

## CLINICAL STUDY

# Clinical studies on the hypolipidemic and antioxidant effects of selected natural substances

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**Abstract:** *Background:* The average blood levels of cholesterol and triacylglycerol in population are generally considered to be indicators of cardiovascular risk.

*Aim of the study:* The aim of our study was to present representative data of selected fractions of blood lipids, and the trend of their levels during the last 35 years in the adult population of Slovakia.

*Results:* This study demonstrates the risk of the elevated average cholesterolaemia and triacylglycerolaemia in men aged 35–39 years and above 60 years, as compared to women's increased blood level that were detected only in the age group above 60 years. Strong gender differences in HDL-cholesterol were found, women having higher values than men, with these differences fading in the age group above 60 years.

The results of two clinical studies present the hypolipemic effects of n-3 polyunsaturated fatty acid in individuals with dyslipidaemia, the hypotriacylglycerolemic effect being dominant ( $p < 0.001$ ). The hypocholesterolemic effect was less significant ( $p < 0.05$ ) than in cases when lyophilized powder of the *Pleurotus ostreatus* was applied ( $p < 0.01$ ). In both cases, a stabilizing effect on serum HDL-cholesterol had been observed. In case of polyunsaturated fatty acid n-3, its increase was borderline with statistical significance ( $p = 0.05$ ).

*Conclusion:* The study emphasises the necessity of improving the average blood level of lipids in the population of Slovakia and the pertinence of using natural substances with a hypolipemic effect in secondary and even primary prevention of atherosclerosis and its serious complications (*Tab. 3, Fig. 4, Ref. 27*). Full Text (Free, PDF) [www.bmj.sk](http://www.bmj.sk).

Key words: cholesterolaemia, triacylglycerolaemia, n-3 polyunsaturated fatty acid, conjugate dienes, antioxidative enzymes.

While the health statistics on population morbidity and mortality from cardiovascular diseases show a decreasing tendency in developed countries, the same is not true for the population of Slovakia.

On contrary, a transitional decrease in 1993–1998 is now followed by an increase which shows the mortality of the Slovak population from cardiovascular diseases in 2004 at 54.6 %, with women being dominant (61.3 %) over men (47.9 %) (27).

This shows to the fact that the above mentioned phenomenon is not genetically determined, but rather subject to the adverse influence of external factors, and is unaffected by existing preventive measures.

Dyslipidaemia and its various forms are generally recognised as the most important risk factors of atherosclerosis and its most common clinical manifestations – ischaemic heart disease.

Despite the expansion of pharmacotherapy through the extensive and successful application of statines in the secondary prevention of dyslipidaemia, the research, conducted on natural

substances that have a hypolipemic effect, remains highly relevant, whether they can be used either as independent diet therapy or complementary to pharmacological treatment.

Mean range of individual lipid fractions, indicating a certain risk rate versus a decreased risk of cardiovascular diseases are unfavourable in several age groups of our population.

It is important to stress that in the last two decades these blood level have not changed noticeably, and thus call for the correction by effective methods of secondary prevention.

The results of our study in subjects with combined dyslipidaemia may be viewed as a relevant groundwork not only for their application in rational diet therapy, but also in primary prevention of dyslipidaemia.

## Subject of the clinical study and their characteristics

The aim of our study was to verify the hypolipemic effect of natural nutrition ingredients PMK n-3, administered through sea fish (mackerel) three times a week in an average daily dose of 120 g/day and lyophilised *Pleurotus ostreatus* powder in amounts of 10 g daily. The two dietetic tests were carried out during 6 weeks in individuals with primarily combined types of dyslipidaemia. Insignificant differences between genders enabled

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**Tab. 1. Average values of blood lipids in adult population in Slovak Republic (Slovak nationwide study 1984–1989) (Kajaba et al.) (n=3175: 1547 women; 1628 men) in summer and autumn period.**

Age (years)	ø Cholesterol blood level (mmol/l)					
	women			men		
	n	M	SD	n	M	SD
19–24	399	4.85	1.26	190	4.39	0.7
25–29	173	4.76	0.84	192	5.17	1.06
30–34	196	5.23	1.28	174	5.23	1.21
35–39	191	5.58	1	197	6.21	1.19
40–49	198	5.47	1.4	387	5.48	1.63
50–59	188	6.18	1.13	338	5.7	0.84
60 and above	202	6.14	1.28	150	5.87	1.24
19–59	1345	5.35	1.15	1478	5.36	1.11
19–60 and above	1547	5.46	1.17	1628	5.44	1.12

Age (years)	ø HDL - chol. s. (mmol/l)					
	women			men		
	n	M	SD	n	M	SD
19–24	399	1.63	0.41	190	1.61	0.41
25–29	173	1.54	0.31	192	1.52	0.31
30–34	196	1.57	0.41	174	1.44	0.41
35–39	191	1.58	0.38	197	1.55	0.38
40–49	198	1.52	0.4	387	1.46	0.4
50–59	188	1.63	0.82	338	1.47	0.82
60 and above	202	1.54	0.49	150	1.58	0.50
19–59	1345	1.58	0.46	1478	1.51	0.46
19–60 and above	1547	1.57	0.46	1628	1.52	0.46

Age (years)	ø Triglycerol blood level (mmol/l)					
	women			Men		
	n	M	SD	n	M	SD
19–24	399	1.23	0.43	190	0.99	0.29
25–29	173	1.04	0.37	192	1.12	0.51
30–34	196	1.24	0.41	174	1.54	0.43
35–39	191	1.35	0.44	197	1.99	0.62
40–49	198	1.34	0.39	387	1.61	0.54
50–59	188	1.62	0.27	338	1.75	0.56
60 and above	202	1.91	0.29	150	1.92	0.63
19–59	1345	1.30	0.39	1478	1.50	0.49
19–60 and above	1547	1.39	0.37	1628	1.56	0.51

us to merge gender groups into one. The group of PMK n-3 dietetic test was composed of 37 individuals (20 women and 17 men) of an average age 39.3 years.

Additionally, 57 individuals (32 women and 25 men) with combined dyslipidaemia were in the group that undertook the dietetic test with *Pleurotus ostreatus*-powder, their average age was 43 years. Activities of the antioxidative enzymes (superoxidismutasis, catalasis, glutation-peroxidasis, glutation, and lipo-peroxidation markers – number of conjugated dienes of polyene fatty acids) apply to 22 individuals (12 women and 10 men) with an average age 35 years, as a sub-group of the basic group of 57 individuals with dyslipidaemia.

## Method and statistics

Clinical and biochemical data were acquired as a part of the autumn representative national study, which examined the dietary condition of more than 7 thousand adult individuals, 50 % of which were studied using laboratory methods – 3175 relatively healthy individuals were thus tested (1547 women and 1628 men).

Basic somatometric examinations, height measurement (while barefoot) using a paper ruler attached to the wall, weight

measurement on a decimal weight. The data was calculated according to the standard formula for body mass index, weight in kg divided by height in meters squared, to give BMI values (in kg/m<sup>2</sup>). The biochemical indicators of the state of nutrition were ascertained from venous blood taken in the morning, on an empty stomach, and preceding a clinical examination.

The presented data were taken from examinations of blood serum, and represent the level of cholesterol, the proportion of HDL-cholesterol, and triacylglycerols through standard laboratory methods using a Vitros 250 automatic analyser, manufactured by Johnson & Johnson, USA.

The activities of antioxidative enzymes (SOD, Kat., GSHPx, and GSH) in erythrocytes were determined spectrophotometrically (23), and conjugated dienes of fatty acids in plasma were found spectrophotometrically according to the published study (19).

Mean values (M), standard error deviation (SED), and standard deviation (SD) were expressed from the clinical and biochemical parameters for the abovementioned data in the studied groups.

The significance was confirmed using the Student's t-test, in which changes in the same individuals were observed using t-tests in matched pairs of values.

## Results and discussion

Average level of blood lipids in population are generally accepted as risk factors of cardiovascular diseases related to atherosclerosis, especially IHD (ischaemic heart disease) (13, 20).

The level of cholesterolaemia is a classic risk marker in this respect (4, 5, 11, 15).

Hypertriglycerolaemia is another major risk factor of atherogenesis (18, 22, 25). Its correlation with other lipid abnormalities significantly increases the risk of cardiovascular disease (21), especially IHD. Current research includes hypertriglycerolaemia among the etiological factors of deep vein thrombosis due to the increase of procoagulative and antifibrinolytic activity caused (24).

The weakening of fibrinolysis during postprandial hypertriglycerolaemia is highlighted in another study (12).

It is generally accepted that a deficient amount of HDL-cholesterol in serum represents an independent risk factor of cardiovascular disease (1, 7), which, in combination with insulin resistency, contributes to the development of metabolic syndrome (16) with a decisive role in the pathogenesis of atherosclerosis and type-2 diabetes mellitus (14, 26).

In this context, we have chosen to present mean data of selected lipid parameters on a representative sample of the adult population of the Slovak republic in Table 1.

The table confirms an increased risk in middle-aged men (35–39 years) with an average cholesterolemia of 6.2 mmol/l. After a drop in two age groups, the risk again increases in the age group of above 60 years, albeit to a lower value of 5.9 mmol/l.

In women of fertile age, such an increase in cholesterolaemia is not observed. It becomes apparent later during menopause (50

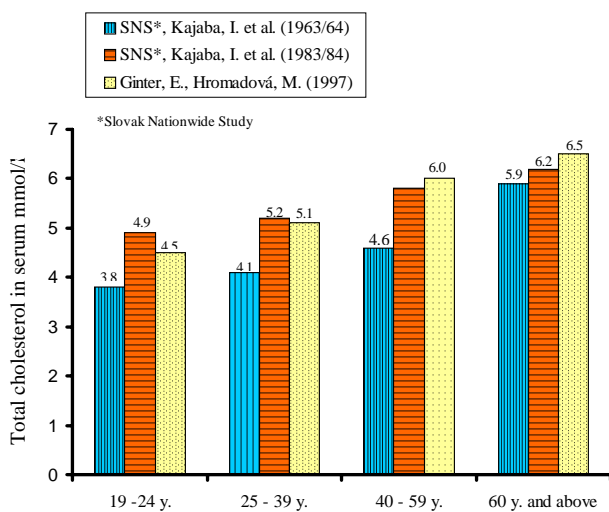


Fig. 1. Trends of average cholesterolemias in groups of adult women between the ages of 19 to 60 and above years in Slovak Republic during three decade period (1963–1997).

to 59 years of age) with a value of 6.2 mmol/l, and remains afterward in the upper age groups.

As we had objective, methodically comparable data on average cholesterolaemia and corresponding age and gender groups at our disposal (6), we present the trends of their levels over almost 35 years in Slovakia (Fig. 1 – women, Fig. 2 – men).

A mild, but steady increase in cholesterolaemia in women was observed in the highest age group, and in two middle-age groups, which are marked by a significant rise in values as compared to the first 20 years. In the last decade, this indicates a stabilisation of average cholesterolaemia values, and only the youngest age group shows a slight decrease in the serum cholesterol.

In the group of men, a similar tendency of cholesterolaemia increase was visible in the 40 to 59 year age group, while the others illustrate a small decrease in values. In the 19–24 year age group, this trend is part of the stabilisation of average cholesterolaemia on a normal level over 35 years in Slovakia.

The fact that the average cholesterolaemia in Slovakia was 5.8 mmol/l (2) in 2004 proves that no cholesterolaemia levels have undergone significant changes in the past few years.

It is appropriate to add data from the Czech Society for Artherosclerosis, according to which during 1985–1998, cardiovascular mortality underwent a major decrease (as much as 27 %) in the Czech Republic, coupled with the decrease of average cholesterolaemias from 6.18 mmol/l to 5.82 mmol/l in women (by 5.8 %) and from 6.21 mmol/l to 5.88 mmol/l in men (by 5.3 %) (4). It must be stated that such favourable changes are yet to come in the Slovak population.

In undertaking steps to achieve the above mentioned data, target values should be reached in the population in phases,

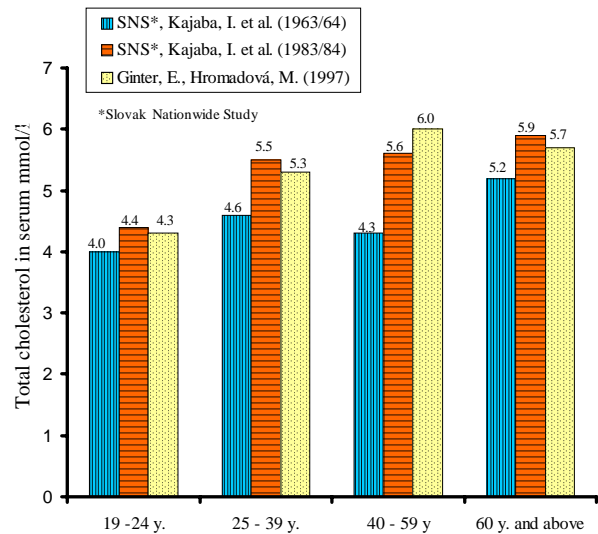


Fig. 2. Trends of average cholesterolemias in groups of adult men between the ages of 19 to 60 and above years in Slovak Republic during three decade period (1963–1997).

beginning with an average cholesterolaemia of 5.5 mmol/l, and progressing towards a value of 5.2, eventually 5.0 mmol/l in accordance with the Lipid consensus in Slovakia (17).

In studying nutritional effects, primarily the effect of anti-oxidative vitamins on serum cholesterol, environmental factors often and justifiably receive much attention. Smokers are a suitable model in this respect, with research being focused on the hazardous effect of smoking on the level of ascorbinaemia and cholesterolaemia (8).

Previously, we justified the inverse correlation between these two biochemical indicators, as well as its direct dependence on seasonal variations of cholesterolemia and the intake of vitamin C (10).

In cases of optimal intake, we found higher ascorbinemias and lower cholesterolaemias in the population (summer and autumn), while the converse was true for the critical winter and spring months.

When studying average triacylglycerolaemias, the potential risk of increased values in correlated age groups of both genders arises, as in cholesterolaemia.

Average level of HDL-cholesterol in serum are considerably higher in women aged 30–34 years and 40–59 years compared to men, and this gender difference fades only in the age group of 60 years and up, as well as subjects aged 19–29 years.

Through a percentage analysis of hyperlipoproteinaemia, found in average bands of serum lipids, we observed general prevalence in about 1/6 of adults aged 19 to 59, and about 1/4 in persons older than 60 years of age. Proportionally, 1 combined form and 2 isolated forms were recorded (an increase in total serum cholesterol 5.5 mmol/l, and serum triacylglycerols >1.9 mmol/l).

**Tab. 2. Influence of 6 week applications PFA n-3 (3x 3g EPA/week/person) on blood lipids in persons with combined dyslipidaemia (n=37: 20 women, 17 men; age 39.3 years).**

	Total cholesterol s. (mmol/l)	Triacylglycerols s. (mmol/l)	HDL-cholesterol s. (mmol/l)	AI Total chol./HDL-cholesterol s.	BMI (kg/m <sup>2</sup> )
Before test					
M	8.5	9.4	1.6	5.6	26.8
SD	1.177	1.609	1.226	1.605	4.095
SEM	0.416	0.692	0.101	0.718	1.448
After test					
M	6.7	4.6	1.7	4.5	free changes
SD	1.581	1.531	0.324	1.046	
SEM	0.559	0.433	0.145	0.468	
Average change	-1.8	-4.8	0.1	-1.0	
Test	p<0.05	p<0.001	P=0.05	NS	

**Tab. 3. Influence of 6 week applications of Pleurotus ostreatus powder (10 g/day/person) on blood lipids in persons with combined dyslipidaemia (n=57: 32 women, 25 men; age 43 years).**

	Total cholesterol s. (mmol/l)	Triacylglycerols s. (mmol/l)	HDL-cholesterol s. (mmol/l)	AI Total chol /HDL-cholesterol s.	BMI (kg/m <sup>2</sup> )
Before test					
M	9.0	8.7	1.2	9.5	27.6
SD	3.122	8.493	0.461	6.801	3.629
SEM	0.806	2.193	0.119	1.756	0.937
After test					
M	7.0	5.6	1.1	7.2	free changes
SD	1.967	4.550	0.399	3.981	
SEM	0.508	1.175	0.103	1.028	
Average change	- 2.0	- 3.1	0.1	- 2.3	
Test	p<0.01	p<0.01	N	P<0.01	

By scrutiny of dyslipidaemia, with respect to decreased values of HDL-cholesterol in serum (women <1.3; men <1.0 mmol/l), the following most common combination was found in adults: increased serum triacylglycerols and, concurrently, a decrease in serum HDL-cholesterol values in more than 1/3 of individuals in the structure of dyslipidaemia prevalence in Slovakia.

The prevalence of decreased serum HDL-cholesterol values in adults proves a rising trend directly proportional to ageing. The trend is more distinctive in women, as men reach a higher percentage (30 %) of decreased values prevalence at younger ages. This percentage approximates values found in men in the USA (35 %), while in women this statistic is significantly lower (15 %), even compared to women in Slovakia (27 %) (9).

Table 2 illustrates the results of the clinical study aimed at observing the hypolipemic effect in middle-aged subjects with

combined dyslipidaemia when consuming sea fish (mackerel) 3 times weekly in amounts of 120 g/day, which represents 3.0 g/day of PMK-EPA for a period of 6 weeks.

Crucially, a 51 % decrease in the amount of triacylglycerols in serum was recorded, though less in total cholesterol, which dropped by 21.2 %. Within this framework, a rise in serum HDL-cholesterol was observed on the borderline of statistical significance (p=0.05).

Table 3 and Figure 3 depict the studied hypolipemic effect of a 6-week period of administration of *Pleurotus ostreatus* powder (10 g/day) as part of the diet of individuals with combined dyslipidaemia. A decrease in total cholesterol and triacylglycerol levels was confirmed with an equal statistical significance (p<0.01), while the stability of HDL-cholesterol in serum remained constant. Figure 4 shows the results of the examination

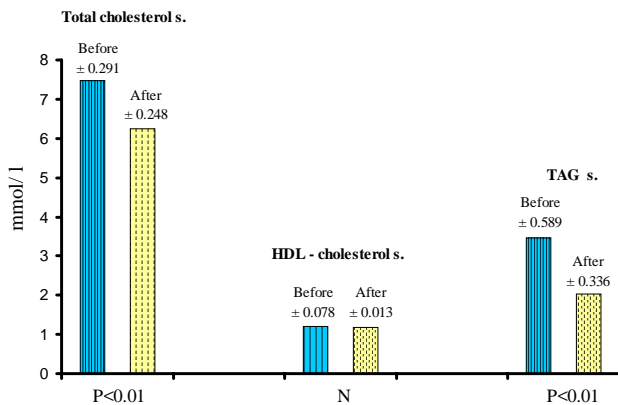


Fig. 3. Changes in serum lipids values by persons with combined dyslipidemia after a 6-week application of *Pleurotus ostreatus* powder (10 g/day/person) (n=57:32 women, 25 men), at the age of 43 y.

of antioxidative enzyme activity in a group of 22 persons (12 women, 10 men) with dyslipidaemia after administering the same amount of pleurotus ostreatus powder in the 6 week diet. Increase in enzymatic activity of glutation peroxidase (GSH-Px) as well as glutation (GSH) at the level of  $p < 0.05$  in both, while the activity of superoxidismutase as well as catalase remain unchanged.

Decrease of lipoperoxidative processes in the organism after the administration of pleurotus ostreatus confirms a significant reduction in the amount of conjugative dienes in plasma  $p < 0.02$ .

## Conclusion

The authors point out the risk of increased average levels of cholesterol in age groups of men aged 35–39 and above, while in women, two higher-age groups are at risk: 50–59 and above 60. The observed trend of cholesterolaemia in the course of almost 35 years confirms the relative stability and with the exception of the youngest age group of 19–24 years and partially 25–39 years, the older age groups remain outside the range of set levels of cholesterolaemia. The increase of average triacylglycerol levels concerns the same age groups of men as in cholesterolaemia, in women only the higher age group of above 60 years. For both sexes, the age group of 60 years and above shows the highest prevalence of dyslipidaemia, on average almost 1/4 of all people. Gender differences in the amount of serum HDL cholesterol display significantly higher levels in age group of women 30–34 years and 40–49 years compared to men. A significant gender difference was not observed in the age group of 60 and above. A clinical study with the application of PMK n-3 for a duration of 6 weeks confirmed a statistically significant hypotriacylglyceroleamic effect by 51 % ( $p < 0.001$ ), a less significant hypocholesteroleamic effect by 21 % ( $p < 0.05$ ) and also a hint of increased serum HDL cholesterol with borderline statistical significance ( $p = 0.05$ ) in individuals with dyslipidaemia. Using pleurotus ostreatus powder during 6 weeks has decreased with the same significance ( $p < 0.01$ ) the level of serum cholesterol

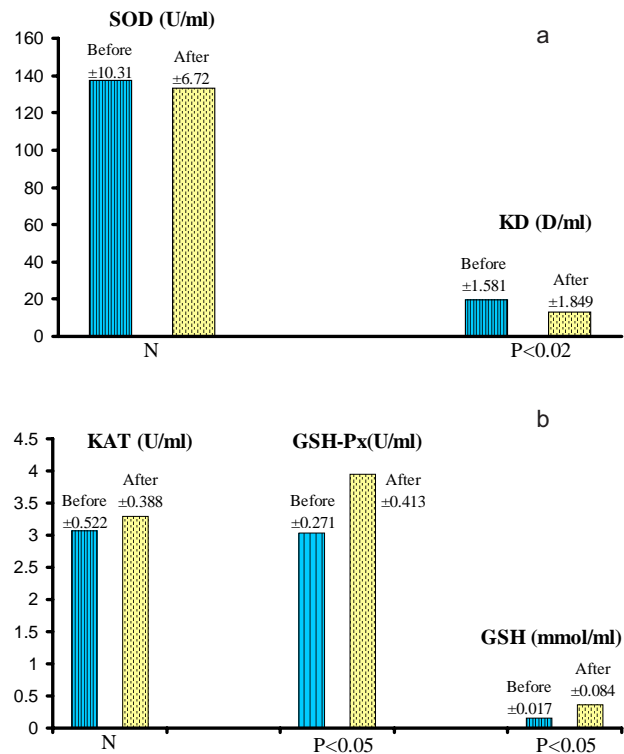


Fig. 4 a, b. Changes in activities of selected antioxidative enzymes and the level of conjugated dienes in plasma important in prevention of atherosclerosis after a 6-week application of *Pleurotus ostreatus* powder (10 g/day/person) (n=22: 12 women, 10 men), at the age of 35 y.

and triacylglycerol while maintaining a HDL-cholesterol-stabilizing effect. The antioxidative effect by increasing GSH-Px and glutation  $p > 0.05$  activity has been most evident, the decrease of lipoperoxidation is expressed as a decrease of the amount of conjugated dienes from PUFA in plasma.

We expect a wider use of the presented findings within the framework of an effective measures of dyslipidaemia and atherosclerosis prevention in population.

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