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EFFECT OF VARIOUSLY OXIDIZED MARINE FISH FAT ON GUINEA PIG ORGANISM*

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The effect of marine fish fat in different stages of oxidation on biochemical and morphological changes in guinea pigs was studied. Oxidized fat was found to adversely affect lipids balance in the organism and reduce the content of antioxidant vitamins (C and E).

Interest in the role of fish fats in human nutrition began in 1970-71 when Dyeberg et al. [6] demonstrated that the low incidence of stenocardia among Eskimos is clearly correlated with their diet. Their observations were soon confirmed by other authors who found that populations whose diets were rich in fish fats and other products of marine origin were less prone to circulatory system diseases widespread in Western industrialized countries. The nutritive value of fish fats and the long-chain polyunsaturated fatty acids of the n-3 family present in them became the object of numerous studies [6, 8, 16, 19, 23, 25, 31-33, 36, 37], and it was found that they are highly beneficial in circulatory system diseases, being antithrombotic and reducing arterial pressure and lipid indices in the blood. They are also hypocholesterolemic. Fatty acids of the n-3 family may contribute to a reduction of coronary disease incidence. However, due to the high content of polyunsaturated fatty acids, fish fats undergo rapid oxidation during storage, the result being free radicals, lipid peroxides and a lot of other fat oxidation products. The peroxides appearing in the first stage of fat oxidation are unstable and readily undergo further transformations which are multidirectional and highly complex. A whole range of oxidation products appear and these may interreact to give new compounds. The substances appearing during oxidation of

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polyunsaturated fatty acids were studied by many authors [7, 9-11, 13-15, 18, 20, 27, 28]. Among those discovered were aldehydes, hydroxyl and hydroxyepoxide compounds, various di- and trihydroxylic esters, cyclic compounds, dimers and polymers, and other secondary products of oxidation, not all of which have been identified. The oxidation of fatty acids in fish tissues is even more complex taking into consideration the interaction of the lipid and protein fractions.

Oxidated fats inhibit the activity of many enzymes [5, 22], and toxic substances may appear in advanced stages of oxidation. It was demonstrated that oxidated fats in the diet cause pathological changes in the mucous membrane of the alimentary tract of animals, probably as a result of peroxides decomposition in intestinal epithelium cells, and also inhibit many enzymes information quoted in [1]) and adversely affect the absorption of folic acid from food, the latter effect being blamed on damage to enzymes of the intestinal content [2]. In vitro experiments [26] showed that linoleic acid peroxides clearly inhibited the activity of pancreatic lipase.

French authors [24] report that free radicals and singlet oxygen appearing during fats oxidation reduce the stability and permeability of cell membranes and inhibit the activity of enzymes present there.

Oxidized fats are also regarded as carcinogenic [1, 3, 4]. French authors suggest that free radicals and active oxygen particles appearing in the organism during oxidation of polyunsaturated fatty acids may attack DNA particles, thereby giving rise to neoplasms.

In view of these possible harmful effects on the human organism of autooxidized marine fish fat, we carried model studies with experimental animals fed marine fish stored for various periods. The main aim of this research was to investigate the effects of variously oxidized marine fish fat on lipids balance in experimental animals fed an atherogenic diet. We wanted to see if consumption of marine fish, despite their oxidized fat content, may positively affect the lipids balance in the organism in conditions of hypercholesterolemia. In our experiments we used guinea pigs in which even small doses of cholesterol induce hypercholesterolemia. These animals provide a good experimental model in studies of nutritional factors affecting atherogenic processes.

MATERIAL AND METHODS

Male guinea pigs initially weighing 530-560 g were divided into four groups: 1) control group I fed granulated LSK stock diet manufactured by "Bacutil" and intended for animals of this kind; 2) control group II fed the granulated LSK diet with 0,1% cholesterol; 3) experimental group I fed diet with 0,1% cholesterol and fresh mackerel (frozen fillets); and 4) experimental group II fed diet containing so called "old" fish i.e. pollack (mintai) stored frozen for one year in minced form augmented with oxidized fish fat adjusting the total fat content to the level in the mackerel diet; this last diet also contained 0,1% cholesterol.

The total fat content in the experimental diets was about 14% kcal, and all

diets, both control and experimental, were suplemented with 20 mg ascorbic acid/100 g diet. The diets and water were supplied to the animals in unlimited quantities. The fish and "oxidized" fish fat were provided by the Institutte of Marine Food Technology of the Agricultural Academy in Szczecin (headed by assistant professor Anna Kołakowska) cooperating in this project. The minced mintai contained 17.56% of protein and 1,06% of lipids; the respective values for mackerel were 16,8 and 11,8%. The peroxide value of fat in frozen mackerel fillets was 5.72 mg O_2/100 g and the TBA test was 0,06 A1/1% 470 nm lipids and 0,33 A1/1% 538 nm lipids. The mackerel was of first-class quality. The oxidized fish fat used in the experiments was obtained by exposing a 2.5-cm layer of mackerel fat to a quartz lamp (14 cm above the layer) for 65 hs the peroxide value of thus treated fat was 23.25 mg O_2/100 g while values for the TBA test were: 0.83 A1/1% 470 nm lipids and 0.94 A1/1% 538 nm lipids.

To ensure equal fat contents in experimental diets, oxidized fat oil was added to the diet with minced pollack (mintai) to adjust its total fat content to that in the mackerel fillets diet.

The animals were fed the various diets for 12 weeks. On the last day of the experiment they were deprived of food for 13 h and after decapitation blood was taken for biochemical assays and internal organs removed for morphological studies.

The following determnations were made in the biological material:

1) haematological indices — hemoglobin and hematocrit — by the conventional method using Drabkin's reagent and hematocritic centrifuge;

2) total cholesterol in blood serum and cholesterol of the high density lipoproteins (HDL) fraction using enzymatic tests from Boehringer; cholesterol in LDL was calculated according to Friedwald's formula [12];

3) triglycerides using enzymatic tests from Boehringer;

4) lipid peroxides in blood serum with Satoh's method [29] by measuring malonyldialdehyde (MDA) produced in the reaction with thiobarbituric acid;

5) total lipids by the sulphophosphovanilline method [38];

6) phospholipids using Boehringer enzymatic test;

7) ascorbic acid in blood serum and tissues by the method of Lowry et al. [21];

8) tocopherol in blood serum by Hashim's method [17];

9) also determined were lipid peroxides in the fat used in experiments, by the sulphocyanide method (BN-74/8020-07), and the TBA test done according to Vyncke [35].

The obtained results were verified statistically with Student's test.

RESULTS

Even small amounts of cholesterol added to the diets had an adverse effect on body weight increase in the experimental animals (Table 1). Fresh fish (mackerel + cholesterol) in the diet did not lead to growth rates better than those observed in animals of control group II fed the stock diet + cholesterol. The lowest

Table 1. Body weight and body weight increase and weight of selected organs (in g and per cent of body weight) of quinea pigs after 12 weeks of experiment

Group	Body weight (g)	Body weight increase (g)	Selected organs									
			liver		lungs		heart		kidneys		testicles	
			g	%b.w.	g	%b.w.	g	%b.w.	g	%b.w.	g	%b.w.
Control I	761.83 ± 88.11	198.33 ± 91.66	21.40 ± 4.29	2.76 ± 0.35	4.25 ± 0.54	0.55 ± 0.05	2.08 ± 0.44	0.27 ± 0.04	4.80 ± 0.28	0.63 ± 0.08	4.12 ± 0.55	0.54 ± 0.10
Control II (with cholesterol)	699.15 ± 64.01	138.67 ± 52.12	20.65 ± 1.89	2.97 ± 0.09	3.90 ± 0.41	0.56 ± 0.03	2.19 ± 0.21	0.32 ± 0.03	4.75 ± 0.77	0.68 ± 0.08	4.58 ± 0.82	0.66 ± 0.08
Fresh fish + cholesterol	679.60 ± 41.27	117.60 ± 53.38	33.00* ** ± 3.80 $p<0.001$	4.65* ** ± 0.61 $p<0.001$	4.24 ± 0.42	0.60 ± 0.09	2.20 ± 0.11	0.31 ± 0.01	4.68 ± 0.26	0.66 ± 0.04	3.98 ± 0.71	0.56 ± 0.09
Fish with oxidizet fat + cholesterol	6.33.00 ± 69.21	102.83 ± 68.65	31.92* ** ± 7.52 $p<0.01$ $p<0.005$	4.98* ** ± 0.65 $p<0.001$	3.95 ± 0.50	0.62 ± 0.03 $p<0.02$ $p<0.01$	2.00 ± 0.16	0.25 ± 0.02	4.53 ± 0.43	0.72 ± 0.06	3.70 ± 0.30	0.59 ± 0.08

* statistically significant compared to control group I

** statistically significant compared to control group II (with cholesterol)

body weights and weight increase were observed in animals eating the diet with oxidized fat.

Measurements of liver, lung, heart, kidney and testicle weights showed that only liver mass increased significantly in animals fed the studied marine fish + cholesterol. The diets with marine fish, especially that with oxidized fat, led to significant drops in hemoglobin and hematocrit contents (Table 2).

Table 2. Hemoglobin and hematocrit levels in quinea pigs after 12 weeks of experiment

Group	Hemoglobin (g/%)	Hematocrit (%)
Control I	11.34 ± 1.25	46.00 ± 2.75
Control II with cholesterol	13.56 ± 1.34	45.33 ± 1.63
Fresh fish + cholesterol	12.74 ± 1.78	42.20 ± 2.28 * ** $p < 0.05$
Fish with oxidized fat + cholesterol	12.15 ± 0.90 * $p < 0.01$	44.16 ± 1.32

* statistically significant compared to control group I

** statistically significant compared with control group II (with cholesterol)

The 0,1% cholesterol addition to the stock diet (control diet II) caused a significant increase of cholesterol in blood serum (Table 3). In animals fed fresh fish + cholesterol, the total cholesterol content in blood serum decreased, while in animals eating fish with oxidized fat + cholesterol this content was higher than in control group II, but the difference was not statistically significant.

The lowest cholesterol levels in LDL lipoproteins (Table 3) were in control group I, significantly higher contents were observed in control group II, and the highest of all in animals fed fish with oxidized fat and cholesterol. The cholesterol content in HDL lipoproteins (Table 3) was lowest in control group I. Higher values were recorded in animals receiving the diet with fresh fish + cholesterol, and also in the group fed fish with oxidized fat + cholesterol.

Triglycerides determinations in blood serum (Table 3) showed the highest values in control group II and considerably lower ones in control group I. The lowest triglycerides values were in animals fed fish diets.

The lowest total lipids contents in blood serum (Table 3) were in control group I, and the highest — in control group II. Additions of marine fish to cholesterol diets significantly reduced total lipids in blood serum. In the group of animals fed oxidized fat + cholesterol the content of total lipids in blood serum was higher than in animals eating fresh fish + cholesterol.

Values for lipid peroxides in blood serum are given in Table 3. The highest values were in animals fed fish with oxidized fat + cholesterol, and slightly lower (with the difference being statistically significant, however) in animals fed fresh fish + cholesterol. The lowest values were in control group I. Cholesterol

Table 3. Total lipids, phospholipids, triglycerids, cholesterol and lipid pergxides in blood serum of guinea pigs after 12 weeks of experiment

Group	Total lipids (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)			Lipid peroxides (nmol/ml)
				total	HDL	LDL	
Control I	282.68 ± 39.58	46.10 ± 12.46	82.80 ± 4.89	92.06 ± 14.77	8.38 ± 1.61	65.67 ± 12.75	1.15 ± 0.22
Control II (with cholesterol)	432.70 ± 56.08* $p<0.001$	71.68 ± 5.71* $p<0.001$	132.70 ± 10.05* $p<0.001$	143.70 ± 12.18* $p<0.0001$	9.59* ± 1.14	111.04* ± 15.60 $p<0.001$	1.48 ± 0.20* $p<0.05$
Fresh fish + cholesterol	312.00 ± 87.98** $p<0.02$	58.32 ± 19.49	59.86 ± 13.70* ** $p<0.01$ $p<0.001$	137.96 ± 49.60	11.11* −2.07 $p<0.05$	113.14 ± 51.33	2.17 ± 0.66* ** $p<0.005$ $p<0.05$
Fish with oxidized fat + cholesterol	383.11 ± 105.22	75.80 ± 12.64* $p<0.002$	54.00 ± 5.60* ** $p<0.001$	172.38 ± 41.68* $p<0.005$	14.14* ** ± 1.67 $p<0.001$	160.60* ** ± 28.51 $p<0.001$	2.47 ± 0.25* ** $p<0.001$

* statistically significant compared with control group I

** statistically significant compared with group II (with cholesterol)

Table 4. Antioxidant vitamines in blood serum in selected tissues of guinea pigs after 12 weeks of experiment

Group	Serum = 6			Ascorbic acid in tissues	
				μ/g	$n = 5$
	tocopherol (mg/dl)	tocopherol (mg/d l total lipids)	ascorbic acid (mg/dl)	adrenals	liver
Control I	0.24 ± 0.07	0.72 ± 0.07	1.05 ± 0.05	1234.1 ± 193.7	119.3 ± 9.2
Control II (with cholesterol)	0.37 ± 0.05* $p < 0.01$	0.73 ± 0.44*	0.91 ± 0.15	1139 ± 120.6	120.3 ± 8.9
Fresh fish + cholestrol	0.41 ± 0.12* $p < 0.01$	1.32 ± 0.44* $p < 0.01$	0.84 ± 0.07* $p < 0.001$	952.0 ± 147.3* $p < 0.05$	107.7 ± 7.4
Fish with oxidized fat + cholesterol	0.15 ± 0.03* $p < 0.02$	0.36 ± 0.05* $p < 0.001$	0.79 ± 0.09* $p < 0.001$	852.1 ± 63.1* $p < 0.05$	114.1 ± 9.3

* statistically significant compared with control group I

additions to the stock diet significantly increased lipid peroxides content in blood serum.

The lowest phospholipids values in blood serum (Table 3) were in animals fed the stock diet, and the highest in the group receiving fish with oxidized fat + cholesterol. Cholesterol additions increased the phosopholipids content in blood serum. Additions of fresh fish + cholesterol to the cholesterol diet significantly reduced phospholipids content in blood serum in comparison to contents in all the other animal groups.

It was also found that consumption of marine fish with oxidized fat reduced the antioxidation potential in the organisms of experimental animals (Table 4). Statistically significant reductions of absolute tocopherol, tocopherol lipids ratio, and ascorbic acid contents were observed in blood serum. There were also statistacally significant drops of ascorbic acid content in adreanals of the experimental animals (Table 4).

DISCUSSION

We have experimentally confirmed that the consumption of mackerel with a high content of polyunsaturated fatty acids of the n-3 family positively affects the organism. Our results also indicate, however, that the consumption of marine fish with oxidized fat is detrimental, leading to disorders in lipids balance and reduced contents of antioxidant vitamins in the organism, among other things. This adverse effect is due to the oxidation of fish fat and the appearance of considerable quantities of peroxides and other toxic compounds. The results we obtained suggest that oxidized fat of marine fish neutralizes the positive biological effects of n-3 fatty acids. The body weight in animals eating fish with oxidized fat were found to be reduced. The liver in these animals was significantly enlarged, and microscopic observations revealed more intense steatosis of this organ.

The liver of herbivorous animals reacts strongly to increased contents of fat in the diet, especially to large amounts of long-chain polyunsaturated fatty acids. In our studies, the most intense liver changes were observed in animals fed oxidized fat.

Haematological indices confirm the adverse effect of the diet's fat on hemoglobin content and hematocritic index in guinea pigs. Particularly interesting results were obtained concerning lipids metabolism. Namely, the blood serum of animals eating oxidized fat was found to contain high amounts of total cholesterol. This is surprising, since one would rather expect an hypocholesterolemic effect in view of the high content of n-3 polyunsaturated fatty acids in the diet. In our previous studies [37] we demonstrated a clear hypocholesterolemic and antiatherogenic effect or fresh fish fat (fish oil) in guinea pigs fed, as in the experiments described here, an atherogenic diet (i.e. one containing an increased amount of cholesterol). Autooxidation of marine fish fat neutralizes the hypocholesterolemic effect of n-3 polyunsaturated fatty acids. The results for

LDL cholesterol confirm the adverse effect of oxidized fat. In animals consuming such fat the ch-TDL values were the highest; also highest were the ch-HDL values, and this once again confirms our view that the content of cholesterol in HDL depends to a large extent on the cholesterol in HDL depends to a large extent on the cholesterol pool in the organism. An adverse effect of oxidized fat on lipids metabolism was also evident in the total serum lipids levels and serum lipiol peroxides. In the group of animals receiving oxidized fat the total lipids levels and lipid peroxides level highest. This was in agreement with our expectations. Worthy of particular notice is our observation that the consumption of marine fish with oxidized fat decreases the content of antioxidant vitamins (C and E) in the organism of experimental animals.

Our experiments demonstrate that the consumption of marine fish containing oxidized fat adversely affects the organisms of experimental animals.

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WPŁYW RYB MORSKICH O RÓŻNYM STOPNIU UTLENIENIA TŁUSZCZU NA ORGANIZM ŚWINEK MORSKICH

Streszczenie

Celem badań było wyjaśnienie wpływu tłuszczu ryb morskich o różnym stopniu utlenienia na organizm świnek morskich w warunkach spożywania aterogennej diety. Chodziło m.in. o uzyskanie odpowiedzi czy spożywanie ryb morskich zawierających tłuszcz utleniony może korzystnie wpływać na gospodarkę lipidową ustroju w warunkach hipercholesterolemii. Badano także zawartość w ustroju witamin antyoksydacyjnych. Doświadczenie przeprowadzono na świnkach morskich samcach, którym podawno przez 12 tygodni określone diety. Grupa kontrolna I otrzymywała granulowaną dietę hodowlaną LSK firmy „Bacutil", grupa kontrolna II dicty j.w. z dodatkiem 0,1% cholesterolu. Grupom badanym podawano ryby morskie z dodatkiem 0,1% cholesterolu — grupie III rybę świeżą, grupie IV rybę z tłuszczem utlenionym. Ogólna zawartość tłuszczu w dietach doświadczalnych wynosiła ok. 14% kcal. Wyniki badań masy ciała i przyrostów masy ciała wykazały, że dodatek cholesterolu do dicty hodowlanej miał ujemny wpływ na ustrój zwierząt, natomiast dodatek do diety ryby świeżej nie wpływał na tempo wzrostu zwierząt w porównaniu z grupą kontrolną II. Najmniejszą masę ciała i przyrosty masy ciała stwierdzono w grupie otrzymującej rybę z tłuszczem utlenionym. W grupach doświadczalnych, tj. otrzymujących badane ryby nastąpiło powiększenie masy wątroby. W tych grupach stwierdzono obniżenie wartości hemoglobiny i hematokrytu. Dodatek do diety hodowlanej cholesterolu (dieta kontrolna II) spowodował istotny wzrost cholesterolu całkowitego w surowicy krwi, natomiast w grupie zwierząt

otrzymujących rybę świeżą nastąpił spadek zawartości cholesterolu w porównaniu z grupą kontrolną II, a w grupie której podawano rybę z tłuszczem utlenionym — wzrost zawartości cholesterolu. Zawartość cholesterolu w LDL była najniższa w grupie kontrolnej I a najwyższa w grupie otrzymującej rybę z tłuszczem utlenionym. Zawartość cholesterolu w HDL była najniższa w grupie kontrolnej I. W grupie otrzymującej rybę świeżą zawartość cholesterolu w HDL była wyższa niż w grupie kontrolnej I i II, a najwyższa w grupie której podawano rybę z tłuszczem utlenionym.

Zawartość nadtlenków lipidowych w surowicy krwi była najwyższa w grupie, której podawano rybę z tłuszczem utlenionym, najniższa w grupie kontrolnej I. Stwierdzono również, że w grupie zwierząt otrzymujących w diecie rybę z tłuszczem utlenionym obniżyła się zawartość tokoferolu i kwasu askorbinowego w surowicy krwi oraz zawartość kwasu askorbinowego w nadnerczach.