EFFECT OF FISH OIL INTAKE ON PLASMA LIPIDS LEVEL IN RATS AND PIGS

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Abstract: The aim of the present study was to compare the effect of diet enriched with 2.5% fish oil (source of polyunsaturated fatty acids) and the effect of diet enriched with 2.5% palm oil (source of saturated fatty acids; control) on plasma lipids level. Two model animals were used: Sus scrofa and Rattus norvegicus. Levels of total plasma cholesterol, triacylglycerol, low-density cholesterol and high-density cholesterol were analysed by the enzymatic-colorimetric method. There were no significant differences between absolute values of the lipid fractions. Plasma lipid concentration in animals which was fed by diet enriched with fish oil was expressed like ratio of the plasma concentration in the other group of animals. In this case dietary fish oil decreased high-density cholesterol less (P<0.01), but low-density cholesterol and triacylglycerols more (P<0.05 and P<0.001) in rat plasma than in pig’s. However, used amount of fish oil added to diet was not able to improve plasma lipid markers in comparison with saturated palm oil.

Key Words: HDL-cholesterol, triacylglycerols, fish oil, Sus scrofa, Rattus norvegicus

INTRODUCTION
Dyslipidemia is one of the most serious risk factor of atherosclerosis which belongs among chronical civilization diseases that causes many premature deaths. Dyslipidemia is inter alia characterized by increased level of triacylglycerol (TAG), higher level of total plasma cholesterol (TC) and low-density cholesterol (LDLC) and decreased amount of plasma high-density cholesterol (HDLCL). Levels of all stated lipids can be modified by dietary intake. There is great difference between two basic groups of fatty acids – saturated and unsaturated. Palm oil belongs to saturated fatty acids known due to its defective influence on health. On the other hand, polyunsaturated n-3 long chain fatty acids (PUFA n-3) have opposite effect. PUFA n-3 decrease plasma level of TAG and increase amount of HDLC (Balk et al. 2006). For example PUFA n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to decrease plasma TAG via activation of peroxisome proliferator-activated receptor α and inhibition of sterol response element-binding protein signalling pathways (Jump 2008). In vivo studies testing EPA and DHA effect on plasma lipids are usually carried out on rodents. However, rodents are not ideal models for humans according to differences in proliferation of peroxisomes (Komprda 2012). Our aim was to compare influence of fish oil intake on plasma lipids levels in pigs and rats. An intention was to carry out an experiment as similar to ordinary human conditions as possible.

MATERIAL AND METHODS
Animals, dietary interventions, analysed tissues
It was used to model animals: thirty-two male rats at the age of eight weeks with the average live weight of 312 ± 23 g (laboratory strain Wistar Albino; Meditox Konárovice, Czech Republic) and thirty-two pigs of both sexes (16 males, 16 females) at the age of eight weeks with the average live weight of 25.5 ± 1.15 kg (Large White x Landrace; Bioprodukt Knapovec a.s., Ústí nad Orlicí, Czech Republic).
The rats were bred in the plastic boxes of four animals in a room standard circumstances, temperature at 23 ± 1 °C, humidity of 60% and 12/12 h of light/dark cycle. The pigs were bred in floored indoor pens of four animals each.

The experiment was performed in compliance with the Czech National Council Act No. 246/1992 Coll. to protect animals against cruelty, the Amended Act No. 162/1993 Coll., and was approved by the “Commission to protect animals against cruelty” of the Mendel University in Brno and of the Ministry of Agriculture of the Czech Republic.

The rats and the pigs were divided into two groups of 16 animals: the experimental group was fed the basic feed mixture with 2.5% of fish oil (F) and the control group was fed the basic feed mixture with 2.5% of palm oil (P). An intention was to use dietary EPA and DHA in amount realistically achievable in human nutrition. The P-diet was used as a control. The animals had free access to the drinkable water and were fed daily \textit{ad libitum} and the leftovers were weighed.

The fattening of rats and pigs lasted for ten weeks. Blood samples in rats were collected by cardiac puncture under anesthesia with isoflurane into the heparin-coated test tubes after the 12-h fasting at the end of the experiment. The pigs were anesthetized by the intramuscular application of the TKX mixture in the total volume of 0.2 mL/kg of the live weight and sacrificed by bleeding. Blood samples were collected (from the aorta) to the heparin-coated test tubes and the liver samples were taken. Both rat and pig blood samples were centrifuged at 200 g for 10 min at 4 °C to obtain plasma for the lipid fractions analysis.

**Plasma lipids determination**

TC, LDLC, HDLC and TAG values were determined by the enzymatic-colorimetric method using an automated chemical analyzer BS-200 (Mindray, Shenzhen, China) and commercial kits (Greiner Diagnostic GmbH, Bahlingen, Germany).

**Statistical evaluation**

Normality of the data distribution was tested by Kolmogorov-Smirnov test. The comparison of the rat and pig model was realized on two levels. The contrasts in absolute amounts plasma lipid concentrations in rats and pigs fed the F- and P-diet were evaluated by one-way analysis of the variance ratio test, including post-hoc Tukey’s test. In order to better recognize eventual inter-species differences, an effect of fish oil on plasma lipid concentrations was evaluated on a relative level. Values related the F-diet were expressed in a given species as percentages of the values measured using the control P-diet in this species. The rat and pig sets were compared using the independent samples t-test. For all evaluations, the STATISTICA 12 package (StatSoft, Tulsa, OK, USA) was used.

**RESULTS AND DISCUSSION**

The comparison of rats and pigs from the aspect of plasma cholesterol and TAG levels as influenced by the diet including either fish oil or palm oil is displayed in Figure 1. The pigs had higher concentration of total plasma cholesterol (TC) than the rats (nearly twice in average, P<0.05) regardless of the dietary intervention (the type of added oil in the diet affected TC neither in rats nor in pigs, P>0.05). As far as the absolute values of the lipid fractions in the rat plasma are concerned, fish oil did not affect TC in comparison with control (P>0.05) not only in the present study, but also in the experiments of Campioli et al. (2012) and Yamazaki et al. (2011).

A similar trend was also established as far as the cholesterol fractions are concerned. The pig samples had higher (P<0.05) concentration of HDL-cholesterol and LDL-cholesterol in plasma compared with the rats. Above that, fish oil in comparison with the control palm oil decreased (P<0.05) HDL-cholesterol in the pig plasma, but not in the rat plasma. Contrary result was established as far as LDL-cholesterol is concerned: dietary fish oil decreased (P<0.05) LDLC compared with the control palm oil in the rat plasma, but not in the pig plasma (P>0.05). No significant difference in HDLC between F- and control rats in the present experiment agrees with the results of Campioli et al. (2012) and Yamazaki et al. (2011), who used as a control a diet with olive oil and a standard feed mixture.
Contrary to our results, Takahashi (2011) reported a decrease of HDLC from 1.56 mmol/L in palm oil-fed rats to 0.58 mmol/L in the fish oil group. Dietary fish oil decreased both TC and HDLC in the rat plasma in comparison with control safflower oil also in our previous experiment (Komprda et al. 2015), where the diets contained 6% of the particular oil.

Contrary to rats, dietary fish oil in comparison with palm oil decreased (P<0.05) HDLC in pigs in the present experiment (Figure 1). Taken from the opposite viewpoint, a surprising ability of saturated fat to increase the favourable HDLC fraction in pigs reported also Puccinelli et al. (2015) after feeding a diet with 20% of lard either continuously or intermittently: HDLC increased from 0.62 mmol/L (control diet) to 1.19 and 2.28 mmol/L, respectively (compare with the values of 1.02 and 1.32 mmol/L in the F- and P-pigs in the present experiment; Figure 1).

Plasma TAG levels were in average more than four-times lower in pigs in comparison with rats (P<0.05). Moreover, type of dietary oil had varying effect on the tested animal species: dietary intervention did not affect plasma TAG in pigs (P>0.05), but fish oil in the diet decreased (P<0.05) plasma TAG in the rats to 56% of the established level when the control diet with palm oil was fed.

An indifference of plasma TAG to the type of dietary oil found in the present study in pigs (contrary to rats; Figure 1) confirms the results of Puccinelli et al. (2015), though the quoted authors used different fats than in the present study with different objectives.

A collation of the tested animal species concerning an effect of fish oil relative to an effect the control palm oil is presented in Figure 2. As far as total plasma cholesterol is concerned, no differences between rats and pigs were established (P>0.05). More favourable effect of fish oil on rats is apparent from Figure 2 concerning cholesterol fractions. Plasma lipid concentration in the F-animals was expressed as a ratio of the plasma concentration in the P-counterparts. In this case dietary fish oil decreased the fraction of HDL-cholesterol less (P<0.01), but the fraction of LDL-cholesterol more (P<0.05) in rats than in pigs. Moreover, fish oil relative to control palm oil decreased plasma TAG in rats substantially more than in pigs (P<0.001; Figure 2).
Figure 2 Concentration of total cholesterol (TC), high-density-lipoprotein cholesterol (HDLC), low-density-lipoprotein cholesterol (LDLC) and triacylglycerols (TAG) in plasma of rats and pigs fed the diet with 2.5% of fish oil (F), relative to the corresponding values established with feeding the control diet with 2.5% of palm oil (P), respectively; pair t-test; n = 16; NS – not significant; * P<0.05; ** P<0.01; *** P<0.001.

Generally speaking, plasma lipid level established in the present experiment in pigs is more similar to humans than to rats; porcine model can therefore be considered superior in the given context. However, using this model, fish oil submitted at a reasonably achievable dose (2.5%; EPA+DHA ingested at an amount of ca 80 mg per kg of live weight and day) was not able to improve plasma lipid markers in comparison with saturated palm oil.

CONCLUSION

The purpose of the present study was to determine the effect of the diet enriched with 2.5% fish and palm oil, respectively on plasma lipids level of animal models (Sus scrofa and Rattus norvegicus). We focused on amount of total plasma cholesterol, triacylglycerol, low-density cholesterol and high-density cholesterol, all of them were analysed by the enzymatic-colorimetric method.

From the point of view of the absolute levels of plasma lipids no significant differences were found between diets, which is not unique result. The consumption of diet enriched with fish oil decrease of HDLC in pig plasma was observed but not in rat one. Diet enriched with palm oil increased levels of LDLC and TAG in rat plasma but not in pig plasma. Plasma lipid concentration in animals which was fed by diet enriched with fish oil was expressed like ratio of the plasma concentration in the other group of animals. In this case dietary fish oil decreased HDL-cholesterol less (P<0.01), but LDL-cholesterol and triacylglycerols more (P<0.05 and P<0.001) in rats than in pigs. The used amount of added fish oil is not able to improve levels of plasma lipids as compared with palm oil.

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REFERENCES


