

Effect of dietary fatty acid composition on plasma lipid levels in rats

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Abstract: The aim of the research was to evaluate in a model organism an effect of different fatty acids on plasma level of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TAG). The findings have been trying to be applied to human nutrition. Forty adult male rats (Wistar Albino) were divided into four groups with ten animals each and were examined for the effect of fatty acids contained in food on animal tissues. All groups were fed the first seven weeks standard feed for mice and rats with the addition of 20% of beef tallow. Fodder was adjusted in the next seven weeks. Basic feed mixture with 5% of safflower oil (n-6 group, SF), fish oil (n-3 group, F), *Schizochytrium* alga oil (n-3 group, DHA), and 20% of beef tallow (T-group). Beef tallow is representative of “atherogenic” saturated fatty acids, 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. At the end of the fattening period, the plasma concentrations of lipids and fatty acid content in the liver tissues was determined. DHA-diet had the most positive (decreasing) effect on TAG levels ($P < 0.05$). It was concluded that dietary *Schizochytrium* microalga oil (with high DHA content) may have the positive potential for decreasing the risk of cardiovascular diseases, but the results obtained in rats should be applied to humans cautiously.

Key-Words: plasma lipids level; liver; dietary fatty acids; beef tallow; fish oil; safflower oil; *Schizochytrium* microalga

Introduction

Fatty acids are considered as the most important component of the lipid in the nutrition. Human nutrition is focused not only on the total lipid content, but also on representation of fatty acids groups (SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids) in foods. Polyunsaturated fatty acids are labelled a fatty acid containing from 2 to 6 double bonds.

Polyunsaturated fatty acids are divided into two groups, n-3 and n-6, characterized by different physiological effects. The group n-3 always has the first double bond on the third carbon, taken from the methyl residue. The same applies to the group n-6 having the first double bond on the sixth carbon [1].

The starting compounds of the series n-3 and n-6 are (essential for humans) α -linolenic acid (ALA; 18:3n-3) and linoleic acid (LA, 18:2n-6). These two essential fatty acids are metabolized by the same set

of enzymes (elongase and desaturase) to long-chain polyunsaturated fatty acids, LC-PUFA.

Physiologically, the most important metabolites of group n-6 and n-3 are arachidonic acid (AA; 20:4n-6), respectively eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [2]. The above-mentioned PUFA families, n-6 and n-3, are essential components of the metabolically active tissues such as liver. The final metabolites of n-6 and n-3 PUFAs are eicosanoids (biologically active substances – prostaglandins, leukotrienes and tromboxanes), playing important roles in regulating different aspects of the inflammatory response [3].

McDaniel et al. [4] describe that diets with high n-6:n-3 ratios, in conjunction with genetic factors, have been associated with the increasing prevalence of chronic inflammatory diseases such as cardiovascular disease. It is generally considered that eicosanoids generated from arachidonic acid, have pro-inflammatory effect, causing platelet aggregation and shrinks the vascular wall. While their

counterparts generated from EPA/DHA are believed to elicit anti-inflammatory effect and ultimately also cardioprotective effect.

Process of atherogenesis is the principle of cardiovascular diseases. One of the risk factors for atherosclerosis (chronic inflammatory process of the vessel wall) is dyslipidemia (high total plasma cholesterol [TC] level, increased low-density lipoprotein cholesterol [LDL-C], decreased high-density lipoprotein cholesterol [HDL-C], high concentration of plasma triacylglycerols [TAG]) [5].

Cholesterol is transported in the blood plasma as part of lipoproteins mostly in the fraction of LDL, HDL less. Changes in the concentration of cholesterol from the perspective of cardiovascular risk are always evaluated in the context of concentrations of total cholesterol, HDL cholesterol, LDL cholesterol and triacylglycerols. The liver and other organs synthesize cholesterol. HDL lipoproteins ensure the removal of excess cholesterol to the liver. LDL lipoproteins ensures transport of cholesterol to the cells. Excess cholesterol contained in LDL particles is deposited in the vessel wall, leads to the formation of atherosclerotic plaques [6].

Material and Methods

Animals and dietary interventions

Forty adult male rats of the laboratory strain Wistar Albino (produced by Bio Test Ltd., Konárovice, Czech Republic) at the age of 10 weeks were used. Animals were reared in the plastic boxes (53.5 x 32.5 x 30.5 cm) of five animals in a room maintained at $23 \pm 1^\circ\text{C}$, humidity 60% and 12/12 h light/dark cycle. 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.

The basic feed mixture, pelletized complete chow for mice and rats (Biokron, Blučina, Czech Republic), composed of wheat, oat, wheat sprouts, soybean meal, extruded soybean, maize, dried milk, dried whey, dried yeast, grounded limestone, monocalcium phosphate, salt, L-lysine hydrochloride and premix of vitamins + minerals, was fed to all animals the first week of the experiment (acclimatization). The animals were fed daily *ad libitum* and had free access to water bottles.

All 40 rats (four groups per 10 animals) were fed (an atherogenic) diet 7 weeks. This diet contained basic feed mixture with beef tallow + evaporated sweetened milk + extra vitamins/minerals premix

(200 + 400 + 20 g.kg⁻¹; T). The next 7 weeks were the second phase of fattening. The rats were randomly divided with following dietary interventions. The first group was fed the (atherogenic) tallow diet continued (T-group). Other groups were divided into groups with 5% of safflower oil (SF-group), fish oil (F-group), *Schizochytrium* alga oil (DHA-group). The content of quantitatively and physiologically important fatty acids in the used feed demonstrates table 1.

At the end of the experiment was carried out taking samples for analysis. Blood samples were collected from all animals into the heparin-coated test tubes (DISPOLAB) and centrifuged at 200 x g for 10 min at 4 °C to obtain plasma. Liver samples were removed for analysis of fatty acids. The liver were stored at -20°C after lyophilization; the main drying at -45°C for 24 hours, the finish drying at -50°C for 3 hours (Freeze Christ Alpha 1-2 LD).

Plasma lipids and fatty acids determination

Total plasma cholesterol and its fractions (LDL cholesterol, HDL cholesterol and triacylglycerols) were determined by the enzymatic-colorimetric method using an automated chemical analyser BS-200 (Mindray, China) and commercial kits (Greiner Diagnostic GmbH, Germany). The determination was carried out at the Department of Chemistry and Biochemistry, Mendel University in Brno.

Fatty acids methyl ester were determined by gas chromatography after fatty acids derivatization referred to in article by Komprda et al. (2013) [7]. The analysis of liver tissue was performed on a gas chromatograph Fisons GC 8000 series with a flame ionization detector, capillary column DB-23 (60 m x 0.25 mm x 0.25 µm, Agilent J & W Scientific, USA) and temperature program 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. Total lipids were extracted (hexane/isopropanol solvent) from the liver tissue by the method based on the extraction method of Hara and Radin (1987) [8]. For each sample, the measurement was carried out concurrently.

The measured data were statistically evaluated by one-way analysis of the variance ratio test, including Tukey's post-hoc test. For the evaluation of the data was used programme Statistica (StatSoft Inc., Tulsa, USA).

Table 1 Content of quantitatively and physiologically important fatty acids in the used feed

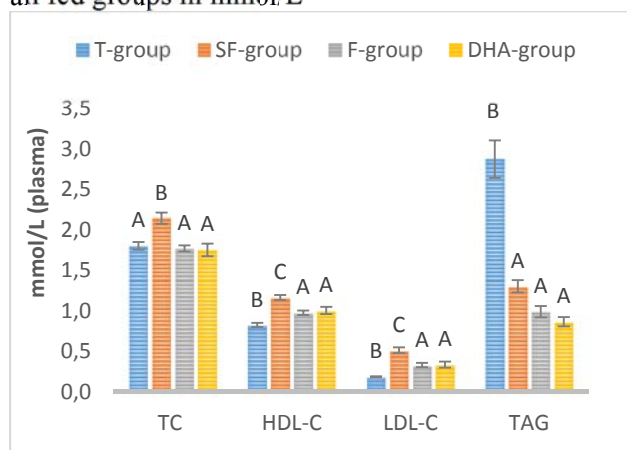
Fatty acid		Content in the diet (% of the sum of fatty acids)			
Name	Designation	T-group	SF-group	F-group	DHA-group
Myristic	14:0	4.7	0.2	3.2	3.1
Palmitic	16:0	17.2	10.5	15.2	16.4
Stearic	18:0	21.0	2.5	2.9	1.7
Oleic	18:1n-9	83.1	19.7	24.6	21.8
Linoleic	18:2n-6	12.5	65.8	26.4	22.8
α -Linolenic	18:3n-3	0.8	0.1	5.2	4.1
Arachidonic	20:4n-6	0.1	0.1	0.4	0.4
EPA	20:5n-3	0.0	0.1	6.6	1.0
DPA	22:5n-3	0.0	0.2	1.1	0.7
DHA	22:6n-3	0.0	0.2	7.7	26.6

Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil (commercial oleum jecoris aselli); DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga

Results and Discussion

The experiment focused on the analysis of fatty acids in liver tissue and plasma lipid fractions determination having regard to the variability of dietary inputs. Indicators of plasma lipid levels are total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TAG).

Fig. 1 The content of plasma lipids level of rats from all fed groups in mmol/L



Legend: TC - total cholesterol, HDL-C - high-density lipoprotein cholesterol, LDL-C - low-density lipoprotein cholesterol, TAG – triacylglycerols; T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the Schizochytrium microalga; a – c: means labelled with different letters differ significantly ($P < 0.05$); one-way analysis of the variance ratio test, including Tukey's post-hoc test, $n = 20$

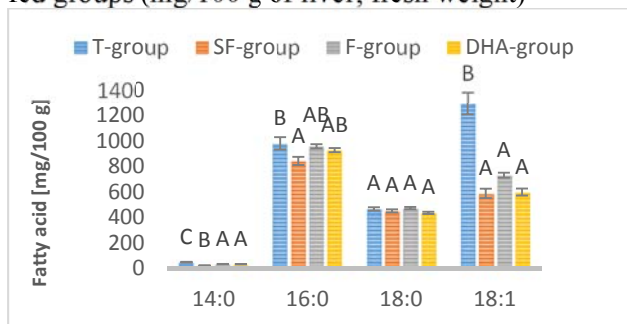
The content of fatty acids measured in liver tissue was converted to mg/100g of fresh weight of liver. The content of plasma lipid fractions was converted to mmol.l⁻¹ of plasma.

As it is apparent from Figure 1, the highest ($P < 0.05$) total cholesterol concentration was established in plasma of rats fed the diet with safflower oil compared with control (T-group). However, it is necessary to add that this cholesterol increasing effect of safflower oil was from the greater part due to the increase in the favourable HDL fraction. The most positive (i.e. decreasing) effect on the plasma levels of TAG rats ($P < 0.05$) was DHA-oil compared with T-group fed the atherogenic diet, which plasma levels of TAG as expected substantially increased ($P < 0.05$). As far as the LDL fraction, the lowering effect was established surprisingly in the T-group with tallow/evaporated milk diet.

It is generally known that a high content of saturated fatty acids and ratio of PUFA n-6/n-3 for the benefit n-6 in the diet increases the risk of cardiovascular disease, while PUFA n-3 reduces the risk of cardio-vascular disease due to favorable physiological effects. Therefore, the concentrations of fatty acids in the liver tissue are divided into various groups in the charts (Figure 2-5).

Regarding saturated fatty acids, the T-group was significantly higher ($P < 0.05$) deposition of myristic acid (14:0) and palmitic acid (16:0) compared with other SF-, F-, DHA-groups. Significant increase ($P < 0.05$) of oleic acid in the feed for the control group (T-group) was expected because, oleic acid has the highest representation content (83.1%) in this compared with other SF-, F-, DHA-group.

Fig. 2 The content of saturated fatty acids and monounsaturated fatty acid in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)

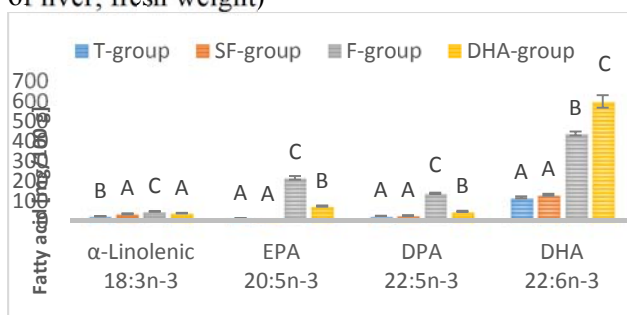


Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the *Schizochytrium microalga*; a – c: means labelled with different letters differ significantly ($P < 0.05$); one-way analysis of the variance ratio test, including Tukey's post-hoc test, $n = 20$

As far as the polyunsaturated fatty acid n-3 series are concerned, higher ($P < 0.05$) deposition of EPA and DHA in animals fed a diet of fish oil (F-groups) and *Schizochytrium* oil (DHA-groups) is interesting in comparison with control (T-group). A significant increase of α -linolenic acid was measured in the group fed fish oil.

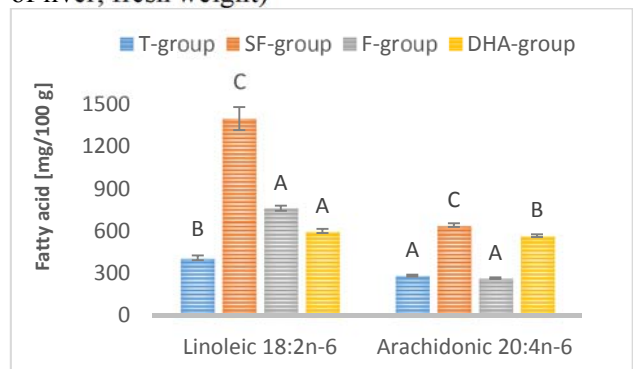
Regarding polyunsaturated fatty acids n-6 series, expected increase ($P < 0.05$) of linoleic acid and arachidonic acid was confirmed in a group of diets with safflower oil (SF-group) compared with control (T-group).

Fig. 3 The content of polyunsaturated fatty acid n-3 series in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the *Schizochytrium microalga*; a – c: means labelled with different letters differ significantly ($P < 0.05$); one-way analysis of the variance ratio test, including Tukey's post-hoc test, $n = 20$

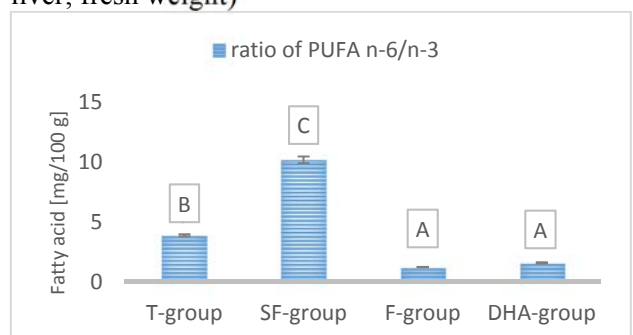
Fig. 4 The content of polyunsaturated fatty acid n-6 series in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the *Schizochytrium microalga*; a – c: means labelled with different letters differ significantly ($P < 0.05$); one-way analysis of the variance ratio test, including Tukey's post-hoc test, $n = 20$

The highest value of the PUFA n-6/n-3 ratio was observed in the group with a safflower oil diet, which was expected due to the high content of linoleic acid in safflower oil. The ratio of PUFA n-6/n-3 in the diet (rich source of PUFA n-3) with fish oil and *Schizochytrium microalga* was measured a significant reduction ($P < 0.05$) compared to control (T-group), while the ratio of PUFA n-6/n-3 in the diet safflower oil is significantly increased ($P < 0.05$) compared with other T-, F-, DHA-groups.

Fig. 5 The ratio of n-6/n-3 polyunsaturated fatty acids in the liver of rats for all fed groups (mg/100 g of liver; fresh weight)



Legend: T-group – basic feed mixture with 20% of beef tallow + 40% of evaporated sweetened milk + 2% of extra vitamins/minerals premix; SF-group – basic feed mixture with 5% of safflower oil; F-group – basic feed mixture with 5% of fish oil; DHA-group – basic feed mixture with 5% of oil extracted from the *Schizochytrium microalga*; a – c: means labelled with different letters differ significantly ($P < 0.05$); one-way analysis of the variance ratio test, including Tukey's post-hoc test, $n = 20$

Figures 1 shows, that the addition of safflower oil in the feed tends to increase ($P < 0.05$) serum total cholesterol as compared to control (T-group), which was found also in the experiment Poudyal et al. (2013) [9]. Chen et al. (2011) reported a significant decrease of plasma total cholesterol in hamsters fed a diet containing *Schizochytrium* lipid extract compared to the control, but this fact is not confirmed in our case regarding DHA diet (Figure1) [10].

HDL fraction increased after the fish oil diet in comparison with control (T-group), which was confirmed also in experimental Popović et al. (2011) [11].

Plasma levels of TAG rats was significantly reduced after DHA-diet containing a high proportion of LC-PUFA n-3 compared to control (T-group) in our experiment. This finding can be explained by the fact that DHA and EPA inhibit the synthesis of diacylglycerol O-acyltransferase (DGAT), fatty acid synthase and acetyl CoA carboxylase enzymes and increase fatty acid β -oxidation via PPAR α -mediated pathway resulting in decreased substrate availability for TAG formation [12].

The results of this experiment obtained in rats have to be interpreted cautiously regarding dietary recommendations for humans, because a better model for humans is a hamster or, better still, a pig than rats or mice [13].

Conclusion

It can be concluded based on our results that, DHA-diet (rich sources of DHA) significantly decrease ($P < 0.05$) the plasma level of TAG, which may have a positive effect on reducing the risk of cardiovascular diseases. It is interesting that the safflower oil (containing about 65% of linoleic from the sum of total fatty acids) in the diet significantly increased the fraction of favorable HDL cholesterol in the rat plasma compared to the control (T-group), wherein said the fact should be related to F- and DHA-groups.

The different composition of fatty acids in the diet has the influence on their deposition in liver tissues. Deposition of linoleic acid in the liver increased ($P < 0.05$) significantly under the diet rich in linoleic acid (SF-group), compared with the control (T-group). Deposition of EPA, DPA, DHA in the liver increased ($P < 0.05$) significantly under the fish oil and *Schizochytrium* oil diet rich in PUFA n-3 compared with control (T-group).

Increased amounts of PUFA n-3 in the diet (F-group, DHA group) affecting desirable increase in the levels of these fatty acids in liver tissues, therefore the ratio of PUFA n-6/n-3 approached almost to an optimum, which is associated with reduced risk of disease occurrence heart and blood

vessels. The ratio of PUFA n-6/n-3 1:1 is considered ideal, but such a relationship can't be achieved in the diet of economically developed countries. Therefore, the World Health Organization recommends a ratio of 5:1-2:1. It is reported that populations with a high ratio of PUFA n-6/n-3 in the diet, such as for example the population of Indian subcontinent has extremely high incidence of cardiovascular diseases. Therefore, it is desirable to increase the consumption of n-3 PUFA diet. In this context it is useful to mention that the higher consumption of PUFA, whose double bond is very susceptible to oxidation, it is recommended to consumption of the lipophilic antioxidant vitamin E higher than the recommended daily dose (10 mg/day), which prevents undesirable oxidation in molecules [14,15].

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