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The Influence of Bioactive Lipid Preparations on Cardiac Muscle and Coronary Vessels State in Rats

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Abstract: Based on elaborated or modified procedures, fish oil was enriched in EPA+DHA fatty acids which content increased from 32.4-83.3% (preparation EFO) while milk fat was enriched in t11C18:1 (VA) and c9, t11C18:2 (CLA isomer) fatty acids from 1.8 and 1.3-16.5 and 12.3%, respectively (preparation EMF). Linoleic acid c9, c12C18:2 contained in grapeseed oil was applied for synthesis of its conjugated dienes of c9, t11 and t10, c12C18:2 configuration in amount of 76.5% (preparation MGO). The composition of Lpid Complex (LC) was elaborated based on these preparations and it contained 28.7% CLA+VA and 27.8% EPA+DHA. An application of lipid preparations in *in vivo* study on the rats considerably limited unprofitable changes within coronary vessels and cardiac muscle induced by the diet rich in saturated fatty acids. Elaborated preparations inhibited a decrease in vascular bed size from 5.7-24.7%, an increase in both cardiac ventricles thickness from 17.3-50.6% and myocytes size from 3.6-34.8%. The lowest decrease in vascular bed size was noted in the groups supplemented with EFO and LC. The thickness of both ventricles increased to the lower degree in groups EMF and LC while the size of myocytes in groups EFO and LC.

Key words: Wistar rats, high-fat diet, enriched lipid preparations, heart morphometric indices, size

INTRODUCTION

The number of patients hospitalized due to cardiovascular system diseases has been increased drastically last years. One of the reasons of this phenomenon is incorrect diet leading to an increase in blood triglycerides and cholesterol content (Cromwell and Otvos, 2004; Halton *et al*., 2006). However, animal and plant origin products contain the range of biologically active compounds which may profitably affect human organism and be used in some diseases prevention and therapy. Such multidirectional activity has been demonstrated in case of ω-3 fatty acids and Conjugated dienes of Linoleic Acid (CLA).

Wider interest in fish oils as a source of ω-3 acids has been noted since the observation that Eskimos from Greenland more rarely suffer from Cardio Vascular Diseases (CVD) and their diet is dominated by fat fish and marine animals with high content of these acids (Connor, 2000). Epidemiological and experimental research have provided further evidences concerning profitable activity of ω-3 acids in CVD prevention (Psota *et al*., 2006; Cundiff *et al*., 2007; Von Schacky and Harris, 2007; Colhoun, 2007).

Formation of atherosclerotic changes and cardiovascular system diseases development may also be counteracted by Conjugated dienes of Linoleic Acid (CLA) (Kritchevsky *et al*., 2002). Conjugated Linoleic Acid (CLA) is a term describing the mixture of positional (8 and 10, 9 and 11, 10 and 12 or 11 and 13) and geometric (cis and trans) isomers of octadecadienoic acid in which unlike in linoleic acid, the double bonds are isolated only with one single bond (i.e. are conjugated) (Fritche and Steinhart, 1998). These compounds may inter alia decrease the level of triglycerides, total cholesterol and low-molecular lipoproteins LDL and also improve LDL/HDL ratio (Lee *et al*., 1994).

Within the study presented, lipid preparations of animal (fish oil and milk fat) and plant origin (grapeseed oil) were enriched in biologically active fatty

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acids. The composition of Lipid Complex (LC) was elaborated based on them and then their influence on cardiac muscle and coronary vessels state was evaluated in *in vivo* study on rats fed with high-fat diet.

MATERIALS AND METHODS

Lipid preparations and Lipid Complex (LC) obtaining: synthesis of conjugated isomers of linoleic acid of c9, t11 and t10, c12 C18:2 configuration. Grapeseed oil containing about 68% (m/m) of linoleic acid c9, c12 C18:2 was used as a raw material. The synthesis involved moreover potassium hydroxide, ethylene glycol, concentrated hydrochloric acid and urea and hexane was used as a solvent.

The synthesis of isomers of linoleic acid C18:2 of c9, t11 and t10, c12 configuration was conducted applying alkaline hydrolysis, free fatty acids acidification, hydrochloric acid, moisture and hexane removing and crystallization with urea according to modified methodology elaborated by Walisiewicz-Niedbalska *et al.* (2009). Fatty acids profile of grapeseed oil before and after Modification process (MGO) is presented in Table 1.

Enrichment of milk fat in linoleic 9c, 11t C18:2 and oleic acid isomer 11t C18:1: Sheep milk fat obtained in a process of double centrifugation (3000 rpm) containing about 1.3% of c9, t11 C18:2 isomer and 1.8% of t11 C18:1 isomer was used as a raw material. Urea, potassium hydroxide, hydrochloric acid as well as also ethylene, methylene and hexane as solvents were used in the process.

Milk fat enrichment in c9, 11t C18:2 and t11 C18:1 isomers was conducted using the following processes: Alkaline hydrolysis, crystallization with urea, freezing out and filtration and solvent and moisture removing according to the methodology elaborated by Walisiewicz-Niedbalska *et al.* (2009). Fatty acids profile of milk fat before and after enrichment process (EMF) is presented in Table 1.

Analytical methods in the previous stages: Methyl esters of fatty acids of grapeseed oil and milk fat were obtained according to AOCS Official Methods Ce2-66 while the analysis was conducted according to AOCS Official Methods Ce1f-96 (AOAC, 2000). For qualitative examinations, the technique of capillary gas chromatography was used in a following conditions: Hewlett Pacard 5890 gas chromatograph with FID detector, SP-2560 column of Supelco company (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) of parameters L×I.D.100 m×0.25 mm, df 0.20 μm, furnace programme: 140°C for 1 min, accretion 1°C min^{-1} up to 180°C, 26 min isotherm, accretion 5°C min^{-1} up to 245°C, 25 min isotherm, FID 255°C detector, feeder-split/splitless -245°C, carrier gas-helium 0.98 mL min^{-1}.

The standards of Sigma (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) and Larodan (Larodan Fine Chemicals AB, Malmo, Sweden) companies and also standard oils: soybean, rapeseed, coconut as well as literature data were used for fatty acids identification (Roach *et al.*, 2002). Heptadecanoic acid C17 of Fluka company (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) was used as an internal standard. Synthesis and enrichment processes and chromatographic analysis were conducted in the Industrial Chemistry Research Institute in Warsaw, Poland.

Table 1: Fatty acids profile (Total fatty acids%) of lipid preparation and lipid complex

	Lipid preparation						
	Grapeseed oil		Milk fat		Fish oil		
Fatty acids	Before	After (MGO)	Before	After (EMF)	Before	After (EFO)	Lipid Complex (LC)
SFA	13.9	1.5	59.1	7.2	15.8	0.8	1.9
MUFA	17.3	19.2	34.9	59.8	39.6	2.5	16.3
PUFA	68.1	77.9	5.4	32.1	43.8	95.8	81.9
Eicosapentaenoic 20:5 (EPA)	-	-	-	-	12.1	19.1	10.6
Docosahexaenoic C22:6 (DHA)	-	-	-	-	20.3	52.2	17.2
EPA+DHA	-	-	-	-	32.4	71.3	27.8
Isomer c9, t 11C18:2	-	34.1	1.3	12.3	-	-	16.8
Isomer t 10, c12C18:2	-	33.4	0.3	0.6	-	-	8.1
CLA	-	66.5	1.6	13.2	-	-	24.9
t11C18:1 (VA)	-	-	2.2	16.5	-	-	3.8

MGO: Modified Grapeseed Oil enriched with c9, t11C18:2 and t10, c12C18:2; EFO: Fish Oil Enriched with C20:5 (EPA) and C22:6 (DHA); EMF-milk fat enriched with c9, t11 C:18:2 and t 11C18: 1; LC-Lipid Complex-composition of MGO, EFO and EMF; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; SFA-ΣC8:0 C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C19:0, C20:0, C22:0; MUFA-Σ C14:1, C16:1, C18:1 c9, C18:1 t9, C18:1 t11, C18:1 other trans isomers, C20:1; PUFA-Σ C18:2 c9c12, C18:2 c9t11, C18:2 t10c12, C18:2 other isomers, C18:3 c9c12c15, C20:5, C22:5, C22:6; CLA-Σ C18:2 c9t11+C18:2 t10c12+C18:2 other isomers

Fish oil Enrichment in C20:5 (EPA) and C22:6 (DHA) acids: Fat from cod liver containing about 12.4% (m/m) of Eicosa Pentaenoic Acid (EPA) and about 20.3% (m/m) of Docosa Hexaenoic Acid (DHA) was used as a raw material. Moreover, potassium hydroxide and hydrochloric acid were used as well as hexane and dichloromethane as the solvents.

The content of EPA and DHA fatty acids in fish oil was increased by an application of the process of saponification, acidification, extraction and washing out on aminopropyl column according to the methodology modified by Usydus *et al.* (2012). Fatty acids profile of fish oil before and after enrichment process (EFO) is presented in Table 1.

Analytical methods for stage of fish oil enrichment: Methyl esters were obtained according to AOCS Official Methods Ce2-66 while the analysis was conducted according to AOCS Official Methods Ce1f-96 (AOAC, 2000). Fatty acids profile was determined using Gas Chromatography Method on Agilent Technologies 6890N chromatograph with an application of FID detector in the presence of standard mixture of Sulpeco company (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). Capillary column Supelco SP 2560 of parameters L×I.D.100 m×0.25 mm, df 0.20 μm was used in the separation process. The samples of 2 μL were dosed in split mode 100:1. The temperature of the feeder was 250°C while that of detector was 260°C. Helium was used as a carried gas and nitrogen as masking one. The flow of a carrier gas by the column was 1.1 mL min^{-1}. Temperature program of the furnace was as follows: initial temperature 140°C (2 min) then temperature accretion of 2°C min^{-1} up to 225°C and 10 min isotherm, accretion 4°C min^{-1} up to 240°C and 10 min isotherm.

Identification and percentage content of particular fatty acids was determined based on retention times and peaks area for the standard mixture 37 FAME mix (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) and additionally for C18:4 and C22:5 fatty acids standards using Aligent Technologies software (Chemstation; 10 Rev A 10.02 (1757)). The reference material NIST 8415 (powdered eggs) was used for the method confirmation.

The enrichment processes and chromatographic analyses were conducted in the accredited laboratory of the Department of Food and Environmental Chemistry, National Marine Fisheries Research Institute in Gdynia, Poland.

Elaboration of Lipid Complex (LC) composition: The composition of bioactive Lipid Complex (LC) was elaborated based on previously elaborated lipid preparations (MGO, EMF, EFO) which were mixed in a suitable ratios using magnetic stirrer and then tocopherol dissolved in methanol was added in an amount of 200 mg/100 g of oil in order to protect unsaturated fatty acids against oxidation process.

Chromatographic analyses were conducted in the Industrial Chemistry Research Institute in Warsaw, Poland and fatty acids content is presented in Table 1. The manner of samples preparation, apparatus and analyses conditions are presented earliear.

Evaluation of lipid preparations and lipid complex on the state of cardiac muscle and coronary vessels

Animals and scheme of experiment: The research material consisted of 60 rats of Wistar breed of body weight of 230±20 g, originating from homozygous breeding of Medical University of Warsaw, Poland. The animals were randomly divided into 6 group, 10 heads in each and were maintained individually in the cages in the Vivarium of the Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland. All rats were fed with standard granulated fodder Labofeed B these from groups II-VI additionally with animal fat while the animals from experimental groups (III-VI) also with elaborated lipid preparations administered using the syringe:

Group I: Control (C)-standard mixture Labofeed.

Group II: Negative control (HFD)-standard mixture+ butter (5 g/head/day).

Group III: Experimental (MGO)-standard mixture+butter (5 g/head/day)+modified grapeseed oil (1 mL/head/day).

Group IV: Experimental (EMF)-standard mixture+butter (5 g/head/day)+enriched milk fat (1 mL/head/day).

Group V: Experimental (EFO)-standard mixture+butter (5 g/head/day)+enriched fish oil (1 mL/head/day).

Group VI: Experimental (LC)-standard mixture+butter (5 g/head/day)+lipid complex (1 mL/head/day).

Research material and analytical methods: According to methodological assumptions, all the rats were subjected to euthanasia after 6 weeks (approval no. 79/2010 of the 2nd Local Animal Ethics Committee, Wroclaw University of Environmental and Life Sciences, Poland). The whole hearts were collected for histological examinations and they were immediately fixed in 4% solution of formalin buffered with CaCOOH (pH7.2-7.4) and after 3 days they were rinsed in water and dehydrated in alcohol series. Paraffin slices of a thickness of 7 μm were cut according to the heart long axis, obtaining the cross-sections of both chambers and atria with respective coronary vessels. The material was stained with hematoxylin and eosin as well as using Azan-Novum Method. Mean thickness of

both heart chambers measured at their half part, average wideness of myocytes, total volume of adipose cells were determined in morphometric examination. The size of vascular bed expressed with respect to heart wall area was calculated. Morphological and morphometric analyses were performed on Nikon Eclipse 80i microscope using Nis Elements Ar Software.

Statistical analysis: Numerical data were elaborated statistically using Statistica 8.0 application for confidence interval of $p \leq 0.05$ and $p \leq 0.01$.

RESULTS AND DISCUSSION

Conjugated dienes of Linoleic Acid (CLA) were synthesized as a result of linoleic acid contained in grapeseed oil alkaline isomerization and crystallization from urea. Process of alkaline isomerization caused a change in double bonds arrangement in unsaturated chains of fatty acids (positional) and the change in radicals arrangement with respect to double bond axis (geometric). This resulted in formation of two not observed naturally in grapeseed oil, conjugated dienes of linoleic acid of configuration cis-9, trans-11 and trans-10, cis-12. In turn, an application of crystallization from urea caused removal in a form of adduct of most of saturated fatty acids increasing thus CLA concentration in the final product (MGO) up to 76.5%. There are also other methods of plant oils isomerization reported in the literature (Walisiewicz-Niedbalska *et al*., 2009).

An application of repeated complexing with urea and dry fractionation caused an increase in Vaccenic Acid (VA) t11C18:1 and linoleic acid c9t11C18:2 concentration in milk fat from 1.8 and 1.3-16.5 and 12.3%, respectively preparation EMF.

Aminopropyl column extraction on solid bed resulted in an increase in ω-3 acids concentration in cod liver oil from about 41-97% and EPA+DHA acids from 32.4-83.3% (preparation EFO) and these values were higher compared to this kind of diet supplements. The literature also provides the information of other methods concerning fish oil enrichment in ω-3 fatty acids (Patkowska-Sokola *et al*., 2008; Bodkowski *et al*., 2011).

The composition of Lipid Complex (LC) containing 98.2% of Unsaturated Fatty Acids (UFA) including 81.9% Polyunsaturated (PUFA) and 16.3% Monounsaturated ones (MUFA) was elaborated based on lipid preparations. The content of EPA+DHA and CLA+VA fatty acids in biopreparation elaborated was 27.8 and 28.7%, respectively (Table 1). The content of fatty acids groups of various saturation degree and selected fatty acids in all elaborated lipid preparations (MGO, EMF, EFO, LC) is presented in Table 1.

The second part of the study involved an evaluation of lipid preparations (MGO, EMO, EFO) and Lipid Complex (LC) effect on the state of coronary vessels and cardiac muscle. The Control group (C) were the rats fed with standard granulated mixture while the negative control (HFD) the rats given butter addition. The experimental groups rats, except high-fat diet were provided with lipid preparations: MGO, EMF, EFO and LC.

Preliminary histological analysis did not reveal any significant pathological changes. No proliferation of connective tissue cells nor intensified collage synthesis and immune system cells infiltration was noted in the groups I-VI. Also, no degenerative changes in muscle cells and no significant changes within coronary vessels were observed. Adipose cells were mainly present within epicardium and around bigger coronary vessels. Unlike in case of morphological examinations when it is difficult to diagnose any significant changes in cardiac muscle structure an influence of high-fat diet (butter addition) and elaborated lipid preparations was visible in morphometric indices values (Table 2 and Fig. 1-4).

Table 2: Value of cardio morphometric indices (mean and SD)

Groups/Lipid preparation	Morphometric indices					
	LV	RV	MLV	MRV	VA	L
C	864.21^{A}	498.28^{A}	12.55^{A}	11.82^{a}	4.81^{A}	708.60^{ACD}
	48.89	19.86	0.56	0.50	0.31	198.95
HFD	1296.88^{B}	906.82^{Ba}	17.32^{Bb}	14.48^{b}	1.64^{Ca}	652.89^{ACD}
	42.26	21.48	1.30	0.79	0.15	196.80
MGO	1069.29^{b}	571.03^{b}	15.67^{b}	13.96	1.86^{BCb}	601.68^{ACD}
	20.80	50.64	1.14	0.89	0.06	236.46
EMF	1072.60	637.39	12.73	13.42	2.09^{BC}	915.36^{C}
	41.50	33.28	0.76	1.09	0.05	205.33
EFO	887.23^{Aa}	518.87^{A}	11.28^{A}	13.08	2.42^{B}	488.84^{D}
	38.87	25.45	0.97	0.40	0.10	213.34
LC	904.88	533.82^{A}	12.76^{b}	11.83^{a}	2.23^{Bb}	1843.62^{B}
	30.39	14.81	0.54	0.68	0.09	351.76

^{aA}Means in rows marked with different superscripts differ significantly at: small letters-$p \leq 0.05$; capitals-$p \leq 0.01$ Groups C (control): Standard granulated mixture; FD (negative control): standard granulated mixture+butter; MGO (experimental): Standard granulated mixture+butter+modified grapeseed oil enriched with c9, t11C18:2 and t10, c12C18:2; EFO (experimental): Standard granulated mixture+butter+fish oil enriched with C20:5 (EPA) and C22:6 (DHA); EMF (experimental): Standard granulated mixture+butter+milk fat enriched with c9, t11 C:18:2 and t11C18:1; LC (experimental): Standard granulated mixture+butter+lipid complex (composition of MGO, EFO and EMF); Morphometric indices: LV: Left Ventricular wall thickness (μm); RV: Right wall ventricular thickness (μm); MLV: Left Ventricular Myocytes size (μm); MRV: Right Ventricular Myocytes size (μm); VA: heart blood vessels size (mm^2); L: volume of Lipocytes in the myocardium (μm^3)

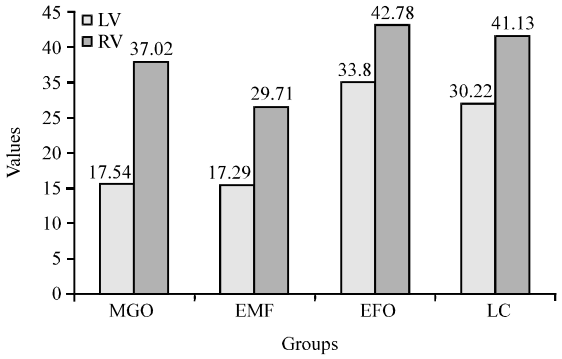

Fig. 1: Inhibition in a decrease in the thickness of Left (LV) and Right (RV) Ventricles walls in the groups receiving lipid preparations compared to the negative control (HFD) (%). LV: Left Ventricular wall thickness (μm); RV: Right wall ventricular thickness (μm)

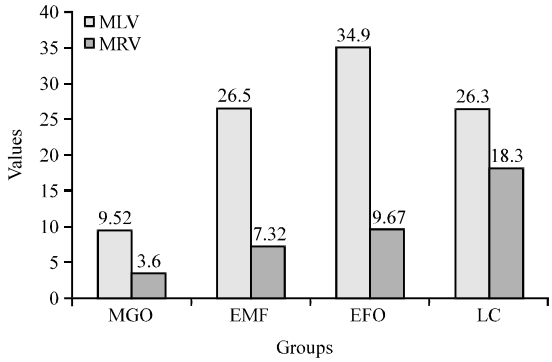

Fig. 2: Inhibition in the growth of Myocytes of the Left (MLV) and Right (MRV) Ventricles of the cardiac muscle in the groups receiving lipid preparations compared to the negative control (HFD) (%). MLV: Left Ventricular Myocytes size (μm); MRV: Right Ventricular Myocytes size (μm)

Compared to the control group, High Fat Diet (HFD-negative control) caused an increase in the thickness of cardiac Left (LV) and Right Ventricle (RV) wall of 50 and 82%, respectively ($p \leq 0.01$) and in myocytes size: MLV of 38% ($p \leq 0.01$) and MRV of 14.5% (Table 2 and Fig. 1, 2). The thickness of both ventricles walls increased, however to a lower degree in most of the experimental groups (high-fat diet+lipid preparations). In case of Left (LV) and Right (RV) Ventricle their walls thickness increased in a following manner of 4.7 and 7.1% in the group receiving LC, of 23.7 and 14.5% in MGO of 24.1 and 27.8% in EMF ($p \leq 0.05$) and of 2.7 and 4% in EFO (Table 2 and Fig. 1). Also, the size of myocytes increased in most of the experimental groups. In case of Left Cardiac Ventricle (MLV) their size increased of 24.9% ($p \leq 0.05$)

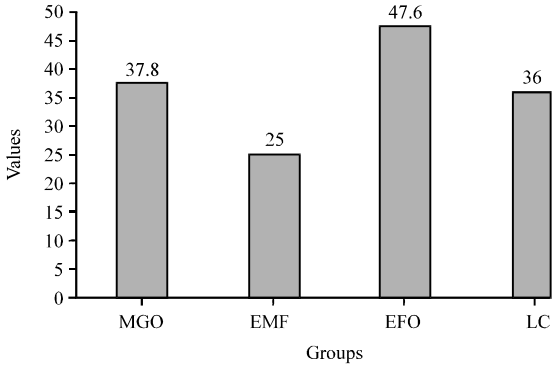

Fig. 3: Inhibition in a decrease in the size of heart blood Vessels (VA) the groups receiving lipid preparations compared to the negative control (HFD) (%). VA: Heart blood vessels size (mm^2)

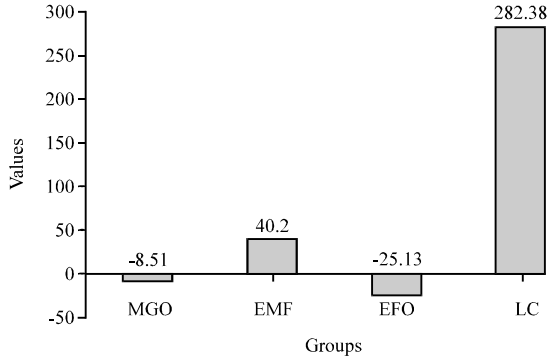

Fig. 4: Changes in the volume of Llipocytes (L) within the myocardium in the groups receiving lipid preparations compared to the negative control (HFD) (%). L: Volume of Lipocytes in the myocardium (μm^3)

in MGO group and of 1.5% in groups LC and EMF while in the Right Ventricle (MRV) of 18.1, 13.4 and 10.7% in groups MGO, EFM and EFO, respectively (Table 2 and Fig. 2). In turn, the size of myocytes was not subject to any changes in group receiving LC (right ventricle) and decreased of 10% in EFO group (left ventricle) (Table 2 and Fig. 2). High-fat diet also affected vascular bed size. In all the groups receiving butter addition their size decreased of 165% (HFD), 161.3% (MGO), 156.5% (EMF), 153.6% (LC) and 149.7% (EFO) compared to the control group ($p \leq 0.01$) (Table 2 and Fig. 3).

Unequivocal results were obtained in turn in case of adipose cells volume. Compared to the control group, their volume increased in the groups receiving LC and EMF addition of 260.2% ($p \leq 0.01$) and 29.2% ($p \leq 0.05$), respectively and decreased in the groups receiving EFO, MGO and HFD of 31, 15.1 and 7.9%, respectively (Table 2 and Fig. 4).

However, the differences in the range of morphometric parameters between the groups fed with animal fat addition (HFD) negative control and the groups additionally receiving lipid preparations (EMF, EFO, MGO, LC) were more significant for the study conducted.

Rats diet supplementation with lipid preparations significantly affected a decrease in both cardiac muscle ventricles thickness and myocytes size. In case of the Left (LV) and Right Ventricle (RV) their thickness was lower of: 33.8 and 42.8% (EFO) (differences significant statistically), 30.2% ($p \leq 0.05$) and 41.1% ($p \leq 0.01$) (LC), 17.5 and 37% ($p \leq 0.05$) (MGO) and 17.3 and 29.7% ($p \leq 0.01$) (EMF) (Table 2 and Fig. 1). In turn, Myocytes size in Left (MLV) and Right Ventricle (MRV) in experimental groups was lower of 34.9% ($p \leq 0.01$) and 9.7% (EFO), 26.3% ($p \leq 0.05$) and 18.3% (LC), 26.5% ($p \leq 0.05$) and 7.3% (EMF) and 9.5 and 3.6% (MGO) (Table 2 and Fig. 2).

Lipid preparations also affected the size of vascular bed. Compared to the negative control (HFD), its area was higher of 47.6% ($p \leq 0.01$) in the group receiving EFO of 37.8% ($p \leq 0.01$) in MGO, of 36% ($p \leq 0.01$) in LC and of 25% ($p \leq 0.01$) in EMF (Table 2 and Fig. 3).

Unequivocal results were however obtained in the range of adipose cells volume. Compared to HFD group their volume decreased of 25.1% ($p \leq 0.05$) and 7.8% in groups EFO and MGO, respectively and increased of 282.4 and 40.2% in groups LC and EMF ($p \leq 0.01$) (Table 2 and Fig. 4).

Except cholesterol and LDL lipoproteins also some diet components contribute in a development of atherogenic lesions in arteries and formation of cardiovascular-system diseases (Bodkowski and Patkowska-Sokola, 2013a). The factors which leads to an increase in the level of atherogenic lipid blood indices, pointing thus strong atherosclerotic activity are saturated fatty acids derived mainly from animal origin fats (Halton *et al*., 2006; Bodkowski and Patkowska-Sokola, 2013b). Since, saturated fatty acids with 12, 14 and 16 carbon atoms cause an increase in the level of cholesterol and its LDL fraction in blood serum they are considered as atherogenic ones while saturated fatty acids with 12, 14 and 18 carbon atoms which favor blood platelets aggregation are considered as thrombogenic ones (Hegsted *et al*., 1993).

This was also confirmed in the study conducted by the researchers who noted 77% higher level of triglycerides, 46% higher level of total cholesterol and 122% higher level of LDL in blood of rats fed with high-fat diet rich in saturated fatty acids (Patkowska-Sokola *et al*., 2008).

The level of blood triglycerides, total cholesterol and lipoproteins LDL may be in turn reduced by polyunsaturated fatty acids of n-3 family, mainly by eicosapentaenoic acid EPA (C20:5) and docosahexaenoic one DHA (C22:6) (Balk *et al*., 2006; Henderson *et al*., 2008; Saito *et al*., 2008; Chang *et al*., 2009; Wergedahl *et al*., 2009). Protective effect of these acids on circulatory system also involves blood coagulation reduction (Mesa *et al*., 2004). They inhibit formation of thromboxane A2 stimulating coagulation as well as adhesion and aggregation of blood platelets and formation of thromboxane B2, next cytokine stimulating coagulation process. EPA acid also initiates the formation of third series prostanoids: prostacyclin PGI_3 and thromboxane TxA_3 which are involved in blood coagulation mechanisms, i.e., decrease it significantly (Wijendran and Hayes, 2004). Omega-3 acids may be thus a factor lowering the risk of coronary arterial disease (Block and Pearson, 2006; Schmidt *et al*., 2006). Antihypercholesterolemic and antiatherosclerotic activity may be also demonstrated in case of Conjugated dienes of Linoleic (CLA) (Lock *et al*., 2005).

Profitable influence of n-3 and n-7 fatty acids in reduction of atherogenic lipid fractions in blood was also confirmed in the results of previous research conducted by the researchers. In rats receiving an addition of lipid preparations enriched in these kind of fatty acids, triglycerides content in blood was lower from 8-32%, total cholesterol from 7-19% while LDL cholesterol from 1.5-28% (Bodkowski *et al*., 2010).

The consequence of long-term high-fat diet rich in saturated fatty acids, except an increase in atherogenic lipid blood indices level are also unprofitable changes observed within cardiovascular system. They are especially dangerous for cardiac muscle (Okere *et al*., 2006). High consumption of saturated fatty acids may lead to some disorders in gene transcription which may cause an incorrect lipids metabolism in muscle cells (Chess *et al*., 2009). High consumption of saturated fatty acids may also lead to disorders in an activity of receptors localized on target cells surface which favors lipids accumulation in intercellular matrix of blood vessels causing their narrowing resulting in atherosclerosis, ischemic heart disease as well as embolisms and myocardial infarction. A transitional stage of these processes is the change in muscle cells volume which at the first stage are subject to hypertrophy and then atrophy as a result of ischemia (Zhang *et al*., 2005; Okere *et al*., 2006).

An unprofitable effect of saturated fatty acids consumption on blood vessels and cardiac muscle state was also observed in this study. This was especially visible in a decrease in a size of vascular bed and an increase in cardiac ventricles thickness and myocytes

size. An increase in left ventricle thickness was accompanied by an increase in myocytes size while in case of the right ventricle it was to a high degree a result of an increased fluid volume in the intercellular space. This means that the cells of left ventricle, performed considerably higher labor compared to the right ventricle as a result of peripheral vessels increased resistance.

Cardioprotective activity was demonstrated in turn by the elaborated lipid preparations which limited a decrease in vascular bed size and inhibited an increase in myocytes size counteracting their hypertrophy and also limited an increase in both ventricles thickness. This was especially distinct in case of an addition of Enriched Fish Oil (EFO) and Lipid Complex (LC).

High consumption of saturated fatty acids may also result in a decrease in adipose cells volume (Okere *et al*., 2006; Chess *et al*., 2009). This is caused by disorders in lipids absorption both by muscle cells and just adipose cells. In this study, adipose cells volume increased as a result of Lipid Complex (LC) application which was presumably an effect of an enhanced endocytosis.

This may lead to a decrease in lipids level in blood limiting the risk of ventricles hypertrophy and ischemic heart disease development. Confirmation of this thesis requires, however, more detailed study, related first of all to the presence and activity of genes responsible for blood lipid balance.

CONCLUSION

An unprofitable effect of the diet rich in saturated fatty acids on the values of morphometric heart indices (vascular bed size, ventricles thickness, myocytes size) was observed as a result of the study conducted. Unprofitable spectrum of the changes within blood vessels and cardiac muscle resulting from high-fat diet application were limited by the elaborated lipid preparations.

Their administration caused a limitation in vascular bed size (from 5.7-24.7%) an inhibition in both ventricles thickness growth (from 17.3-50.6%) and myocytes size (from 3.6-34.9%). A decrease in vascular bed size was limited to the highest degree by Enriched Fish Oil (EFO) and Modified Grapeseed Oil (MGO). In turn, an increase in both ventricles thickness was considerably limited by Lipid Complex (LC) and Enriched Fish Oil (EFO) while Modified Fish Oil (MFO) and Lipid Complex (LC) were the most effective in case of an increase in myocytes size growth.

The most profitable activity among the elaborated lipid preparations in the range of all examined morphometric features was demonstrated for the Lipid Complex (LC) which probably results from synergistic activity of biologically active fatty acids contained in it.

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