EXPERIMENTAL MODELS REGARDING THE EFFECTS OF POLYUNSATURATED FATTY ACIDS ON BIOCHEMICAL BLOOD PROFILE IN GUINEA PIGS

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Summary

Serum cholesterol concentration is an important causal factor in the development of atherosclerosis and its complications in the form of coronary heart disease. One of the possible ways to reduce the risk of cardiovascular disease is consuming more polyunsaturated fatty acids (PUFA), particularly n-3 fatty acids. This study was undertaken to investigate the relationship between blood cholesterol, triglycerides and PUFA after feeding a high cholesterol diet or dietary PUFAs. Twenty-one Guinea pigs (12 weeks old) were randomly assigned to and fed a normal, high fat (0.5 % cholesterol) without (CD) or with Omega-3 (COD) diet for 12 weeks. The CD diet increased serum triglycerides, total and LDL cholesterol levels by 50.28%, 50.51% and 40.98 % respectively. Adding Omega-3 (0.6 mg/kg) in diet reduced the serum level of total cholesterol (59mg/dl vs. 39 mg/dl), triglycerides (94 mg/dl vs. 41 mg/dl) and LDL-C (45 mg/dl vs. 31 mg/dl).

Key words: diet, coronary diseases, fatty acids, serum level, Omega-3

In this era of multiple pharmacological treatments for cardiovascular disease many believe that simple dietary interventions or nutritional supplements may be a more natural and acceptable method of providing benefits. The n–3 fatty acids of particular interest for the prevention of cardiovascular diseases include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (11). These very-long-chain n–3 fatty acids are found predominantly in fish and fish oils. Both health professionals and the public are increasingly interested in Omega 3 fatty acids from fish and fish oils ant their role in the prevention and management of coronary heart disease (2). Reducing intake of saturated fat and dietary cholesterol and avoiding excess calories, remain the cornerstone of the dietary approach to decreasing risk of atherosclerotic vascular disease. During the past 20 years, however, there has been renewed interest in other dietary components that might favorably improve lipid profiles and reduce risk of coronary heart disease (CHD) (10). Dietary intake of n-3 polyunsaturated fatty acids (PUFA) mostly derived from fish or as pharmacological supplements, is associated epidemiologically with cardiovascular protection. Randomized intervention trials have shown that n-3 PUFA reduce mortality endpoints, specifically sudden death and fatal myocardial infarction (1,5).

Many studies have reported a negative relation between intake of omega-3 fatty acids (FAs) and CVD incidence and/or mortality (3,7,8). Omega-3 PUFAs generally exert their cardio protective effects through changes in lipids and lipoproteins. In addition, Omega-3 FAs especially EPA and DHA contribute benefits through their antiarrhythmic, anti-inflammatory, antithrombotic effects. Moreover, EPA and DHA also improve vascular endothelial function and help lower blood pressure, platelet sensitivity (12).

The aim of this study was therefore to prove from a biochemical point of view the supposed protective role of the Omega 3 fatty acids on serum cholesterol and triglycerides after feeding a high cholesterol diet or dietary PUFAs, using experimental models, based on a controlled diet.

Materials and methods

Materials

Concentrated fish oil was extracted from Omacor capsules (Pronova, Norway), cholesterol was obtained from sheep wool powder, ~95% (GC), (Sigma-Aldrich), and the standard chow for Guinea pigs was obtained from Pannonmill Takarmany Kft., Hungary.

Animals and Diets

Twenty-one Guinea pigs were used for this study, which were randomized to three different dietary regimens: a standard chow diet, a high fat (0.5 % cholesterol) diet (CD) and high fat (0.5 % cholesterol) with Omega-3 (0.6 mg/kg) diet (COD). Guinea pigs were chosen because they are excellent models to evaluate the mechanisms by which diet interventions and drug treatments alter plasma lipids and lipoprotein metabolism (4). Guinea pigs were housed five per cage, in a temperature-controlled environment with a 12-h light–dark cycle, and allowed free access to food and water. Body weight was recorded once a week. The animals were sacrificed after 12 weeks on the experimental diet.

Serum determinations

Fasting serum samples were obtained for cholesterol, triglycerides and omega-3 determinations at baseline and after 6 and 12 weeks on the experimental diet. The blood was collected from the retro orbicular sinus in serum separator tubes, and centrifuged immediately. Serum was then stored at 0-4 °C until analysis. The samples were analyzed with Konelab 20i clinical chemistry analyzer in order to determine total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides and with Shimadzu GC-17 A gas-chromatograph with a FID detector for fatty acids determinations.

Results and discussion

Cholesterol and triglycerides determinations

In the case of the control group all values of the parameters analyzed were according to the standard limits characteristic to this specie and didn't present any significant changes during the entire experiment (Table 1). Therefore, the diet with an optimal lipid concentration (the combined diet administrated to this group contained 2.39% fat) does not determine an overstepping of the superior limits in the biochemical parameters.

After 6 weeks, during which the guinea pigs were administrated a high fat diet with 0.5% cholesterol, the values of the parameters studied in the case of the positive group raised as following: total cholesterol from 48mg/dl to 89 mg/dl, LDL cholesterol from 36 mg/dl to 47 mg/dl, HDL cholesterol from 4 mg/dl to 9 mg/dl, and triglycerides from 88 mg/dl to 129 mg/dl. Although their values have risen compared to the initial ones, HDL cholesterol and LDL cholesterol values are in between the normal limits. The total cholesterol and triglyceride values moderately overstep the standard limits. These results are in accordance with the other data from studies done in this field, Lin et al. (6), obtaining similar results. After 12 weeks, the values of the parameters studied are higher also from baseline as well as from day 45. It can be noticed that the cholesterol and triglyceride values have doubled compared to the initial ones, and the HDL cholesterol value has overstepped the superior limit. It is known as a well fact that the successive administration of cholesterol over a long period is directly proportional to the raising of the biochemical values in time. Therefore, we can observe that at 6 weeks from the diet's administration with add of 0.5% cholesterol the values are slightly higher, at 12 weeks, these values have doubled compared to the initial ones. The atherogen dyslipidemia includes the triad: high triglyceride level, a lower HDL cholesterol value, and the increase of the LDL cholesterol level. The triglyceride value is a risk factor independent for cardiovascular diseases. The raising of this value can be due to an endothelial dysfunction (9).

In the case of the Omega-3 group it can be noticed that the administration of a diet with add of 0.5% cholesterol at the same time with the diet supplement Omacor has led to a decrease in the majority of the parameters studied. At 6 weeks it can be seen the decrease in the total cholesterol value from 59 mg/dl at 56 mg/dl, LDL cholesterol from 45 mg/dl to 39mg/dl, and triglycerides from 94 mg/dl to 73 mg/dl. The exception remains in the HDL value which remains constant. The parameters values from the 12^{th} week are lower compared to the values obtained in the 6^{th} week as well as baseline, exception making the HDL cholesterol which presents a growing from 11 mg/dl to 15 mg/dl. This fact is explained by the benefic action that the polyunsaturated fatty acids with long chain have in the organism: they enrich the lipid rapport from blood decreasing the total cholesterol, triglycerides and VLDL, and at the same time increasing the HDL (benefic cholesterol). It can be noticed that the unsaturated fatty acids with long chain

Omega – 3 types, acts especially on the serum level of the triglycerides, their value reducing to half. This fact can be explained by the step by step accumulation of these acids in the organism.

Table 1

The values of the biochemical parameters

Diet	Total cholesterol			Triglycerides		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Standard diet	51 mg/dl	58 mg/dl	65 mg/dl	82 mg/dl	90 mg/dl	120 mg/dl
CD diet	48 mg/dl	89 mg/dl	97 mg/dl	88 mg/dl	129 mg/dl	177 mg/dl
COD diet	59 mg/dl	56 mg/dl	39 mg/dl	94 mg/dl	73 mg/dl	41 mg/dl

Diet	LDL cholesterol			HDL cholesterol		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Standard diet	48 mg/dl	42 mg/dl	52 mg/dl	6 mg/dl	17 mg/dl	19 mg/dl
CD diet	36 mg/dl	47 mg/dl	61 mg/dl	4 mg/dl	9 mg/dl	9 mg/dl
COD diet	45 mg/dl	39 mg/dl	31 mg/dl	11 mg/dl	11 mg/dl	15 mg/dl

* Values are given as mean, in mg/dl

Fatty acids determination

During the experiment was monitored the evolution of the fatty acids concentration in blood, given the fact that they were given as a dietary supplement. Although before the study the diet of guinea pigs was not supplemented with polyunsaturated fatty acids, their presence at a rate of 14.82% is explained by the possible feeding plants containing linoleic and alpha-linolenic fatty acids.

In order to express their protective effect, the ratio between omega-3 fatty acids and omega-6 fatty acids has to be at least 1:4. The values from chromatogram indicates a higher concentration of linoleic acid, which is part of omega-6 family, in contrast to alfa-linolenic acid concentration, which is an omega-3 acid. Thereby, the obtained ratio is 1:3.5, with respect for omega-6 acids. These values are very similar to those found in humans due to their increasing occidental diet habits.

At the second determination, it can be observed the presence of long chain omega-3 fatty acids – EPA and DHA (fig. 1, red arrow). Their presence is explained by the supplementation of guinea pigs diet with Omacor. The values of serum fatty acids concentration are shown in table 2. Beside the higher concentration of

polyunsaturated fatty acids, it can be seen the improvement of the n-3/n-6 ratio. Thus, after 6 weeks the n-3/n-6 ratio is 1.34:1 with respect to n-3 fatty acids.

The serum identification of EPA and DHA can be correlated with the cholesterol and triglycerides values obtain from the same serum samples. In this way, it can be confirmed the effects of omega-3 fatty acids in lowering the total cholesterol and triglycerides and improvement of lipoprotein fractions ratio.

Table 2

Fatty acids concentration in serum – COD diet

No.	**Fatty acid**	**Abbreviate**	**Baseline (%)**	**6 weeks (%)**	**12 weeks (%)**
1.	Palmitic	16:0	27.74	7.43	12.55
2.	Palmitoleic	16:1	18.19	0.45	0.79
3.	Heptadecanoic	17:0	-	1.92	0.98
4.	Stearic	18:0	2.54	3.05	5.35
5.	Oleic	18:1	24.73	7.38	14.22
6.	Vaccenic	18:1 Izomer	7.26	0.63	1.23
7.	Linoleic	18:2	11.53	7.17	12.47
8.	Alfalinolenic	18:3	3.29	4.95	3.27
9.	Cis-11-Eicosenoic	20:1	1.64	6.05	1.88
10.	Arahidonic	20:4	-	6.64	2.81
11.	Cis-5,8,11,14,17-Eicosapentaenoic	20:5	ND	6.45	7.56
12.	Cis-4,7,10,13,16,19-Docosahexanoic	22:6	ND	7.16	9.77
13	Total SFA		30.28	12.40	18.88
14	Total MUFA		51.82	14.06	17.33
15	Total PUFA		14.82	32.37	33.83
16	SFA/UFA		0.58	0.88	1.08
17	n-6/n-3		3.5	0.74	0.74

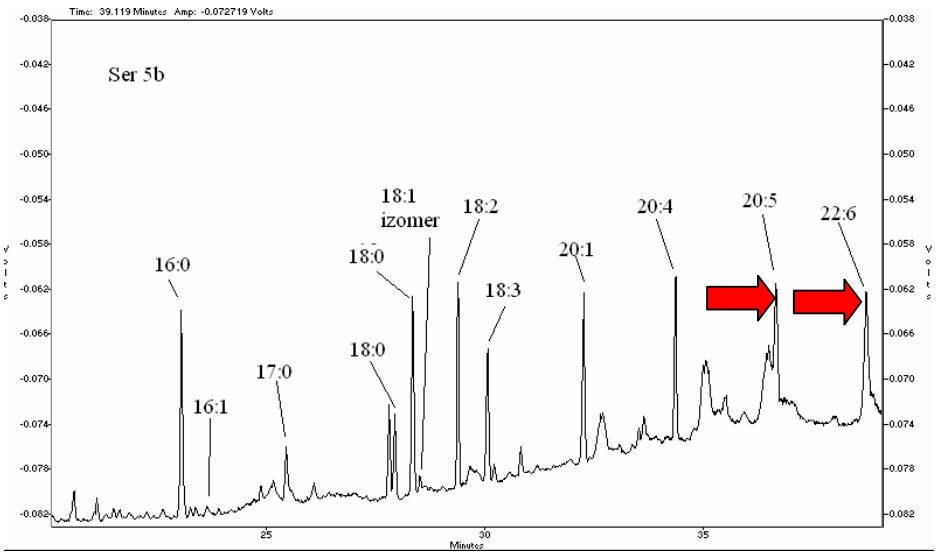

Fig. 1. Fatty acids chromatogram after 6 weeks, COD diet

After 12 weeks, it can be seen that even if the polyunsaturated fatty acids values remain relatively constant, the concentrations of the very long chain fatty acids – EPA and DHA – are higher, compared to those obtained in the 6th week. The relatively constant value is explained by the alfa-linolenic acid low concentration.

Conclusions

The cholesterol's sanguine concentration grows in direct proportion to the period of the high fat diet's administration (0.5% cholesterol adding).

Dietary cholesterol in high percents determines the increase of the biochemical parameters involved in the cardiovascular pathogenesis, especially the total cholesterol (with 50.51%) and the triglycerides (with 50.28%).

In what concerns the biochemical parameters, the Omega – 3 influences clearly in the most obvious way the level of the triglycerides and total cholesterol, in decreasing their levels with 56.38%, and respectively 33.33%.

Omega-3 intervenes in the modification of the lipoprotein fraction values, increasing the HDL- cholesterol and decreasing the LDL- cholesterol

Acknowledgments

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