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Does Omega-3 Supplementation have Beneficial Effects on Blood Pressure, Left Ventricular Geometry and Arterial Function and Arterial Properties in Patients with Chronic Renal Disease Stage 1-3?

Agnieszka Pluta[1*], Paweł Strozecki[2], Jacek Kesy[3], Magdalena Krintus[4], Beata Sulikowska[2], Grazyna Odrowaz-Sypniewska[4] and Jacek Manitius[2]

[1]Division of Community Nursing, Faculty of Health Sciences, Ludwik Rydygier Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń, Poland Łukasiewicza 1 Street, 85-821 Bydgoszcz, Poland

[2]Department of Nephrology, Hypertension and Internal Diseases, Faculty of Medicine, Ludwik Rydygier Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń, M. Skłodowskiej-Curie 9 Street, 85-094 Bydgoszcz, Poland

[3]Chair of Plant Physiology and Biotechnology, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University in Toruń, Lwowska 1 Street, 87-100 Toruń, Poland

[4]Department of Laboratory Medicine, Faculty of Pharmacy, Ludwik Rydygier Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń, M. Skłodowskiej-Curie 9 Street, 85-094 Bydgoszcz, Poland

[*]**Corresponding author**: Agnieszka Pluta, Division of Community Nursing, Faculty of Health Sciences, Ludwik Rydygier Collegium, Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń, Łukasiewicza 1 Street, Bydgoszcz, Poland, Tel: +48 52 5855813; E-mail: agnieszkapluta@poczta.onet.pl

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Abstract

Background: Chronic kidney disease (CKD) is characterized by unfavorable cardiac and vascular remodeling. The study aimed at evaluating effects of 6-month supplementation with omega-3 on blood pressure, left ventricular geometry and function, and arterial properties in patients with CKD 1-3.

Methods: Six-month supplementation with omega-3 acids (2 g/day) was completed in 87 CKD patients, and in 27 individuals without CKD, hypertension or overt cardiovascular disease. At baseline and after supplementation, an echocardiographic examination was performed, evaluating left ventricular mass index (LVMI), left ventricular relative wall thickness (RWT), and ejection fraction (EF). Ultrasound imaging of the common carotid artery with intima media thickness (IMT), aortic pulse wave velocity measurement (PWV) and 24-hour blood pressure monitoring (ABPM) were performed. Serum concentration of omega-3 acids: eicosapentaenoic (EPA), docosahexaenoic (DHA), and alpha linolenic (ALA) was determined using gas chromatography.

Results: After six-month omega-3 supplementation, ALA concentration increased in CKD patients and in reference group, while EPA and DHA did not change. PWV and IMT values did not change significantly. Posterior wall thickness (PWd) (p=0.018) and RWT decreased (p=0.035), while LVMI and EF did not change in CKD group. ABPM did not change.

Conclusion: Supplementation with omega-3 acid resulted in beneficial left ventricular remodeling, despite the absence of changes in ABPM and arterial properties.

Trial registration: The study was registered in Clinical Trials.gov. Identifier: NCT 02147002.

Keywords: Omega-3 acids; Chronic kidney disease; Left ventricular hypertrophy

Introduction

Fatty acids consist of a chain of carbon atoms with a methyl group at one end of the chain and an acid group at the other end. There are saturated and unsaturated fatty acids. There are only single bonds between carbon atoms in saturated fatty acids. Unsaturated fatty acids, on the other hand, have one or more double bonds between carbon atoms [1]. Among polyunsaturated fatty acids, a special role is played by omega-3 or n-3 acids (alpha-linolenic acid 18: 3, ALA) and omega-6 or n-6 (linoleic acid 18: 2; LA). These acids differ in the position of the first double bond on the methyl side. In omega-3 acids, this bond is located at the third carbon atom, while in the omega-6 it is at the sixth carbon atom, counting from the last carbon atom farthest away from the carboxyl group, denoted as omega [2]. Omega-3 acids are an important component of cell membranes and are not synthesized in the human body [3]. The parent omega-3 fatty acid in the omega-3 family is ALA, and in the omega-6 family-LA. The human body is able to transform LA into arachidonic acid (AA) and ALA into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Desaturase and elongase enzymes, for which the LA and ALA metabolites compete, participate in these two pathways [4]. Omega-3s have protective effects on the cardiovascular system in both the general population [5,6] and patients in various stages of CKD [7-9]. These effects are the result of, among other things, anti-inflammatory, anti-atherosclerotic, anticoagulant activity.

Treatment of chronic kidney disease (CKD) consists in treating the underlying cause, that is the disease which has caused renal damage, but also in actions aimed at inhibiting the progression of this damage,

that is nephroprotective treatment. The sooner chronic kidney disease is diagnosed, irrespective of its cause, the more effective the said procedures will be. There are many indications that omega-3 polyunsaturated fatty acids (Omega-3 PUFAs) may be part of the nephroprotective process [7,10-13] through inhibiting the progression of CKD [14-16]. Kidney disease and heart disease are inextricably linked. Early onset of CKD increases cardiovascular morbidity and mortality rates, which further increase with the degree of impairment of glomerular filtration [17]. The risk of cardiovascular death in patients with CKD is more than 10 times higher than in the general population. Moreover, in the group of patients under the age of 35, it is almost 1,000 times higher than in the general population [18]. The development of cardiovascular changes in the CKD population is caused by traditional risk factors such as sex, smoking, hypertension, lipid disorders, and diabetes, as well as conditions specific to this patient group: anemia, malnutrition, oxidative stress, chronic inflammation hyperhomocysteinemia or uremic toxemia [19].

The aim of the paper was to evaluate the effect of a six-month supplementation with omega-3 on blood pressure, left ventricular geometry and function, and arterial properties in patients with chronic kidney disease stage 1-3.

Materials and Methods

The study was conducted between September 2012 and November 2014 upon approval by the Bioethics Committee of the Nicolaus Copernicus University in Toruń Ludwik Rydygier Collegium Medicum in Bydgoszcz (KB 305/2012). All patients included in the study gave written consent to participation.

The study population consisted of patients with CKD treated in the Nephrology Outpatient Clinic of the University Hospital No. 1 in Bydgoszcz. The criteria for inclusion in the study were: diagnosed chronic kidney disease stage 1-3, the written consent of the patient to participate in the study, and age over 18 years. *The criteria for the diagnosis of chronic kidney disease were based on the guidelines of* Kidney Disease Outcome Quality Initiative 2012. Exclusion criteria were: immunosuppressive therapy, diabetes and lack of consent to participate in the study. Participation in the study was offered to 130 patients, 40 of whom refused to participate. The reference group included n=30 individuals without CKD, hypertension or overt cardiovascular disease. Exclusion criteria from the study in the reference group: hypertension, CKD, overt cardiovascular disease and lack of written consent for participation in the study. The reference group included n=30 patients without CKD, hypertension or overt cardiovascular disease.

In the analyzed population of CKD patients, 30 patients were in stage 1, 33 in stage 2, and 27 in stage 3 of chronic kidney disease. Six people-3 from the CKD group and 3 from the reference group did not complete the study according to the protocol: 2 women were excluded from the study due to pregnancy and 4 patients did not report for a follow-up examination after 6 months. For the purposes of this study, the results of 87 patients with CKD and 27 patients from the reference group were analyzed both before and after supplementation.

The underlying causes of kidney disease in the CKD group were: chronic glomerulonephritis confirmed by kidney biopsy (n=16; 18.4%), hypertensive nephrosclerosis (n=3; 3.5%), polycystic kidney disease (n=28; 32.2%), gouty nephropathy (n=5; 5.7%), nephrolithiasis (n=23; 26.4%), loss of one kidney due to injury (n=1, 1.1%). In 11 (12.6%) cases, the cause of the disease was not determined.

Each patient participating in the study was subjected to a 6-month supplementation with omega-3 (Gold Omega 3) at a dose of 2 x 1000 mg. One capsule of Gold Omega3=1000 mg contains 65% omega-3 acid, including 330 mg eicosapentaenoic acid (EPA), 220 mg docosahexaenoic acid (DHA), and 100 mg of other acids, including alpha-linolenic acid (ALA). These acids are of fish origin.

In all persons participating in the study, the following measurements were performed before and after supplementation with omega-3: determination of creatinine concentration, estimation of eGFR, determination of serum concentration of ALA, EPA and DHA by gas chromatography, 24-hour ambulatory blood pressure monitoring, echocardiography, ultrasound imaging of the common carotid artery intima-media thickness (IMT) and measurement of aortic pulse wave velocity (PWV).

Serum levels of creatinine and total cholesterol, HDL cholesterol and LDL cholesterol were determined using the Horiba ABX Pentra 400 biochemical analyzer.

Laboratory tests

Material used for the study was venous blood serum. Fasting blood was collected from the median cubital vein into two dry glass tubes without additives, in the Vacutainer closed vacuum system under standard conditions; between 7.00 and 9.00 in the morning. After collection, blood samples were left at room temperature for 30 minutes for clotting. One tube was used for the enzymatic creatinine assay using the Horiba ABX Pentra 400 biochemical analyzer. Estimated glomerular filtration rate (eGFR) was then estimated on the basis of CKD-EPI [20]. The second tube of coagulated blood was centrifuged for 15 minutes at 4000 RPM. After centrifugation, serum was separated from the blood clot. The separated serum at a volume of about 2 ml was stored in an Eppendorf tube at -80. For the analysis of ALA, EPA, and DHA, a PerkinElmer (USA) gas chromatograph equipped with flame ionization detector (GC-FID) was used. Separation of FAMEs was carried out on an Equity-5 (Supelco) capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness) using Hydrogen as the carrier gas.

100 μl of human serum were saponified in 5 ml PTFE screw-capped glass tubes containing 10 μg of tridecanoic acid as internal standard, and 1 ml of 0.5% (w/v) sodium methylate. The samples were heated for 15 min at a temperature of 100and, after cooling to room temperature, esterified with 1.5 ml of BF3 in methanol (also at 100) for 10 min. Again after cooling of the tubes, 1 ml of n-hexane was added to extract the fatty acid methylesters. The contents of the tubes were then shaken for 1 min and 1 ml of saturated sodium chloride solution was added. Afterwards, the tubes were centrifuged for 5 min at 2200× g. The clear hexane top layer was transferred into an injection vial, evaporated to dryness under a stream of nitrogen and then redissolved in 100 μl of hexane. 1 μl of the final solution was applied into the GC injector. The method of FA analysis was adopted from Bondia-Pons et al. [21].

The identities of sample methyl ester peaks were determined by comparing their relative retention times with those of well-known FAMEs standards. Quantification was based on the amount of the internal standard recovered. The results were expressed in mg/100 ml of serum.

Concentration of KT=S KT/S ST x M ST [mg/100 ml]

Where: S KT-fatty acid peak area

S ST-internal standard peak area

M ST-amount of internal standard in μg.

Echocardiographic examination

All subjects underwent an outpatient echocardiographic examination including measurements of the left atrium dimension (LAD1), left ventricular internal diameter at end diastole (LVIDd), intraventricular septal thickness at diastole (IVS.d), left ventricular posterior wall thickness (PWd). The measurements were performed according to recommendations of the American Society of Echocardiography (ASE) [22]. Left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were assessed using the long-axis area-length method. Ejection fraction (EF) was calculated subsequently. Left ventricular relative wall thickness (RWT) was also calculated (RWT=2*PWd)/LVIDd). Left ventricular mass was calculated using the formula developed by Devereux and colleagues: LVM=0.8 [1.04 (IVS.d+LVIDd+PWd)3–LVIDd3]+0.6 (g); subsequently, the left ventricular mass index was calculated (LVMI=LVM/BSA-where BSA stands for body surface area) [23].

In the pulsed-wave Doppler ultrasound with the gate placed at the top of the mitral valve flap, the maximum infiltration was determined by a color-coded test and the following were measured: the maximal velocity of the early mitral inflow (E max), the maximal velocity of the inflow caused by atrial contraction (A max), and the E/A index=E max/A max. The velocities are expressed in [m/s].

The isovolumic relaxation time (IVRT) was also measured as the time from the closing of the aortic valve to the opening of the mitral valve. The deceleration time of the early mitral inflow wave (E dec) was measured by pulsed blood flow through the valve. It was determined by measuring the drop in E wave velocity from its peak to zero.

Impairment of left ventricular systolic function was identified when EF<55%. The criterion for LVH identification was LVMI >95 g/m² for women and LVMI >115 g/m² for men [24]. On the basis of LVMI and RWT, 4 types of left ventricular geometry were determined:

Normal structure of the left ventricle: without LVH and RWT ≤ 0.42,

Concentric remodeling: without LVH and RWT>0.42,

Concentric hypertrophy: LVH and RWT>0.42,

Eccentric hypertrophy: LVH and RWT ≤ 0.42.

All echocardiographic examinations were performed by the same investigator.

Blood Pressure Measurement

24-hour ambulatory blood pressure monitoring (ABPM) was performed using the A&D TM-2430 device. Cuff size was adapted to a patient's arm circumference. BP measurements were taken every 30 minutes. The patients were instructed in the operation principles of the apparatus. In the evaluation of BP results obtained by ABPM, mean values of SBP, DBP, MAP, PP and heart rate for the entire period of 24 hours were analyzed. Subsequently, mean arterial pressure measured by ABPM (MAP ABPM) was calculated from the formula MAP ABPM=DBP ABPM+1/3 (SBP ABPM-DBP ABPM) (mmHg) and pulse pressure measured by ABPM (PP ABPM) was calculated from the formula PP ABPM=SBP ABPM-DBP ABPM (mmHg).

The clinical assessment included the calculation of body mass index (BMI).

Ultrasonographic measurement of the common carotid artery and aortic pulse wave velocity

Each subject underwent an ultrasonographic measurement of common carotid artery IMT. The patients were examined in the supine position, after a 5-minute rest. IMT was measured 10-30 mm below the carotid bifurcation in 3 points free from atherosclerotic plaque both on the left and right side. Arithmetic mean was then calculated from the obtained results. Participants of the study had their PWV measured between the carotid artery and the femoral artery using the Complior device (Artech Medical, Pantin, France). Testing was performed under fasting conditions, in a quiet room, after a 10-minute rest, in the supine position, on an outpatient basis. One sensor was placed at a palpable pulse site on the right carotid artery, while the second sensor was placed at a palpable pulse site on the right femoral artery. Time (t) between the occurrence of pulse wave in the carotid and femoral arteries was measured automatically in 10 subsequent cycles and averaged. Pulse wave distance (d) was accepted as the distance between sensor attachment sites on the carotid and femoral arteries multiplied by coefficient 0.8 in accordance with current guidelines [25]. PWV was calculated using the equation PWV=d/t and expressed in [m/s]. PWV was measured twice in each subject, one measurement taken directly after another, and mean values were calculated.

Statistical analysis

The obtained results were analyzed statistically using the STATISTICA software from StatSoft Inc. The distribution of variables with normal distribution was analyzed using the Shapiro-Wilk test. If the variable had a normal distribution, it was represented as the mean ± standard deviation (SD). For variables of non-normal distribution, both the median and the top and bottom quartiles were given. Normally distributed variables were compared using the t-Student test. For variables that did not have a normal distribution, the Mann-Whitney U test was used. The ANOVA test was used to compare more than 2 variables. The post hoc analysis used the Tukey test. The assessment of the correlations between the test indicators was carried out using the Pearson's linear correlation coefficient (for normal distribution tests) and the Spearman correlation coefficient (for non-normal distributions). The statistical significance level was accepted as $p<0.05$.

Results

During the supplementation with omega-3 acid, the tolerance of the Gold Omega 3 preparation was good. The occurrence of side effects in the form of belching and nausea was observed in 2 patients (1.7%). The symptoms were transient and did not require the discontinuation of therapy.

Clinical characteristics and laboratory results in patients with CKD stage 1-3 and in the reference group before and after 6 months of supplementation with omega-3 acid are shown in Table 1. In both groups, a statistically significant increase in ALA was observed after 6 months, with no change in EPA or DHA. In the CKD group, the eGFR did not change significantly (74.9 ± 23.5 vs. 72.3 ± 25.5 ml/min/1.73 m², p=0.055), while in the reference group it decreased statistically significantly (96.3 ± 14.8 vs. 89.9 ± 14.9 ml/min/1 73 m²; p=0.011). Patients with CKD had a statistically significant decrease in PWd (1.03 ± 0.16 vs. 0.99 ± 0.16 cm, p=0.018) and RWT (0.41 ± 0.07 vs. 0.40 ± 0.07; p=0.035) with no differences in LVMI. In the reference group,

PWd increased (0.87 ± 0.14 vs. 0.92 ± 0.15 cm; p=0.025) with no change in both RWT and LVMI. After supplementation, PWV decreased in the reference group (8.60 ± 1.70 vs. 8.23 ± 1.83 m/s, p=0.044), while in the CKD group it remained unchanged. IMT in both populations did not change significantly during the 6-month follow-up period.

The prevalence of arterial hypertension (AH) in the study population of patients with CKD was 79% and it was statistically significantly different between stage 1 (55%), stage 2 (84%) and stage 3 (96%). During omega-3 fatty acid supplementation there were no significant changes in blood pressure as measured by ABPM (Table 2).

Before initiation of supplementation with omega-3, abnormal left ventricular geometry was diagnosed in 53 (61%) patients with CKD, including left ventricular hypertrophy (LVH) in 37 (43%) and left ventricular remodeling in 16 (18%) patients. Features of left ventricular concentric hypertrophy were found in 19 (22%) and features of left ventricular eccentric hypertrophy in 18 (21%) patients. After 6 months of supplementation, abnormal left ventricular geometry was found in 45 (52%) patients, including LVH in 31 (36%) and concentric remodeling of the left ventricle in 14 (16%) patients (NS). Features of left ventricular concentric hypertrophy were found in 16 (18%) patients with CKD and features of left ventricular eccentric hypertrophy in 15 (17%) patients.

In echocardiographic examination (Table 3) after 6-month omega-3 supplementation in patients with CKD stage 2 had, a statistically significant reduction in PWd (1.02 ± 0.16 vs. 0.97 ± 0.16 cm, p <0.05) and RWT (0.42 ± 0.08 vs. 0.38 ± 0.07; p<0.01) in the absence of significant changes in LVMI and EF. Deficient indicators of structure (IMT) and stiffness of large arteries (PWV) with the progression of CKD were observed. There was also a deterioration of left ventricular diastolic function in the absence of statistically significant changes in systolic function (EF), depending on the CKD stage. Prior to omega-3 acid supplementation, the maximum velocity of arterial contraction (A max) (p=0.001851) increased while the E max/A max ratio decreased with the progression of CKD (p=0.00891). The increase in LVMI with the degree of CKD both before and after omega-3 supplementation did not reach statistical significance.

Duration of hypertension and antihypertensive therapies and statin therapy in groups of patients with CKD 1-3 are shown in Table 4. AH duration was significantly statistically longer in patients with CKD3 compared to patients with CKD1 and 2 (p<0.001, p<0.05). The number of antihypertensive medications used increased along with advancing stages of CKD and presented as follows: in CKD1 the median was 1.0 (range 0-4), in CKD2 the median was 1.0 (range 0-5) and in CKD3 the median was 2.0 (range 0-5).

Parameter	**Reference (n=27)**		**P**	**CKD (n=87)**		**P**
	Baseline	**After 6 months**	**1 vs. 2**	**Baseline**	**After 6 months**	**3 vs. 4**
	-1	**-2**		**-3**	**-4**	
Sex (women/men)	18-Sep	18-Sep		40/47	40/47	
BMI (kg/m²)	24.5 ± 2.9	24.7 ± 2.8	0.05	27.2 ± 3.9	27.2 ± 3.8	0.78
SBP ABPM (mmHg)	121.6 ± 8.3	121.7 ± 8.1	0.97	126.1 ± 13.6	126.8 ± 13.9	0.74
DBP ABP (mmHg)	73.4 ± 4.7	73.9 ± 5.2	0.68	75.7 ± 8.5	76.1 ± 8.3	0.59
MAP ABPM (mmHg)	90.1 ± 5.3	89.8 ± 5.7	0.81	91.9 ± 10.0	93.0 ± 9.6	0.25
PP ABPM (mmHg)	48.4 ± 6.7	47.6 ± 6.2	0.49	50.4 ± 9.1	50.7 ± 9.3	0.91
HR ABPM (beats/min)	71 ± 5	71 ± 5	0.79	71 ± 8	70 ± 10	0.24
Creatinine (mg/dl)	0.75 ± 0.20	0.82 ± 0.21	0.0097	1.05 ± 0.35	1.12 ± 0.46	0.3
eGFR CKD-EPI (ml/min/1.73 m²)	96.3 ± 14.8	89.9 ± 14.9	0.011	74.9 ± 23.5	72.3 ± 25.5	0.055
ALA (mg/100 ml)	1.52 (1.17;2.11)	2.48 (2.06;3.38)	0.0008	1.8 (1.11;2.64)	3.0 (2.24;3.96)	0.0001
EPA (mg/100 ml)	6.64 ± 3.42	7.25 ± 3.51	0.52	7.6 ± 3.83	8.46 ± 4.95	0.2
DHA (mg/100 ml)	8.21 ± 4.67	8.31 ± 3.36	0.92	9.09 ± 4.48	8.95 ± 3.84	0.81
LAD1 (cm)	3.54 ± 0.50	3.60 ± 0.50	0.44	3.79 ± 0.53	3.84 ± 0.56	0.33
IVS.d (cm)	0.87 ± 0.14	0.91 ± 0.14	0.07	1.08 ± 0.20	1.07 ± 0.21	0.58
LVIDd (cm)	4.78 ± 0.51	4.80 ± 0.39	0.77	4.98 0.50	4.99 ± 0.48	0.86
LVIDs (cm)	3.20 ± 0.41	3.15 ± 0.36	0.58	3.30 ± 0.47	3.28 ± 0.45	0.58
PWd (cm)	0.87 ± 0.14	0.92 ± 0.15	0.025	1.03 0.16	0.99 ± 0.16	0.018
EF (%)	65 ± 7	65 ± 7	0.76	64 ± 8	62 ± 7	0.08

RWT	0.37 ± 0.08	0.38 ± 0.06	0.26	0.41 ± 0.07	0.40 ± 0.07	0.035
LVMI (g/m²)	81.3 ± 17.7	86.7 ± 18.2	0.07	103.3 ± 25.9	101.3 ± 26.6	0.26
IVRT (ms)	88.7 ± 22.3	95.1 ± 22.7	0.1	96.6 ± 25.9	99.5 ± 28.2	0.12
E dec (ms)	206 ± 58	185 ± 35	0.053	206 ± 53	198 ± 60	0.36
E max (m/s)	0.74 ± 0.20	0.66 ± 0.18	0.023	0.65 ± 0.15	0.65 ± 0.16	0.91
A max (m/s)	0.69 ± 0.15	0.64 ± 0.15	0.011	0.69 ± 0.17	0.68 ± 0.16	0.54
E max/A max	1.12 ± 0.40	1.08 ±0.40	0.41	1.00 ± 0.37	1.01 ± 0.36	0.96
PWV (m/s)	8.60 ± 1.70	8.23 ± 1.83	0.044	9.27 ± 2.21	9.16 ± 2.08	0.51
IMT (mm)	0.63 ±0.13	0.65 ± 0.16	0.24	0.66 ± 0.14	0.66 ± 0.14	0.59

Abbreviations: BMI: Body Mass Index; SBP ABPM: Systolic Blood Pressure Measured by 24-Hour Ambulatory Blood Pressure Monitoring; DBP ABPM: Diastolic Blood Pressure Measured by 24-Hour Ambulatory Blood Pressure Monitoring; MAP ABPM: Mean Arterial Pressure Measured by 24-Hour Ambulatory Blood Pressure Monitoring; PP ABPM: Pulse Pressure Measured by 24-Hour Ambulatory Blood Pressure Monitoring; HR ABPM: Heart Rate Measured by 24-Hour Ambulatory Blood Pressure Monitoring; eGFR: Estimated Glomerular Filtration Rate; LAD1: Left Atrium Dimension; IVS.d: Interventricular Septal Thickness in Diastole; LVIDd: Left Ventricular Internal Dimension in Diastole; LVIDs: Left Ventricular Internal Dimension in Systole; PWd: Posterior Wall of the Left Ventricle; LVIDs: Left Ventricular Internal Dimension in systole; ESV: End-Systolic Volume; EF: Ejection Fraction; RWT: Relative Wall Thickness of the left ventricle; LVMI: Left Ventricular Mass Index; IVRT: Isovolumic Relaxation Time; E dec: E Wave Deceleration Time; E max: Peak Velocity of Early Mitral Inflow; A max: Peak Velocity of Late Mitral Inflow; PWV: Pulse Wave Velocity; ALA: Alpha-Linolenic Acid; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid.

Table 1: Characteristics of the study group of patients with CKD and the reference groupand post intervention with Omega-3.

After supplementation with omega-3 fatty acid, there was a statistically significant increase in ALA in the whole study group (n=114): 1.72 (1.12; 2.27) mg/100 ml vs. 2.83 (2.18; 3.88) mg/100 ml; p<0.001.

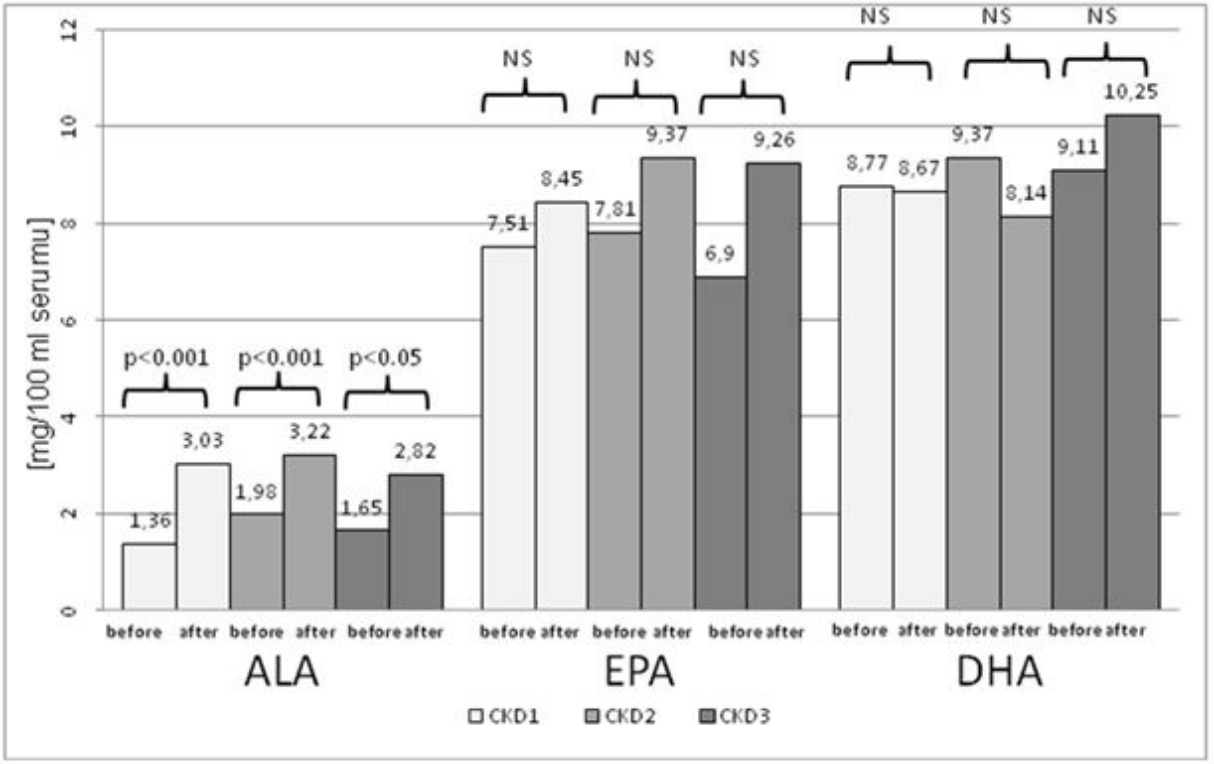

Figure 1: Concentration of ALA, EPA and DHA in patients witch CKD 1, CKD 2, CKD 3and post intervention with omega-3.

This increase was also demonstrated in patients with stage 1 CKD: 1.36 (0.96; 2.47) mg/100 ml vs. 3.03 (2.07; 3.83) mg/100 ml; P<0.001, stage 2 CKD: 1.98 (1.48; 2.57) mg/100 ml vs. 3.22 (2.31; 4.79) mg/100 ml; P<0.001, and stage 3 CKD: 1.65 (1.11; 2.77) mg/100 ml vs. 2.82 (2.24; 3.56) mg/100 ml; p<0.02. In contrast, EPA and DHA concentrations did not change statistically significantly. Changes in ALA, EPA, and DHA levels in patients with CKD are shown in Figure 1.

Parameter	CKD 1 (n=29)	CKD 2 (n=32)	CKD 3 (n=26)	ANOVA (p)
Gender				
women/men	14/15	15/17	Nov-15	
Age (years)				
Baseline	50 ± 11	58 ± 111	63 ± 72	0.000035
Pos intervention	51 ± 11	59 ± 111	64 ± 72	0.000035
BMI(kg/m²)				
Baseline	26.4 ± 4.0	27.6 ± 3.9	27.6 ± 3.7	0.44
Post intervention	26.7 ± 3.9	27.3 ± 3.7	27.6 ± 4.0	0.67
SBP ABPM (mmHg)				
Baseline	123.5 ± 11.1	125.2 ± 12.8	130.2 ± 16.4	0.17
Pos intervention	124.8 ± 14.2	124.7 ± 11.2	131.7 ± 15.8	0.15
DBP ABPM (mmHg)				
Baseline	75.6 ± 8.5	74.9 ± 7.8	76.8 ± 9.6	0.71
Pos intervention	77.0 ± 8.7	74.6 ± 8.0	77.1 ± 8.3	0.45
MAP ABPM (mmHg)				
Baseline	91.2 ± 9.0	90.7 ± 10.0	94.2± 10.9	0.37
Post intervention	92.9 ± 10.3	91.3 ± 8.8	95.3± 9.7	0.36
PP ABPM (mmHg)				
Baseline	47.9 ± 5.7	50.3 ± 7.7	53.4 ± 12.5	0.07
Post intervention	47.8 ± 7.7	50.1 ± 65.9	54.6 ± 12.73	0.022
HR ABPM (beats/min)				
Baseline	72.9 ± 7.9	71.6 ± 8.9	67.8 ± 6.5	0.052
Post intervention	71.1 ± 7.6	70.0 ± 9.7	68.5 ± 11.5	0.45
Creatinine (mg/dl)				
Baseline	0.75 ± 0.17	1.00 ± 0.162	1.46 ± 0.291,3	0.001
Post intervention	0.78 ± 0.16	1.04 ± 0.241	1.58 ± 0.511,5	0.0001
eGFR CKD-EPI (ml/min/1,73m²)				
Baseline	101.1 ± 9.5	74.2 ± 9.02	46.6 ± 8.12,4	0.001
Post intervention	97.3 ± 12.3	72 ± 17.22	44.7 ± 13.42,4	0.001
1p<0.01 vs CKD 1; 2p<0.001 vs CKD 1; 3p<0.05 vs CKD 1; 4p<0.001 vs CKD 2.				

Table 2: Clinical characteristics of the study groups of patients with CKD 1, CKD, CKD3 and post intervention with omega-3.

Parameter	CKD 1 (n=29)	CKD 2 (n=32)	CKD 3 (n=26)	ANOVA (P)
LAD1 (cm)				
Baseline	3.68 ± 0.43	3.79 ± 0.56	3.95 ± 0.58	0.26
Post intervention	3.76 ± 0.45	3.84 ± 0.59	3.94 ± 0.64	0.49
IVS.d (cm)				
Baseline	1.02 ± 0.19	1.07 ± 0.19	1.16 ± 0.213	0.036
Post intervention	1.01 ± 0.20	1.05 ± 0.20	1.16 ± 0.213	0.027
LVIDd (cm)				
Baseline	5.04 ± 0.51	4.95 ± 0.45	4.95 ± 0.57	0.76
Post intervention	5.00 ± 0.45	4.99 ± 0.53	4.97 ± 0.47	0.97
LVIDs (cm)				
Baseline	3.32 ± 0.41	3.18 ± 0.43	3.42 ± 0.54	0.16
Post intervention	3.26 ± 0.44	3.21 ± 0.47	3.38 ± 0.44	0.11
PWd (cm)				
Baseline	0.97 ± 0.16	1.02 ± 0.16*	1.10 ± 0.153	0.014
Post intervention	0.97 ± 0.16	0.97 ± 0.16	1.05 ± 0.16	0.11
EDVI (ml/m²)				
Baseline	30.4 ± 9.0	27.6 ± 7.6	29.9 ± 8.4	0.37
Post intervention	31.2 ± 8.9	29.4 ± 10	30.6 ± 9.3	0.75
EF (%)				
Baseline	65 ± 5	64 ± 8	62 ± 10	0.42
Post intervention	64 ± 6	62 ± 6	60 ± 8	0.11
RWT				
Baseline	0.39 ± 0.07	0.42 ± 0.08	0.44 ± 0.061	0.008954
Post intervention	0.39 ± 0.07	0.39 ± 0.07**	0.42± 0.06	0.1
LVMI (g/m²)				
Baseline	98.4 ± 25.1	99.8 ± 19.8	113.2 ± 31.2	0.06
Post intervention	96.1± 23.6	98.4 ± 25.0	110.8 ± 29.9	0.09
IVRT (ms)				
Baseline	89 ± 19	98 ± 27	106 ± 313	0.033213
Post intervention	87 ± 16	101 ± 27	112 ± 373	0.018345
E dec (ms)				
Baseline	190 ± 37	203 ± 53	219 ± 61	0.1
Post intervention	180 ± 38	191 ± 64	226 ± 673	0.015247
E max (m/s)				
Baseline	0.69 ± 0.13	0.65 ± 0.14	0.62 ± 0.18	0.17

Post intervention	0.70 ± 0.16	0.64 ± 0.16	0.62 ± 0.17	0.13
A max (m/s)				
Baseline	0.61 ± 0.15	0.71 ± 0.13[1]	0.75 ± 0.201	0.001851
Post intervention	0.63 ± 0.15	0.69 ± 0.15	0.73 ± 0.18	0.1
E max/A max				
Baseline	1.20 ± 0.36	0.93 ± 0.253	0.87 ± 0.422	0.00891
Post intervention	1.16 ± 0.34	0.97 ± 0.333	0.87 ± 0.363	0.00925
PWV (m/s)				
Baseline	8.70 ± 2.03	9.22 ± 2.11	10.07 ± 2.41	0.11
Post intervention	8.48 ± 1.76	9.19 ± 1.94	10.0 ± 2.353	0.025812
IMT (mm)				
Baseline	0.59 ± 0.13	0.68 ± 0.123	0.72 ± 0.133	0.00154
Post intervention	0.60 ± 0.12	066 ± 0.12	0.71 ± 0.163	0.019095
*p<0.05 baseline vs. post intervention; **p<0.01 baseline vs. post intervention; 1p<0.01 vs. CKD 1; 2p<0.001 vs. CKD 1; 3p<0.05 vs. CKD 1.				

Table 3: Parameters of left ventricular structure and function, PWV in patients with CKD 1, CKD, CKD 3 and post intervention with omega-3.

	CKD1 (n=29)	**CKD 2 (n=32)**	**CKD3 (n=26)**	**Test chi²**
Duration of hypertension	Median 3	Median 10.5	Median 15	<0.001
Median (range)	(range:0-30)	(range:0-35)	(range:0-35) [1,2]	
ACE inhibitor n (%)	12 (41%)	14 (44%)	17 (66%)	NS
ARB n (%)	4 (14%)	6 (19%)	4 (15%)	NS
Calcium antagonists (%)	6 (21%)	13 (41%)	15 (58%)	<0.05
Beta blocker n (%)	4 (14%)	15 (47%)	11 (42%)	<0.05
Diuretic n (%)	4 (14%)	9 (28.1%)	8 (31%)	NS
Other medication for high blood pressure n (%)	0 (0%)	4 (13%)	6 (23%)	<0.05
Statin n (%)	5 (17%)	11 (34%)	11 (42%)	NS
Abbreviations: ACE: Angiotensin Converting Enzyme; ARB: Angiotensin Receptor Antagonist 1p<0.001v CKD1; 2p<0.05v CKD2(test Kruskala-Wallisa)				

Table 4: Duration of hypertension and antihypertensive and statin therapies in groups of patients with CKD1-3.

Before supplementation with omega-3 acids, there were the statistically significant correlations in the whole group of 114 subjects between EPA concentrations and: SBP ABPM (r=-0.20; p<0.05), DBP ABPM (r=-0.20; p<0.05), MAP ABPM (r=-0.19; P<0.05) and PWV (r=-0.21; p<0.05). No statistically significant correlation was found of the concentrations of EPA, DHA, and ALA with echocardiographic parameters.

After supplementation with omega-3 acids, no statistically significant correlations were found between EPA, DHA, ALA and BP ABPM, PWV, IMT, and echocardiographic parameters.

Discussion

Many recent publications highlight the importance of supplementation with omega-3 fatty acids or dietary supplementation with products containing these acids in reducing the risk of cardiovascular disease as well as the progression of CKD [11,12,14,15,17,26-28]. Potential mechanisms through which omega-3 fatty acids may have beneficial effects on the cardiovascular system include anti-inflammatory, anticoagulant, and anti-atherogenic activity, lowering the levels of cholesterol and triglycerides and enhancing endothelial function [5,11,28]. ALA acid, which is the precursor of omega-3 acids, is synthesized exclusively by plants and

must be supplied with food to human diet [29]. Its rich source in the diet can be found in vegetable oils. Under natural conditions, EPA and DHA acids are found in marine algae and phytoplankton, which synthesize these fatty acids [30]. Valuable sources of these acids are fats from saltwater fish species, feeding on plankton or fish. The American Heart Association recommends the consumption of 1 g of fish oil per day for patients with heart disease, and 1g of omega-3 fatty acids per day for patients with high cardiovascular risk [31]. Omega-3 fatty acids reduce cardiac hypertrophy and fibrosis in the cardiovascular system and also inhibit the effects of angiotensin II on vascular smooth muscle [32]. If similar metabolic effects occur in the myocardium, hypertrophy induced by angiotensin II may be inhibited [33].

The prevalence of arterial hypertension and cardiovascular disease is high in patients with CKD [17,19]. Arterial hypertension leads to myocardial hypertrophy and remodeling. In the study population of patients with CKD, arterial hypertension was found in 69 patients (79%) and left ventricular abnormalities in 53 patients (61%). After supplementation with omega-3 fatty acids, left ventricular abnormalities were found in 45 (52%) patients. LVMI did not change statistically significantly in either the study group as a whole or in individual CKD stages. The incidence of AH as well as left ventricular remodeling rates were analyzed in the previous authors' work [34]. There are no data publishing changes in the epidemiology of AH and cardiovascular disease in patients with CKD.

In the present study, after a 6-month supplementation with omega-3 fatty acids, ALA levels increased both in the CKD and in the reference group. However, significant changes in EPA and DHA levels were not observed. The ability of the body to produce metabolites of the n-3 series depends on the activity of enzymes-desaturases. Conversion of ALA to EPA is catalyzed by 6 and 5-desaturases. EPA then undergoes the processes of elongation and desaturation, resulting in the formation of DHA [4,35] which is partially retro-convertible. The retro-conversion of DHA to EPA occurs after its release from the phospholipid structure with the participation of phospholipase A2. It is estimated that about 10% of DHA can be converted to EPA through β-oxidation of DHA [36].

The level of activity of desaturases depends on many factors such as age, dietary factors, hormonal factors, cardiovascular diseases or viral infections [35,37,38]. It can be assumed that supplementation with omega-3 acids leads to the activation of desaturases (5 and 6) in the formation of alpha-linolenic acid conversion products, with a simultaneous decrease in n-6 acid conversion activity, which is related to the phenomenon of competition between acids of both families with respect to 6 and 5 denaturizes [39]. Factors which may be responsible for the lack of elevated serum EPA and DHA levels in the study population of CKD patients may include age and cardiovascular disease. Another factor causing the inhibition of omega-3 fatty acid metabolism is the intake of statins [40]. In the present study, 31% of CKD patients received statin therapy. No statistically significant changes in serum EPA and DHA levels in this study may also indicate a relatively rapid capture of these acids and their incorporation into cell membrane structures.

The results of this study did not show significant changes in blood pressure measured with ABPM. The results of previous studies analyzing the effect of omega-3 supplementation on blood pressure are inconclusive. The meta-analysis performed by Cabo et al. [41] highlighted the beneficial effect of omega-3 fatty acids at >3 g/day on blood pressure regulation especially in older adults and persons with untreated arterial hypertension. In a population of peritoneal dialysis patients, supplementation with 3 g of omega-3 fatty acids for 8 weeks significantly reduced SBP values (148.6 ± 18.3 vs.. 126.4 ± 14.3 mmHg, p<0.0001) and DBP (85.1 ± 14.2 vs. 73.3 ± 10.1 mmHg; 0.001) [42]. The beneficial effect of 4 g of omega-3 fatty acid supplementation over a period of 8 weeks on SBP and DBP ABPM was demonstrated in patients with CKD taking antihypertensive drugs [5]. In those patients, eGFR was between 15 and 60 ml/min/1.73 m². The values of SBP ABPM and DBP ABPM decreased significantly (119.8 vs.. 116.4 mmHg, 72.15 vs. 70.55 mmHg, p<0.0001 for both). It is worth emphasizing that cardiovascular events or blood pressure values >170/100 mmHg in a period of 3 months before supplementation constituted criteria for exclusion from the study. In a study by Svensson et al. [43], no beneficial effects on BP ABPM values were found in two groups of CKD patients, one taking omega-3 at a dose of 4 g/day for 8 weeks, and the other group taking capsules with olive oil from olives also for a period of 8 weeks. In the said study, AH was diagnosed in 78% of patients receiving omega-3 acids and their baseline ABPM values were 131/73 mmHg, while in the second group, AH was diagnosed in 83% of patients and their baseline ABPM BP values were 141/80. Also, a regular consumption of fish meals for a year in the AH group did not show any effect on SBP and DBP ABPM values, irrespective of the ratio of polyunsaturated to erythrocyte saturated fatty acids [44]. No change in SBP and DBP ABPM values in the present study may be associated with optimal BP values in patients with CKD. The mechanism of hypotensive activity of omega-3 acids is related, inter alia, to increased levels of prostacyclin and vasodilating agents. It is also important to inhibit the synthesis of thromboxane A2 (TXA2) and prostaglandin E2 (PGE2), which is responsible for the secretion of renin and the resorption of sodium ions [30,45]. These mechanisms may be disturbed in CKD patients.

The present study did not show any effect of omega-3 supplementation on HR ABPM. Mori et al. [5] reported a beneficial effect of omega-3 supplementation on HR ABPM (66.9 ± 1.5 beats/min vs. 65.6 ± 0.5 beats/min, p<0.001) in patients with CKD and AH. The mean eGFR value of 33.45 ml/min/1.73 m² in that group of patients indicated the progression of CKD as compared to patients in the present study (eGFR 74.9 ml/min/1.73 m²). It should be stressed that the degree of sympathetic activation correlates positively with the progression of renal disease [46]. The authors of the aforementioned study conclude that omega-3 acids may reduce cardiovascular risk in non-diabetic patients with moderate-to-severe CKD [5].

Omega-3 fatty acids have a beneficial effect on the cardiac muscle in patients after myocardial infarction [47]. The authors of this publication concluded that a 6-month supplementation with omega-3 fatty acids (the authors did not specify the dose) significantly reduced the left ventricular end-systolic volume (p=0.0001) while increasing the left ventricular ejection fraction (p=0.0001) and the stable value of left ventricular diastolic volume. In a group of patients with chronic heart failure, the increase in left ventricular ejection fraction during a 3-month period of omega-3 supplementation was most favorable at a dose of 4 g per day [48]. In patients with cardiomyopathy, omega-3 supplementation at 2 g/day during a 12-month period positively influenced EF [49]. In the present study, EF did not change significantly both in the CKD group and in the whole study group. This may be due to the fact that in the majority of 81 (93.1%) patients with CKD left ventricular systolic function was normal prior to omega-3 supplementation. The absence of effects of supplementation with omega-3 fatty acids on the increase of left ventricular ejection fraction may also be associated with the small, 2-gram dose of acid or the relatively short 6-month period of supplementation. It should be

noted that in this study, patients with stage 3 CKD prior to omega-3 supplementation showed statistically significant positive correlations of DHA and EPA with EF (r=0.42; p<0.04, r=0.47; p<0.02), which was not confirmed after the supplementation. The positive correlation between DHA and EF (β=0.009; p=0.05) in the men-only group was demonstrated by other authors in a prospective cohort study [50].

In a multicenter study ALFA OMEGA, analyzing the frequency of cardiovascular events in 4837 patients after myocardial infarction, a diet enriched in small doses of polyunsaturated fatty acids (400 mg EPA and DHA, 2 g ALA) was shown not to reduce the incidence of cardiac events during 40 months of observation [51]. The authors concluded that the lack of positive results of the supplementation program was associated with optimal pharmacological therapy including antiplatelet, antihypertensive and statin drugs [51]. In the present study, patients were subjected to only 6 months of cardiovascular monitoring and no cardiovascular events occurred in the patients during this period. In a recent published study, there were no statistically significant differences in the risk of major vascular events over an average of 2.5 years between the group receiving 1 g of omega-3 and the placebo-treated group in 15 480 diabetic patients without cardiovascular disease [52].

According to the guidelines of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC), increased intima-media thickness of the carotid artery>0.9 mm is a manifestation of organ damage in patients with arterial hypertension. Value of IMT>1.5 mm indicates atherosclerotic plaque [24]. In this study, IMT thickening was found in 6 (6.9%) patients with CKD stage 1-3 before supplementation with omega-3 acids and in 4 (4.6%) patients after supplementation. Supplementation with omega-3 did not affect the statistically significant change in IMT, whose mean values in both the reference group and the CKD group were within the normal range. In another prospective study of patients with AH, a regular consumption of fish meals over a year significantly reduced the value of IMT (1.00 ± 01 vs.. 0.81 ± 0.19 mm, p<0.001), but only in patients with increased ratio of polyunsaturated to erythrocyte saturated fatty acids [44]. Japanese researchers investigating correlations between EPA and DHA levels in serum and the cardiovascular status of hypertensive fishermen and farmers treated on an outpatient basis demonstrated that the levels of EPA and DHA were significantly higher in fishermen [53]. Patients involved in fishing were also found to have lower statistically significant values of IMT. The authors linked this difference with the balance of consumption between fats of land and sea origin and concluded that further research was needed to clarify the mechanisms underlying these relationships. No association between the concentration of omega-3 in the blood and the value of IMT in a population of both healthy women and women with diabetes or arterial hypertension was demonstrated by Monge et al. [54]. A beneficial effect of a 6-month supplementation with omega-3 fatty acids at a dose of 3 g/day on the value of IMT was found in 27 hemodialysis patients (0.79 ± 0.21 mm vs. 0.65 ± 0.18 mm, p<0.001) [55].

Arterial stiffness is defined as reduced arterial elasticity. PWV is the "gold standard" for assessing arterial stiffness and a commonly accepted indicator of cardiovascular risk. Clinical data indicate that omega-3 fatty acids improve vascular flexibility [56,57]. Nestel et al. [57], who studied patients with dyslipidemia receiving 3 g of EPA, 3 g of DHA or placebo daily for 7 weeks, showed that EPA supplementation improved arterial elasticity by 36% and DHA by 27%. In the study groups, total vascular resistance decreased. The authors concluded that the improved arterial flexibility was associated with the effect of omega-3 acids on endothelial function and smooth muscle tone. Polyunsaturated omega-3 fatty acids alter prostaglandin metabolism in the direction of increasing the level of vasodilatory prostaglandins while reducing that of vasoconstrictor prostaglandins [4]. The beneficial effect of omega-3-fortified diets on vascular regulation and blood pressure has been demonstrated in a population of hypertensive patients [58]. In a 6 month follow-up, the PWV value changed significantly in the reference group (8.60 ± 1.70 vs. 8.23 ± 1.83 m/s, p=0.044). In the CKD group, the PWV value did not change significantly. This remains in line with the results of a study by Krantz et al. [59], where in a population of patients with AH, 48% of whom were diagnosed with diabetes, a 3-month supplementation with omega-3 at a dose of 3.36 g daily did not contribute to the statistically significant decrease in PWV, whose initial value was 1602 ± 324 cm/s. However, is a study of patients with AH, a 3-month supplementation with omega-3 at a dose of 1800 mg/day statistically significantly reduced the value of PWV (10.4 ± 0.4 vs. 9.4 ± 0.3 m/s, p=0.021), but only in patients with high cardiovascular risk of ≥ 7.5% [60].

In conclusion, it should be emphasized that due to changes in the cardiovascular system already present in the early stages of CKD, the patients have taken a number of drugs that affect the cardiovascular system, such as angiotensin-converting-enzyme inhibitors, beta blockers or statins. The doses of these drugs during the 6-month follow-up period were not modified. The changes that were observed were attributed to the omega-3 supplementation.

Critical comments about the material and methods: The present study has several limitations. They result from a relatively small study group, a short period of supplementation with omega acids, as well as the daily dose of omega-3 fatty acids. Extending the observation period to 12 months could increase the opportunity to demonstrate the effect of omega-3 fatty acids on cardiac remodeling. Also, the use of more precise LVMI measurement methods, such as magnetic resonance imaging, could increase the reliability of the test. The value of the study would also be higher if it involved control and CKD groups receiving placebo and a random selection of patients into the omega-3 and placebo groups.

To better understand the relationship between omega-3 fatty acids and the cardiovascular system, the tissue content of omega-3 fatty acids should also be analyzed, not just the serum concentration of these substances.

Despite the limitations mentioned above, the results of this study allow us to formulate the following conclusions.

Conclusions

Six-month omega-3 supplementation exerts favorable effects on serum ALA concentration, but not on its metabolites-EPA and DHA. Supplementation with omega-3 acid resulted in beneficial left ventricular remodeling, despite the absence of changes in blood pressure and arterial properties. There was no deterioration in renal function in CKD group.

Conflict of Interests

The authors declare no conflict of interests.

Comments

The results of the present study were presented in the form of an abstract during the 22th Session of the Polish Society of Nephrology held on 18-20 June 2015 in Kołobrzeg, Poland.

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Author Contributions

Agnieszka Pluta-writing-original draft, data curation , Paweł Stróżecki-formal analysis, Jacek Kęsy-methodology, Magdalena Krintus-data curation, Beata Sulikowska-methodology, Grażyna Odrowąż-Sypniewska-methodology, Jacek Manitius-conceptualization.

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