and as % change from baseline, (Δ%, Mean±SEM); p<0,05, compared to baseline.

Abbreviations: T2DM — type 2 diabetes mellitus, CAN — cardiovascular autonomic neuropathy, SDNN — standard deviation of all NN intervals, SDANNi — standard deviation of the means of all RR intervals for all 5-minute segments of the entire recording, RMSSD — the square root of the mean of the sum of the squares of differences between adjacent NN intervals, pNN50 — percentage of differences greater that are 50 ms between adjacent sinus RR intervals.

Table 2

Spectral heart rate variability parameters during the active period after 3 months of ω-3 polyunsaturated fatty acids therapy (Δ%, Mean±SEM)

Parameter	Patients with T2DM and advanced stage of CAN (n=36)				
	Groups	Baseline	After treatment	% change from baseline	p
VLF (ms^2)	Control (n=15)	1113,6±98,1	1103,9±72,8	+1,81%±2,63%	>0,05
	Treatment (n=21)	1075,9±47,8	1208,9±48,7	+13,6%±3,29%	>0,05
LF (ms^2)	Control (n=15)	390,8±21,98	388,5±14,74	+1,6%±3,72%	>0,05
	Treatment (n=21)	349,1±18,8	437,1±16,4 [b]	+30,2%±6,42%	<0,01
HF (ms^2)	Control (n=15)	242,1±18,5	244,5±13,6	+4,1%±4,15%	>0,05
	Treatment (n=21)	223,9±11,9	254,1±8,8 [a]	+18,1%±6,02%	<0,05
LF/HF	Control (n=15)	1,66±0,06	1,63±0,06	-1,6%±3,07%	>0,05
	Treatment (n=21)	1,58±0,06	1,76±0,06 [a]	+14,4%±7,16%	<0,05

Note: the results are presented as absolute values and as % change from baseline, (Δ%, Mean±SEM); p<0,05; <0,01 compared to baseline.

Abbreviations: T2DM — type 2 diabetes mellitus, CAN — cardiovascular autonomic neuropathy, VLF — very low frequency power, total spectral power of all NN intervals between 0,003 and 0,04 Hz, LF — low frequency power, total spectral power of all NN intervals between 0,04 and 0,15 Hz, HF — high frequency power, total spectral power of all NN intervals between 0,15 and 0,4 Hz, LF/HF — ratio of low to high frequency power.

motes to statistically significant increase in SDNN (Δ=+12,2%±2,64% (p<0,05)); SDANNi (Δ=+16,13%±2,82% (p<0,05)); RMSSD (Δ=+23,0%±3,69% (p<0,05)); pNN50 [Δ=+31,9%±4,98% (p<0,05)]. Investigated parameters did not change significantly in the control group (p>0,05).

Changes of the spectral HRV parameters during the active period of the day in patients with T2DM and advanced stage of CAN after 3 months of ω-3 PUFAs therapy are given in table 2.

As can be seen from table 2 prescription of ω-3 PUFAs to patients with T2DM and advanced stage of CAN promotes statistically increase in LF [Δ=+30,2±6,42% (p<0,01)], HF [Δ=+18,1%±6,02% (p<0,05)]; LF/HF [Δ=+14,42%±7,16% (p<0,05)] and at the same time does not affect VLF parameters (p>0,05) during the active period of day.

The features of spectral HRV parameters during the passive period of the day in patients with T2DM and advanced stage of CAN after treatment with ω-3 PUFAs are given in table 3.

As can be seen from table 3 prescription of ω-3 PUFAs to patients with T2DM and advanced stage of CAN promotes statistically significant increase in LF [Δ=+30,8%±4,95% (p<0,01)], HF [Δ=+18,9%±4,72% (p<0,05)], LF/HF ratio [Δ=+10,5%±2,1% (p<0,05)]; and at the same time does not affect VLF parameters [Δ=+10,6%±1,79% (p>0,05)] during the passive period of day. Investigated parameters did not change significantly in the control group (p>0,05).

So, development of advanced CAN is accompanied by sympathicotonia with increased LF/HF ratio and decreased RMMSD parameters. Prescription of ω-3 PUFAs to patients with T2DM and advanced CAN lead to improvement of subclinical arrhythmogenic myocardial impairment: increase of SDNN, SDANNi, RMSSD and pNN50; LF- and HF-components, LF/HF ratio during the active and passive periods of day.

Table 3

**Spectral heart rate variability parameters during the passive period after 3 months
of ω-3 polyunsaturated fatty acids therapy (Δ%, Mean±SEM)**

Parameter	Patients with T2DM and advanced stage of CAN (n=36)				
	Groups	Baseline	After treatment	% change from baseline	p
VLF (ms^2)	Control (n=15)	1441,1±106,7	1439,3±84,7	+1,83%±2,47%	>0,05
	Treatment (n=21)	1405,6±73,0	1544,6±74,5	+10,6%±1,79%	>0,05
LF (ms^2)	Control (n=15)	509,1±17,82	502,1±13,0	-0,6%±2,55%	>0,05
	Treatment (n=21)	492,9±28,7	631,6±33,2[b]	+30,8%±4,95%	<0,01
HF (ms^2)	Control (n=15)	340,5±25,3	326,7±19,01	-1,9%±3,38%	>0,05
	Treatment (n=21)	335,5±18,6	388,6±17,7[a]	+18,9%±4,72%	<0,05
LF/HF	Control (n=15)	1,57±0,08	1,59±0,07	+1,6%±1,92%	>0,05
	Treatment (n=21)	1,49±0,05	11,63±0,04[a]	+10,5%±2,1%	<0,05

Note: the results are presented as absolute values and as % change from baseline, (Δ%, Mean±SEM); p<0,05; <0,01 compared to baseline.

Abbreviations: T2DM — type 2 diabetes mellitus, CAN — cardiovascular autonomic neuropathy, VLF — very low frequency power, total spectral power of all NN intervals between 0,003 and 0,04 Hz, LF — low frequency power, total spectral power of all NN intervals between 0,04 and 0,15 Hz, HF — high frequency power, total spectral power of all NN intervals between 0,15 and 0,4 Hz, LF/HF — ratio of low to high frequency power.

Discussion

Several large-scale, randomized clinical trials have shown that dietary intake of ω-3 PUFAs improves the prognosis of patients with symptomatic heart failure or recent MI. Therefore, dietary consumption of ω-3 PUFAs is recommended in international guidelines for the general population to prevent CHD. However, the precise mechanisms underlying the cardioprotective effects of ω-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 Ca^{2+} channels, to prevent lethal arrhythmias. Moreover, ω-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 ω-3 PUFAs were recently identified as anti-inflammatory mediators. These ω-3 metabolites may contribute to the beneficial effects against cardiovascular diseases that are attributed to ω-3 PUFAs [10, 11].

HRV is a non-invasive measurement that indirectly reflects the cardiac autonomic regulation [6, 9]. Overweight persons with an increased risk of T2DM have an impaired HRV. In a randomized, double-blind, parallel comparison, 65 overweight volunteers consumed DHA 1,56 g/day and EPA 0,36 g/day or sunflower-seed oil (placebo) for 12 weeks. In 46 of these subjects HRV was assessed in the frequency domain using 20 min ECG recordings. Omega-3 PUFAs supplementation improved HRV by increasing HF power, representing parasympathetic activity, and it also reduced HR at rest and during submaximal exercise. Thus, the authors concluded that dietary supplementation with DHA-rich fish oil reduced HR and modulated HRV in a favorable way in these overweight subjects with a high risk of CVDs [12].

Heart rate variability was examined in 43 type 1 and 38 T2DM patients and related to ω-3 PUFAs content in platelet membranes. In T1DM patients HRV increased with increasing levels of DHA. Furthermore, this positive correlation between HRV and platelet DHA was more pronounced in patients with T1DM solely receiving insulin therapy and without signs of diabetic complications. However, this study could not demonstrate a significant association between ω-3 PUFAs and HRV in the patients with T2DM. In contrast, a small Italian study found that 6 months of ω-3 PUFAs treatment in a group of 13 T2DM patients partially improved HRV in the frequency domain [12].

Both nervous tissue and heart tissue have a high content of ω-3 PUFAs (especially DHA) and this may be consistent with the finding that this marine ω-3 PUFAs may modulate cardiac autonomic function as assessed by HRV. The incorporation of ω-3 PUFAs in synaptic membranes could potentially influence the autonomic control of the heart. Thus, ω-3 PUFAs may modulate HRV both at the level of the ANS and the heart. Most of the data support that ω-3 PUFAs beneficially modulates cardiac autonomic control thereby possibly reducing the risk of arrhythmias [11, 12].

Omega-3 PUFAs have potent effects on ion channels and calcium regulatory proteins. Circulating (acute administration) ω-3 PUFAs affect ion channels directly while incorporation (long-term supplementation) of these lipids into cell membranes indirectly alter cardiac electrical activity *via* alteration of membrane properties. Omega-3 PUFAs reduce baseline heart rate and increase HRV *via* alterations in intrinsic pacemaker rate rather than from changes in cardiac autonomic neural regulation [13].

So, cardiovascular benefits of omega-3 PUFAs [11-14] are: antidysrhythmic effects (reduced sudden death; possible prevention of atrial fibrillation; possible protection against pathologic ventricular arrhythmias; improvement in HRV; antiatherogenic effects (reduction in non-high-density lipoprotein cholesterol (HDL-C) levels; reduction in triglyceride and very low-density lipoprotein cholesterol (VLDL-C) levels; reduction in chylomicrons; reduction in VLDL and chylomicron remnants; increase in HDL-C levels; "improvement" (increase) in LDL and HDL particle size; plaque stabilization; antithrombotic effects (decreased platelet aggregation; improved blood rheologic flow); anti-inflammatory and endothelial protective effects (reduced endothelial adhesion molecules and decreased leukocyte adhesion receptor expression; reduction in proinflammatory eicosanoids and leukotrienes; vasodilation); mild decreased systolic and diastolic blood pressure.

Conclusion

Prescription of the ω-3 PUFAs in patients with T2DM and advanced stage of CAN was accompanied by a statistically significant increase of the time-domain HRV parameters (SDNN, SDANNi, RMSSD and pNN50). It also contributed to significantly positive changes in the spectral HRV parameters (LF, HF, LF/HF) during the active and passive periods compared with the control group. Our results suggest that the efficacy of ω-3 PUFAs is not associated with improved glycemic control of T2DM in patients with advanced stage of CAN, but is rather the result of a direct effect of the pharmacological agent on the investigated metabolic indexes.

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