EFFECT OF N-3 AND N-6 POLYUNSATURATED FATTY ACIDS ON PLASMA CHOLETSTEROL IN TISSUES OF RATS

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ABSTRACT

The aim of the research was to test the hypothesis effect of long-term consumption of n-3 PUFA on plasma cholesterol levels in animal model. The findings have been trying to be applied to human nutrition. Three groups of rats were examined for the effect of fatty acids contained in food on animal tissues. The sources of PUFA n-3 and n-6 were added to standard feed for mice and rats. The animals were divided into several groups: with 6% addition of safflower oil (n-6, control group, SA), with 6% addition fish oil (n-3, FO) and with 6% addition DHA oil (n-3, DHA). The animals were fed for 40 days ad libitum. Each group was composed of 10 animals. DHA oil and fish oil have high representation in proportion of n-3 fatty acids and safflower oil has high representation in proportion of n-6 fatty acids. The experiment blood samples were taken from the animals in heparin tubes, which were analyzed for the concentration of total cholesterol, HDL cholesterol, LDL cholesterol and triacylglycerols at the end of the experiment. The analytical determination of the content of fatty acids was found in the liver tissues. The diet enriched eicosopentaenoic acid and docosahexaenoic acid has led to a significant decrease in non-esterified fatty acids and inhibition of LDL in the blood.

Key words: cholesterol, fatty acids, gas chromatogramy, rats, liver, n-3 fatty acids, n-6 fatty acids

Acknowledgments: This project was made with support of Internal Grant Agency of The Faculty of Agronomy Mendel University in Brno, TP8/2013 the effect of polyunsaturated fatty acids on blood plasma cholesterol in pigs.

INTRODUCTION

Lipids with specific proteins create macromolecular complexes called lipoproteins. These complexes enabled transport of lipids in the body. An important component of the lipid is cholesterol, which constitute inner lipoproteins with triacylglycerols. Phospholipid and free cholesterol are on the surface of the lipoprotein. Low density lipoproteins (LDL) are synthesized in the liver, they distribute cholesterol to peripheral tissues. They are responsible for cholesterol deposition in tissues. High density lipoproteins (HDL) transported cholesterol from peripheral tissues to the liver where they are catalysed (Zehnálek, 2007).

Polyunsaturated fatty acids (PUFA) have affected the activity and functional status of blood vessels and process of atherogenesis which caused cardiovascular disease. Eicosanoids (PG2, TA2) are metabolites of PUFA n-6 and they act pro-inflammatory, vasoconstrictor, causing platelet aggregation. On the other side, eicosanoids of PUFA n-3 (PG3, TA3) act anti-inflammatory, vasodilator and anti- platelet aggregation. PUFA n-3 ultimately reduce the risk of cardio- vascular disease, autoimmune diseases and cancer (Komprda, 2003).

The influence of n-3 polyunsaturated fatty acids on the regulation of blood lipids, including cholesterol is the subject of numerous scientific publications. It is assumed that n-3 PUFA act as modulators of gene transcription. The affect transcription factors involved in the metabolism of lipids, cholesterol, as well as carbohydrates. The most important transcription factors are PPAR (Peroxisome Proliferator-Activated Receptor) and SREBP-2 (Sterol Regulatory Element-Binding Protein). Intake of EPA and DHA significantly affect the expression of PPARα and SREBP-2 gene. They are playing a key role in cholesterol homeostasis (Mourek, 2003).

In our project, we have dealt with the impact of income n-3 and n-6 fatty acids on cholesterol and its fraction in experimental groups of rats. The aim was tested the hypothesis about effect of long-term consumption of n-3 PUFA on plasma cholesterol levels in model animals and apply this knowledge in human nutrition.

MATERIAL AND METHODS

We were studied the effect of fatty acids in food on represenation fatty acids in animal tissues. We added sources of PUFA n-3 and n-6 to standard feed for mice and rats (Biokron). Animals were divided into group with 6% addition of safflower oil (n-6, control group, SA), group with 6% addition fish oil (n-3, FO) and group with 6% addition DHA oil (n-3, DHA). The animals were fed for 40 days ad libitum and had ad libitum intake of water. Each group was composed of 10 animals. DHA oil and fish oil are rich in proportion of n-3 fatty acids, safflower oil is rich of n-6 fatty acids. The composition of n-6 and n-3 fatty acids of used oils is shown in Fig. 1.

	DHA oil (%)	Fish oil (%)	Safflower oil (%)
linoleic (n-6)	5,9	9,5	61,7
linolenic (n-6)	0,3	0,4	0,7
linolenic (n-3)	0,4	1,4	0,4
arachidonic (n-6)	0,7	0,8	0,5
EPA (n-3)	0,9	8,5	0,5
DHA (n-3)	32,3	11,2	1,4

Fig.1 The composition of n-6 and n-3 fatty acids in used oils (%)

Blood samples were taken from all animals to heparin tubes (DISPOLAB) at the end of the experiment. Blood samples were analyzed for the concentration of total cholesterol (TL), HDL-cholesterol (HDLC), LDL cholesterol (LDLC) and triacylglycerols (TAG). The analytical

determination of fatty acids was defined on the liver tissues. Total cholesterol a its fraction were perfomed by spectrophotometry on blood plasma at Department of Chemistry and Biochemistry, Mendel University in Brno. The determination of fatty acids was performed after extraction and derivatization (Rozíková, 2010). The evaluation of liver samples was on gas chromatograph Fisons GC 8000 series, capillary column DB-23 (60 m x 0.25 mm x 0.25µm, Agilent J & W Scientific, USA). The injector was heated to 250˚ C and detector (FID) to 260˚ C. Temperature program was 140˚ C/ 1 min, gradient 5˚ C/ min to 200˚ C/ 1 min, gradient 3˚ C/ min to 240˚ C held for 15 min. The carrier gas was used nitrogen, flow rate of 1.5 ml/ min, the pressure of 200 kPa and a split ratio of 20:1.

RESULT AND DISCUSSION

In the project we had focused on determining the effect of fatty acids in the diet on the level of total cholesterol and its fractions. A higher intake of unsaturated fatty acids should reduce total cholesterol in blood. Polyunsaturated fatty acids should be increased HDL fraction and the LDL fraction should be reduced. Otherwise, a higher consumption of saturated fatty acids would decrease HDLC and increase LDLC and total cholesterol. The content of fatty acids measured in liver were converted to mg/100g of liver weight. The addition of oil to the diet significantly did not affect the final weight of rats, daily weight growth and final weight of the liver in the experimental animals.

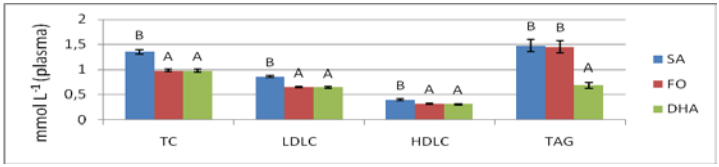

Fig. 2 The content of total cholesterol, LDLC, HDLC a TAG in blood of rats in all tested groups

The groups with the addition of fish oil and DHA oil had significantly decreased values of total cholesterol, LDLC and HDLC to the control group.The group with the addition of DHA oil had significantly reduced TAG content. It was halved compared to SA and FO.

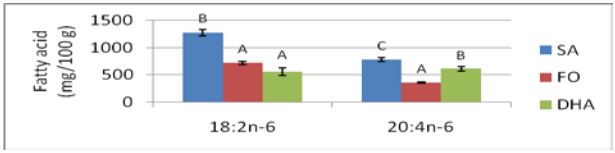

Fig. 3 The content of linoleic and arachidonic acid in the liver of rats for all tested groups

The control group was significantly higher content of linoleic acid and arachidonic acid compared with groups with addition of fish oil and DHA oil. FO group had significantly lower content of arachidonic acid group than DHA group.

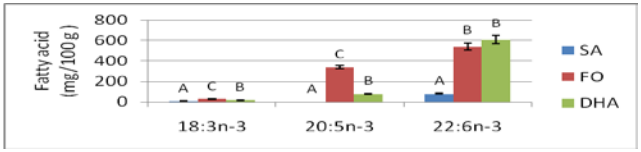

Fig. 4 The contents of α-linolenic, EPA and DHA in the liver of rats for all tested groups

High acid content of α-linolenic acid was detected in the FO group but the group also had significantly higher DHA content in the liver compared to the control group. EPA content was much higher in Group NP than in the control group and DHA. Proof was the increase in EPA than DHA group FO. Significant increase of DHA from the control group was measured FO group and DHA. A significant difference between the group of DHA and FO was found, only a tendency to increase the DHA group.

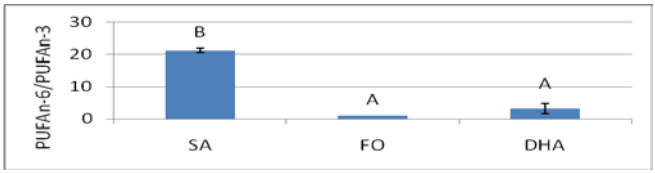

Fig. 5 The ratio of n-6/n-3 fatty acids in the liver of rats in all tested groups

The ratio of n-6 fatty acids to total n-3 fatty acids was much higher in the control group than in the groups FO and DHA. Significant difference was observed in the increase in the ratio of n-6/n-3 the DHA group than in the group FO.

The content of linoleic acid was significantly positively correlated with levels of plasma cholesterol and its fractions (ranging from R=0.44 to 0.65, $P < 0.05$) in the liver of rats. The content of the most important metabolite of linoleic acid, arachidonic acid was similarly positively correlated TC and its fractions. Relationship of arachidonic acid to TAG was detected ($P > 0.05$). The content of α -linolenic acid and its metabolites (EPA, DHA) was significantly negatively correlated to TC, HDLC and LDLC (correlation coefficient ranged from -0.42 to -0.82, $P < 0.05$). The content of DHA in the liver was significantly negatively correlated to the concentration of TAG levels ($R = -0.44$, $P < 0.05$). Ratio n-6/n-3 acids in the liver was positively correlated with the level of these lipid fractions (correlation coefficient ranged from 0.31 to 0.84, $P < 0.05$).

The unexpected result is a decrease HDLC fraction in the group with addition of DHA and FO oil. A study by König et al. (2007), cholesterol in plasma decreased as a result of activation of PPARα and reduction of SREBP -2, leading to a reduction in cholesterol biosynthesis. Transcription factors modulate the signaling pathway of EPA and DHA. Another explanation for this decrease is that the fish oil facilitates the secretion of bile acids in the liver transer cholesterol (Takahashi, 2011). In other studies, there was a decrease in HDL cholesterol in the tested mice fed soybean oil (Kamisako et al., 2012). Zhang et al. (2009) in their study reported in this context that hamsters are better than rat experimental models to test for cholesterol lowering, as synthesize and secrete cholesterol and bile acids ways more similar to human.

CONCLUSIONS

Diet enriched (2 g/day) of eicosopentaenoic acid and docosahexaenoic acid has led to a significant decrease in non-esterified fatty acids in the blood and inhibition of LDL (Mourek, 2007). The experiment was achieved by reducing total cholesterol levels in feeds containing higher proportion of n-3 acids than in the control group. Individual fractions of cholesterol (TL, LDLC, TAG) showed values to our predispositions. HDLC cholesterol decreased value compared to the control group. The composition of fatty acids had the influence on tissues in oils. Safflower oil increased n-6 fatty acids in liver tissue. Fish oil and DHA oil had positive effect of increasing n-3 fatty acids (EPA, DHA) in liver tissues.

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