Czech J. Anim. Sci., 59, 2014 (9): 391–398

Original Paper

Effect of dietary eicosapentaenoic and docosahexaenoic acid on expression of rat liver genes controlling cholesterol homeostasis and on plasma cholesterol level

T. Komprda[1], G. Zorníková[1], A. Knoll[2,3], Z. Vykoukalová[2], V. Rozíková[1], O. Škultéty[2], R. Krobot[4]

[1]Department of Food Technology, Mendel University in Brno, Brno, Czech Republic

[2]Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno, Brno, Czech Republic

[3]CEITEC MENDELU, Mendel University in Brno, Brno, Czech Republic

[4]Department of Animal Nutrition and Forage Production, Mendel University in Brno, Brno, Czech Republic

ABSTRACT: A hypothesis that eicosapentaenoic acid + docosahexaenoic acid (EPA+DHA) lower plasma cholesterol via increased expression of the *Insig-1* gene with ensuing decrease of expression of genes coding for 3-hydroxy-3-methyl-glutaryl-CoA reductase (*Hmgcr*) and low density lipoprotein receptor (*Ldlr*) was tested in rats fed a diet with 3% of fish oil (FO). Expression of the *Insig-1* gene in the liver of the FO-fed rats was 730% ($P < 0.05$) of the control. However, contrary to the hypothesis, expression of the *Hmgcr* gene and *Ldlr* gene was 165% and 210% of the control ($P > 0.05$). Nevertheless, FO in the diet decreased ($P < 0.05$) plasma cholesterol of rats by 10% (from 1.19 to 1.07 mmol/l); it was therefore concluded that the cholesterol-lowering effect of EPA+DHA is at least partly based on mechanisms other than tested in the present experiment.

Keywords: PPARα; SREBP-2; *Insig-1*; cholesterol; PUFAn-3; rats

INTRODUCTION

Effect of the principal components of fish oil (FO), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) on cardiovascular diseases risk decrease has been repeatedly reported (Givens and Gibbs 2008). The underlying biochemical and molecular mechanisms that can explain cardioprotective effects of EPA and DHA in the rodent models and humans have recently been reviewed (Komprda 2012). The effect of EPA/DHA on blood lipid level is based on the action of these PUFAs n-3 as ligands of the various isoforms of peroxisome proliferator-activated receptor (PPAR), and on modulation of the signalling pathway of the transcription factor sterol response element-binding protein (SREBP) (Jump 2008).

Regarding plasma triacylglycerol (TAG) levels, the protective effect of EPA/DHA is sufficiently explained: PPARα activation and inhibition of the SREBP-1 signalling pathway stimulates fatty acid (FA) β-oxidation and inhibits FA synthesis with the final result of decreased serum TAG (Jump 2008).

The situation is much less clear as far as cholesterol is concerned (Komprda 2012). A principal transcription factor binding the promoter region of the genes coding for proteins controlling cholesterol homeostasis (3-hydroxy-3-methyl-glutaryl-CoA reductase HMG-CoA-R, low density lipoprotein receptor LDL-R) is SREBP-2 (Nakamura et al. 2004). SREBP-2 released from the endoplasmic reticulum and consequently its activation in the Golgi apparatus is affected by an amount of protein INSIG (insulin-induced gene), product of the *Insig*

gene (Sato 2010). SREBP-2 is not directly ligated by EPA/DHA; a relationship, still not unequivocally explained, between PPARα ligation and SREBP-2 activation is presumed (Luci et al. 2007). Konig et al. (2007) suggested presence of a PPAR-responsive sequence in the *Insig-1* gene promoter.

Still not fully explained molecular mechanisms controlling cholesterol homeostasis are complemented by contradictory results regarding studies evaluating effects of EPA/DHA on plasma total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and low density lipoprotein-cholesterol (LDL-C) both in rats and in humans. Fish oil (FO) in the rat diet decreased plasma TC in most of the recent studies (Lu et al. 2011; Ferramosca et al. 2012; Xiao et al. 2012). On the other hand, regarding studies in humans, EPA/DHA either reduced TC and LDL-C (Lopez-Huertas 2009) or increased both TC and LDL-C and HDL-C (Maki et al. 2003) or no change in TC and slight increases in HDL-C and LDL-C, respectively were established (Eslick et al. 2009).

Following hypothesis (based e.g. on the data of Konig et al. 2007) was tested in the present experiment: EPA+DHA (FO constituents), which are natural ligands of PPARα, ingested by rats in an amount achievable within an ordinary way of nutrition activate this transcription factor, which leads to an increased expression of the *Insig-1* gene with a consequence of decreased amount of the nuclear form of SREBP-2; a decreased expression of the *Hmgcr* and *Ldlr* genes ensues, with a consequence of decreased cholesterol synthesis and increased plasma cholesterol clearance.

MATERIAL AND METHODS

Animals, diets, analyzed tissues. Adult (56 days) male rats of the laboratory strain Wistar Albino (produced by Bio Test Ltd., Konárovice, Czech Republic) were used. Rats were randomly divided into three groups with ten animals each and housed in plastic boxes (53.5 × 32.5 × 30.5 cm) per five animals in a room maintained at 23 ± 1°C, humidity 60%, and 12/12 h light-dark cycle (maximum intensity of 200 lx). The experiments were 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 were approved by the Commission to protect animals against cruelty of the Mendel University in Brno.

Table 1. Composition of the diets

Composition		Diet		
		C	F	P
Component (g/kg)	basic feed mixture[1]	933	970	970
	maize starch	67		
	salmon oil		30	
	palm oil			30
Nutrients (g/kg)	crude protein	219	228	228
	fat[2]	25	56	56
	crude fibre	47	49	49
	nitrogen-free extractives	709	667	667
ME (MJ/kg)		15.2	15.2	15.2

C = control diet, F = experimental diet with fish oil, P = negative control with palm oil, ME = metabolizable energy
[1]complete chow for mice and rats (composition: wheat, oat, wheat sprouts, soybean meal, extruded soybean, maize, dried milk, dried whey, dried yeast, grounded limestone, monocalcium phosphate, feed salt, L-lysine hydrochloride, vitamins, minerals)
[2]hexane/2-propanol extract

The basic feed mixture, pelletized complete chow for mice and rats (Biokron, Blučina, Czech Republic), was composed of wheat, oat, wheat sprouts, soybean meal, extruded soybean, maize, dried milk, dried whey, dried yeast, grounded limestone, monocalcium phosphate, feed salt, L-lysine hydrochloride, and premix of vitamins and minerals. The experimental diet was formed by admixing of 3% of salmon oil to the chow (F). The chow with 3% of palm oil (P) served as a negative control with a presumed cholesterol-increasing effect. The chow with an adequate amount of maize starch to render the diet isocaloric was designated as a control (C). Composition of the diets is shown in Table 1; fatty acid content is presented in Table 2.

The mean initial (56 days) live weight of the C-, F-, and P-rats was 368 ± 4, 366 ± 4, and 373 ± 4 g, respectively. The animals were fed daily *ad libitum* and had free access to drinking water. Feed consumption was measured daily; animals were weighed in weekly intervals. At the end of the experiment lasting 48 days, after the 12-h fasting, blood samples were collected by cardiac puncture under anesthesia with isoflurane into the heparin-coated test tubes and centrifuged at 200 *g* at 4°C for 10 min to obtain plasma. Liver was removed and RNA was isolated immediately from an aliquot of 1 g.

Quantification of the gene expression in the liver. Total RNA was isolated using RNeasy Lipid

Table 2. Fatty acid content in the diets and daily intake by rats

Fatty acid	Content in the diet (% of the sum of fatty acids)			Intake (mg per kg of live weight and day) (mean ± standard error of the mean)		
	C	F	P	C	F	P
14:0	2.9	3.8	1.5	41[B] ± 2	65[c] ± 2	30[A] ± 1
16:0	22.1	15.2	33.8	373[B] ± 17	311[A] ± 10	766[C] ± 30
18:0	5.1	4.2	4.7	79[B] ± 4	73[A] ± 2	103[C] ± 4
18:1n-9	25.9	35.0	38.2	440[A] ± 20	739[B] ± 25	866[C] ± 34
18:2n-6	40.4	27.9	20.5	691[C] ± 32	585[B] ± 19	465[A] ± 19
18:3n-3	3.6	4.8	1.3	54[B] ± 2	84[C] ± 3	30[A] ± 1
20:5n-3	0.0	2.9	0.0	0[A] ± 0	45[B] ± 2	0[A] ± 0
22:5n-3	0.0	2.0	0.0	0[A] ± 0	26[B] ± 1	0[A] ± 0
22:6n-3	0.0	4.2	0.0	0[A] ± 0	73[B] ± 2	0[A] ± 0

C = standard chow for mice/rats (control), F = standard chow for mice/rats supplemented with 3% of fish oil, P = standard chow for mice/rats supplemented with 3% of palm oil

[A–C]means with different superscripts in lines differ at $P < 0.05$

Tissue Mini Kit (Qiagen GmbH, Hilden, Germany). The quality of isolation was checked on the 1.2% RNA gel visualized by ethidium bromide. Concentration of isolated RNA was measured on Nano-Drop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, USA). Isolated RNA was stored at –80°C.

One μg of the isolated RNA was reverse transcribed using Omniscript RT Kit (Qiagen) and oligo-dT primers. Obtained cDNA was used for quantitative PCR with specific primers for the rat *Insig-1* gene (fw TCTTCCCGGACGAGGTGATAG, rev AGCTGCACATTATTGGCGAAAT), *Hmgcr* gene (fw AAGGGGCGTGCAAAGACAATC, rev ACACGGCACGGAAAGAACCATAGT), *Ldlr* gene (fw GGACAAGTCGGACGAGGAGAA, rev AGCTGATGCACTCCCCACTGT), *PPARα* gene (fw GCCTTGTCCCCACATATTCG, rev AGAGGAGAGTTCCGGAAG), *SREBP-2* gene (fw ATCCGCCCACACTCACGCTCCTC, rev GGCCGCATCCCTCGCACTG), and housekeeping gene *Actb* (fw AGAGGGAAATCGTGCGTGAC, rev GTTTCATGGATGCCACAGGATT).

The reaction mixture was as follows: 1 μl of cDNA, 0.2 μl of AmpErase® Uracyl N-glycosylase (Applied Biosystems, Foster City, USA), 10 μl of Power SYBR® Green PCR Master Mix (Applied Biosystems), 0.2 μl of each primer (mol/μl), 8.4 μl of H_2O. All analyses were carried out on the 7500 Real-Time PCR System (Applied Biosystems) under the following conditions: 2 min of UNG incubation at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, 30 s at specific annealing temperature that

was either 65°C (expression of the *Insig 1* and *Ldlr* gene) or 60°C (expression of both remaining genes), and 30 s at 60°C.

Effectivity of each reverse transcription reaction was calculated based on the standard curve method using decimal dilution of the input cDNA. The specificity of each PCR fragment was verified by the sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI PRISM® 3100-Avant Genetic Analyzer (Applied Biosystems). The measured C_T data were analyzed by considering the basal condition as the reference value for relative amount of the gene expression determined under each condition.

Changes in the gene expression were calculated on relative basis in relation to the level of expression of a given gene in the liver of the control rats.

Cholesterol determination. Total plasma cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), and plasma triacylglycerol (TAG) were determined by the enzymatic-colourimetric method using an automated chemical analyzer BS-200 (Mindray, Shenzhen, China) and commercial kits (Greiner Diagnostic GmbH, Bahlingen, Germany).

Fatty acid determination. Total lipid extraction from the liver, fatty acid derivatization, and fatty acid determination were performed strictly according to the protocol described in the paper of Komprda et al. (2013).

Statistical evaluation. One-Way Analysis of the Variance ratio test, including Tukey's post-hoc test (STATISTICA, Version 8, 2007), was used for evaluation of differences between the dietary

interventions. Relationships between tested traits were assessed using correlation analysis.

RESULTS

Feed intake, weights. Average daily feed intake did not differ ($P > 0.05$) between dietary groups and was 25 ± 2, 23 ± 1, and 24 ± 2 g/day in the C-, F-, and P-rats. Dietary intervention had no significant effect ($P > 0.05$) either on the average daily weight gain (2.99 ± 0.19, 3.01 ± 0.12, and 3.25 ± 0.19 g) or on the final live weight that reached the value of 512 ± 13, 511 ± 9, and 529 ± 11 g in the C-, F-, and P-rats, respectively.

Gene expression. Relative expression of the genes coding for *PPARα* and *SREBP-2* in the liver of the FO-fed rats was 47% and 57% as compared to the control; due to the great variability of the C_T values, the differences were insignificant ($P > 0.05$).

Relative expression of the *Insig-1* gene in the liver of rats fed the diet with 3% of fish oil was 730% of expression of this gene in the control rats ($P < 0.05$); however, an assumption that an over-expression of the *Insig-1* gene leads to down-regulation of the *Hmgcr* gene and *Ldlr* gene, respectively was not confirmed (Figure 1). Expression of the *Hmgcr* gene and *Ldlr* gene in the liver of the FO-fed rats was 165% and
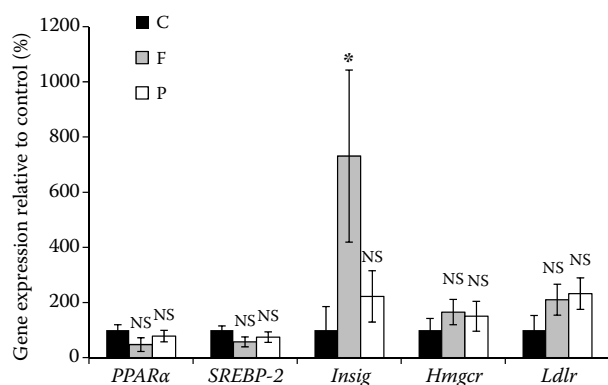

Figure 1. Expression of the genes presumably controlling cholesterol homeostasis

C = rats fed the control diet (n = 10), F = rats fed the control diet with 3% of fish oil (n = 10), P = rats fed the control diet with 3% of palm oil (n = 10), *PPARα* = peroxisome proliferator-activated receptor α, *SREBP-2* = sterol response element-binding protein 2, *Insig-1* = insulin-induced gene-1, *Hmgcr* = 3-hydroxy-3-methyl-glutaryl-CoA reductase, *Ldlr* = low density lipoprotein receptor

*amount of mRNA in the sample differed from the control ($P < 0.05$), NSamount of mRNA in the sample did not differ from the control ($P > 0.05$)
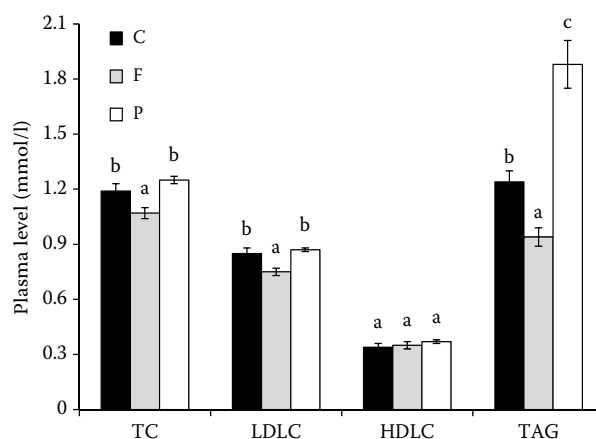
Figure 2. Plasma cholesterol and triacylglycerols (TAG) of rats fed the control diet (C) and the control diet with either 3% of fish oil (F) or 3% of palm oil (P), respectively (n = 10)

TC = total cholesterol, LDLC = low density lipoprotein cholesterol, HDLC = high density lipoprotein cholesterol
a–cmeans with different letters within a given trait differ at $P < 0.05$

210% relative to the expression of these genes in the liver of the control rats; again, the differences were insignificant ($P > 0.05$) due to the great variability of the C_T values within each tested group of rats.

Plasma cholesterol. Plasma levels of total cholesterol and its fractions in LDL and HDL are shown in Figure 2 (including TAG; however, this marker was not analyzed in a more detail from the reasons mentioned in Introduction).

FO in the diet decreased ($P < 0.05$) total plasma cholesterol and LDL cholesterol in rats by 10 and 12%, respectively as compared to the control diet; HDL-C was not changed by the dietary intervention.

Liver fatty acids: relationships to plasma cholesterol and gene expression. Fatty acid content in the rat liver is shown in Table 3.

Relative level of expression of the *Insig-1* gene in the liver was in a positive relationship ($P < 0.05$) to EPA and DPA (docosapentaenoic acid) content in this tissue (r = +0.27 in both cases). EPA content in the liver of the rats fed the diet with fish oil was 13 times higher ($P < 0.05$) than in the liver of the control rats (Table 3). These data correspond with the differences ($P < 0.05$) in the *Insig-1* gene expression between the F- and C-group of rats. On the other hand, no relationship ($P > 0.05$) between DHA content in the liver and the *Insig-1* gene expression was established. Negative correlation ($P < 0.05$) to the *Insig-1* gene expression in the liver was found in the case of myristic acid (14:0; r = –0.37) and oleic acid (18:1n-9; r = –0.37).

Table 3. Fatty acid content in the liver of rats fed the control diet (C) and the control diet with either 3% of fish oil (F) or 3% of palm oil (P) (mean ± standard error of the mean)

Component	Diet		
	C	F	P
Total lipid (%)[1]	$3.1^A \pm 0.1$	$3.3^{AB} \pm 0.2$	$3.7^B \pm 0.1$
14:0	$21^{AB} \pm 0.5$	$19^A \pm 1$	$23^B \pm 1$
16:0	$687^{AB} \pm 11$	$623^A \pm 37$	$749^B \pm 13$
18:0	$360^A \pm 5$	$343^A \pm 22$	$425^B \pm 10$
18:1n-9	$454^A \pm 11$	$469^A \pm 26$	$685^A \pm 16$
18:2n-6	$384^A \pm 14$	$536^B \pm 30$	$495^B \pm 13$
20:4n-6	$476^B \pm 10$	$343^A \pm 25$	$496^B \pm 14$
18:3n-3	$7^A \pm 0.6$	$21^B \pm 1$	$9^A \pm 0.4$
20:5n-3	$6^A \pm 0.4$	$79^B \pm 5$	$4^A \pm 0.3$
22:5n-3	$24^A \pm 1$	$80^B \pm 5$	$22^A \pm 0.7$
22:6n-3	$104^A \pm 2$	$228^B \pm 14$	$104^A \pm 3$

Fatty acid (mg/100 g)

[1]hexane/2-propanol extract

[A, B]means with different superscript in lines differ at $P < 0.05$; Tukey's post-hoc test

Relative expression of the *Hmgcr* gene correlated positively ($P < 0.05$) with the liver content of stearic acid (18:0; $r = +0.24$), linolenic acid (18:2n-6; $r = +0.31$), and DHA ($r = +0.23$). Positive correlation ($P < 0.05$) with liver stearic acid ($r = +0.25$) and linolenic acid ($r = +0.22$) and negative correlation with liver myristic acid ($r = −0.24$) was found in the case of the *Ldlr* gene expression.

As far as plasma cholesterol is concerned, both total cholesterol and LDL cholesterol was negatively ($P < 0.05$) correlated with liver content of all tested PUFAn-3 ($r = −0.41$ to $−0.47$) and also of linoleic acid ($r = −0.26$).

Regarding the relationship between expression of the tested genes and plasma cholesterol, the only significant ($P < 0.05$) correlation found was that between the *Insig-1* gene and LDL cholesterol ($r = −0.26$).

DISCUSSION

Feed intake. No significant effect of the FO addition in the diet on daily weight gain or final live weight of rats found in the present experiment was reported also by Yamazaki et al. (2011) and Campioli et al. (2012). On the other hand, FO decreased body weight of rats in an experiment of Lu et al. (2011). Moreover, FO can have an antiobesity effect in obese mice (Arai 2009a).

Daily FO intake in F-group of rats (1.57 g per kg of live weight and day) established in the present study was slightly higher than in the experiment of Yamazaki et al. (2011), 1 g/kg of live weight and day, which the authors labelled as a "lower-dose FO supplementation".

EPA+DHA intake in the F-group of rats (23 + 37 = 60 mg/day) was substantially lower in the present study in comparison with the results of Lu et al. 2011 (169 mg/day), but comparable with an experiment of Popovic et al. 2011 (75 mg/day).

Gene expression. *Actb* gene was used as an endogenous control in quantitative real-time PCR in the present study based on the results of the similar experiments measuring an effect of dietary PUFA n-3 on expression of genes controlling cholesterol homeostasis (Densupsoontorn et al. 2007; Fernandez-Alvarez et al. 2011; Lecker et al. 2011; Lu et al. 2011): none of the quoted authors reported any change of this housekeeping gene due to the addition of n-3 PUFA.

The fact that, similarly to our data, *PPARα* gene expression was not induced in EPA-fed mice in the experiment of Sugiyama et al. (2008) was surprising. *PPARα* mRNA in the n-3 PUFA-fed rats was similarly unaltered in the experiment of Lu et al. (2011). According to Takahashi (2011), n-3 PUFA are able to directly bind PPARα and stimulate its activity; an increase of the *PPARα* gene expression is therefore not essential to stimulate activities of the target enzymes. Nevertheless, Takahashi (2011) reported higher amount of *PPARα* mRNA in FO-fed rats as compared to palm oil, the results opposite to the present study. Hirako et al. (2010) found *PPARα* gene expression in FO-fed mice 175% of the control.

Regarding SREBP, Caputo et al. (2010), using PPARα antagonist, argued that an effect of EPA/DHA on SREBP-1c expression is not mediated through PPARα action. On the other hand, Fernandez-Alvarez et al. (2011) reported that PPAR/RXR heterodimer activated SREBP-1c promoter and a PPARα agonist increased SREBP-1c expression in the rat liver.

Though the *SREBP-2* mRNA in the liver of the FO-fed rats was less than 60% of the control in the present study, the differences were not significant, similarly to an experiment of Hirako et al. (2010), where *SREBP-2* expression was not influenced by FO in mice. On the other hand, both Arai et al. (2009a) and Arai et al. (2009b) reported a decrease of *SREBP-2* mRNA in EPA/DHA-fed mice. Similarly, *SREBP-1c* and *SREBP-1* mRNA decreased in the liver of the FO-fed rats (Takahashi 2011) and n-3 PUFA-fed rats (Lu et al. 2011), respectively.

SREBP-2 protein was not quantified in the present study, similarly to the experiments of Arai et

al. (2009a, b) and Hirako et al. (2010). However, Sugiyama et al. (2008), who also reported no effect of EPA on *SREBP-2* gene expression in mice, found highly reduced protein amount of the mature SREBP-2; the authors (Sugiyama et al. 2008) admitted their unsuccessful identification of the key molecules mediating EPA function on SREBP-2 processing, but suggested post-transcriptional suppression of SREBP-2 by EPA in the presence of PPARα. Lu et al. (2011) found a decrease of an amount of both the precursor and the nuclear SREBP-1 protein in their experiment.

It is worthy to mention in this context an effect of concentration: Caputo et al. (2010) reported a decrease of both *SREBP-1* mRNA and SREBP-1 protein in human hepatoma treated with EPA/DHA, but only in concentration of 50µM and not in concentration of 25µM.

The up-regulation of the *Insig-1* gene by FO in the present experiment (Figure 1) agrees with the data of Konig et al. (2007) who found increased *Insig-1* gene expression in the rat hepatoma cell line after activation of PPARα. Similarly, Botolin et al. (2006) reported transiently induced *Insig-1* mRNA in rat hepatocytes after DHA treatment. On the other hand, the recent experiments testing an effect of FO on the gene expression in mice (Arai et al. 2009a, b; Hirako et al. 2010) found decreased *Insig-1* gene expression.

The results of the present experiment regarding rat liver *Hmgcr* gene (Figure 1) do not agree with the data found in mice, where *Hmgcr* gene expression was decreased by EPA/DHA (Arai et al. 2009a), by menhaden oil (EPA) (Arai et al. 2009b), by FO (Hirako et al. 2010) or by EPA alone (Sugiyama et al. 2008). On the other hand, only tuna oil (DHA), but not menhaden oil (EPA) decreased expression of *Ldlr* in the study of Arai et al. (2009b).

Plasma cholesterol*.* Plasma TC level of the FO-fed rats in the present experiment (1.07 mmol/l; Figure 2) is approximately in the middle of the range of the results of similar experiments (FO-fed rats; all data recalculated to mmol/l): 3.22 (Lu et al. 2011) → 2.45 (Ferramosca et al. 2012) → 2.36 (Xiao et al. 2012) → 2.04 (Campioli et al. 2012) → 1.01 (Yamazaki et al. 2011) → 0.98 (Takahashi 2011) → 0.54 (Popovic et al. 2011).

The same is true regarding plasma LDL-C in the present experiment (0.75 mmol/l; Figure 2): the results of the similar experiments with the FO-fed rats are between 1.84 mmol/l (Xiao et al. 2012) and 0.23 mmol/l (Popovic et al. 2011), the value

reported by Campioli et al. 2012 (0.81 mmol/l) being very similar to our data.

Published data regarding HDL-C also vary conspicuously between 1.11 mmol/l (Ferramosca et al. 2012) and 0.17 mmol/l (Popovic et al. 2011), with the level found in the present experiment (0.35 mmol/l) approximately in the middle.

Taking into account possible methodical incompatibilities of the published papers (direct determination of all cholesterol fractions using preparative ultracentrifugation or calculation of the LDL-C fraction; recalibration of the commercial apparatuses usually designed for determination of human samples for the rat plasma samples or lack of thereof), more important than the comparison of the absolute values are likely the differences between plasma markers of the FO-fed rats and the control rats found within a given experiment.

Similarly to the present study (Figure 2), FO in the rat diet decreased plasma TC also in most of the recent studies (Lu et al. 2011; Takahashi 2011; Campioli et al. 2012; Ferramosca et al. 2012; Xiao et al. 2012). However, Yamazaki et al. (2011) and Campioli et al. (2012) did not find differences in TC between the FO-fed rats and the control rats. A possible reason in the case of the experiment of Yamazaki et al. (2011) was a low FO intake (1 g per kg of live weight and day); however, FO intake was only slightly higher in the present experiment (1.57 g per kg of live weight and day).

According to Konig et al. (2007), plasma cholesterol in rats is decreased due to the PPARα activation and ensuing SREBP-2 reduction, which leads to a decreased cholesterol synthesis; Sugiyama et al. (2008) mention a TC decrease in mice only in the presence of PPARα (in the wild-type mice, but not in the *PPARα* null mice).

Another explanation of the plasma cholesterol reduction in rats by FO is the possibility that FO facilitates in the liver cholesterol secretion into bile acids due to the up-regulation of the cholesterol transporters (Takahashi 2011). Sugiyama et al. (2008) concluded that apart from cholesterol biosynthesis, many other mechanisms participate in cholesterol homeostasis (bile acid synthesis, bile secretion) and the mechanism of the hypocholesterolemic effect of EPA is not fully understood (though it is different from fibrates).

Moreover, a TC decrease in some experiments of this type (n-3 PUFA-fed rats) need not be due to n-3 PUFA at all, but e.g. due to different qualities of micronutrients: addition of the cold-pressed flaxseed

oil to a rat diet decreased plasma TC in comparison with the control rats fed a diet with totally refined flaxseed oil in an experiment of Xiao et al. (2012).

Conclusions of Ramprasath et al. (2012) that PUFA-rich diets decrease plasma total cholesterol (in this case in humans) independently of changes in cholesterol absorption or synthesis are in agreement with our data, namely the FO tendency to up-regulate rat liver *Ldlr* and *Hmgcr* gene, respectively (Figure 1).

Because the method of LDL-C determination is not clear from most of other similar papers, only HDL cholesterol fraction is discussed.

In summary, the results of experiments evaluating an effect of FO on HDL-C in rodents are ambiguous. Both Yamazaki et al. (2011) and Campioli et al. (2012) did not find a difference in plasma HDL-C in the FO-fed and the control rats, similarly to our data. On the other hand, Popovic et al. (2011) and Xiao et al. (2012) reported an increase of this marker in the rat plasma after FO intake. Contrary to the above-mentioned results, Takahashi (2011) found a decrease of this parameter from 1.56 mmol/l (palm oil-fed rats) to 0.58 mmol/l (FO group). Similar conclusions reported also Kamisako et al. (2012) in mice fed a diet with FO in comparison with soybean oil-fed control: FO decreased plasma HDL-C from 1.50 to 0.56 mmol/l.

As far as an interspecies comparison is concerned, an increase of HDL-C due to EPA/DHA intake follows from human clinical studies (metaanalysis of Jacobson et al. 2012). Zhang et al. (2009) suggest in this context that hamsters are a better model than rats for studying plasma cholesterol-lowering effect of functional foods because they synthesize and excrete cholesterol (and bile acids) in a manner similar to humans.

CONCLUSION

Regarding the hypothesis tested in the present experiment (EPA+DHA increase expression of the *Insig-1* gene in the rat liver, which leads to suppression of the *Hmgcr* gene and *Ldlr* gene with a consequence of decreased plasma cholesterol), only the first and the last step was confirmed. Therefore, it can be concluded that the cholesterol lowering effect of fish oil is at least partly based on mechanisms other than tested here. However, the inconclusive results of the present study agree with often contradictory literature data discussing all steps of the putative signalling pathway from EPA/DHA to plasma cholesterol both in rodents and in humans, including the statement that mechanism of a hypocholesterolemic effect of EPA/DHA in rodents (and humans) has still not been fully understood.

REFERENCES

Arai T., Kim H.J., Chiba H., Matsumoto A. (2009a): Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. Journal of Atherosclerosis and Thrombosis, 16, 674–683.

Arai T., Kim H.J., Chiba H., Matsumoto A. (2009b): Interaction of fenofibrate and fish oil in relation to lipid metabolism in mice. Journal of Atherosclerosis and Thrombosis, 16, 283–291.

Botolin D., Wang Y., Christian B., Jump D.B. (2006): Docosahexaenoic acid (22:6, n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk- and 26S proteasome-dependent pathways. Journal of Lipid Research, 47, 181–192.

Campioli E., Rustichelli C., Avallone R. (2012): N-3 dietary supplementation and lipid metabolism: differences between vegetable- and fish-derived oils. Journal of Functional Foods, 4, 207–212.

Caputo M., Zirpoli H., Torino G., Tecce M. (2010): Selective regulation of UGT1A1 and SREBP-1c mRNA expression by docosahexaenoic, eicosapentaenoic, and arachidonic acids. Journal of Cellular Physiology, 226, 187–193.

Densupsoontorn N., Worgall T.S., Seo T., Hamai H., Deckelbaum J. (2007): Fatty acid supplied as triglyceride regulates SRE-mediated gene expression as efficiently as free fatty acids. Lipids, 42, 885–891.

Eslick G.D., Howe P.R.C., Smith C., Priest R., Bensoussan A. (2009): Benefits of fish oil supplementation in hyperlipidemia: a systematic review and meta-analysis. International Journal of Cardiology, 136, 4–16.

Fernandez-Alvarez A., Alvarez M.S., Gonzalez R., Cucarella C., Muntane J., Casado M. (2011): Human SREBP1c expression in liver is directly regulated by peroxisome proliferator-activated receptor α (PPARα). Journal of Biological Chemistry, 286, 21466–21477.

Ferramosca A., Conte L., Zara V. (2012): A krill oil supplemented diet reduces the activities of the mitochondrial tricarboxylate carrier and of the cytosolic lipogenic enzymes in rats. Journal of Animal Physiology and Animal Nutrition, 96, 295–306.

Givens D.I., Gibbs R.A. (2008): Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. Proceedings of the Nutrition Society, 67, 273–280.

Hirako S., Kim H.J., Arai T., Chiba H., Matsumoto A. (2010): Effect of concomitantly used fish oil and cholesterol on lipid metabolism. Journal of Nutritional Biochemistry, 21, 573–579.

Jacobson T.A., Glickstein S.B., Rowe J.D., Soni P.N. (2012): Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. Journal of Clinical Lipidology, 6, 5–18.

Jump D.B. (2008): N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. Current Opinion in Lipidology, 19, 242–247.

Kamisako T., Tanaka Y., Ikeda T., Yamamoto K., Ogawa H. (2012): Dietary fish oil regulates gene expression of cholesterol and bile acid transporters in mice. Hepatology Research, 42, 321–326.

Komprda T. (2012): Eicosapentaenoic and docosahexaenoic acids as inflammation-modulating and lipid homeostasis influencing nutraceuticals: a review. Journal of Functional Foods, 4, 25–38.

Komprda T., Zornikova G., Rozikova V., Borkovcova M., Przywarova A. (2013): The effect of dietary *Salvia hispanica* seed on the content of n-3 long-chain polyunsaturated fatty acids in tissues of selected animal species, including edible insects. Journal of Food Composition and Analysis, 32, 36–43.

Konig B., Koch A., Spielmann J., Hilgenfeld C., Stangl G.I., Eder K. (2007): Activation of PPARα lowers synthesis and concentration of cholesterol by reduction of nuclear SREBP-2. Biochemical Pharmacology, 73, 574–585.

Lecker J., Matthan N.R., Billheimer J.T., Rader D.J., Lichtenstein A.H. (2011): Changes in cholesterol homeostasis modify the response of F1B hamsters to dietary very long chain n-3 and n-6 polyunsaturated fatty acids. Lipids in Health and Disease, 10:186.

Lopez-Huertas E. (2009): Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies. Pharmacological Research, 61, 200–207.

Lu J., Borthwick F., Hassanali Z., Wang Y., Mangat R., Ruth M., Shi D., Jaeschke A., Russel J.C., Field C.J., Proctor S.D., Vine D.F. (2011): Chronic dietary n-3 PUFA intervention improves dislipidaemia and subsequent cardiovascular complications in the JSR:LA-cp rat model of the metabolic syndrome. British Journal of Nutrition, 105, 1572–1582.

Luci S., Konig B., Giemsa B., Huber S., Hause G., Kluge H., Stangl G.I., Eder K. (2007): Feeding of a deep-fried fat causes PPARα activation in the liver of pigs as a non-proliferating species. British Journal of Nutrition, 97, 872–882.

Maki K.C., Van Elswyk M.E., McCarthy D., Seeley M.A., Veith P.E., Hess S.P., Ingram K.A., Halvorson J.J., Calaguas E.M.,

Davidson M.H. (2003): Lipid responses in mildly hypertriglyceridemic men and women to consumption of docosahexaenoic acid-enriched eggs. International Journal for Vitamin and Nutrition Research, 73, 357–368.

Nakamura M.T., Cheon Y., Li Y., Nara T.Y. (2004): Mechanisms of regulation of gene expression by fatty acids. Lipids, 39, 1077–1083.

Popovic T., Borozan S., Arsic A., Debeljak-Martacic J., Vucic V., de Luka S., Milovanovic I., Trbovic A., Glibetic M. (2011): Effects of n-3 supplementation on plasma and liver phospholipid fatty acids profile in aged Wistar rats. Croatica Chemica Acta, 84, 73–79.

Ramprasath V.R., Jones P.J.H., Buckley D.D., Woollett L.A., Heubi J.E. (2012): Decreased plasma cholesterol concentrations after PUFA-rich diets are not due to reduced cholesterol absorption/synthesis. Lipids, 47, 1063–1071.

Sato R. (2010): Sterol metabolism and SREBP activation. Archives of Biochemistry and Biophysics, 501, 177–181.

Sugiyama E., Ishikawa Y., Li Y., Kagai T., Nobayashi M., Tanaka N., Kamijo Y., Yokoyama S., Hara A., Aoyama T. (2008): Eicosapentaenoic acid lowers plasma and liver cholesterol levels in the presence of peroxisome proliferators-activated receptor alpha. Life Sciences, 83, 19–28.

Takahashi Y. (2011): Soy protein and fish oil independently decrease serum lipid concentrations but interactively reduce hepatic enzymatic activity and gene expression involved in fatty acid synthesis in rats. Journal of Nutritional Science and Vitaminology, 57, 56–64.

Xiao Y., Qianchun G., Jiqu X., Fenghong G., Qingde H., Zhihua Y., Jine Y. (2012): Effects of cold-pressed and vitamin E-enriched flaxseed oils on lipid profile and antioxidant status in high-fat fed rats. European Journal of Lipid Science and Technology, 114, 461–468.

Yamazaki R.K., Brito G.A.P., Coelho I., Pequitto D.C.T., Yamaguchi A.A., Borghetti G., Schiessel D.L., Kryczyk M., Machado J., Rocha R.E.R., Aikawa J., Iagher F., Naliwako K., Tanhoffer R.A., Nunes N.A., Fernandes L.C. (2011): Low fish oil intake improves insulin sensitivity, lipid profile and muscle metabolism on insulin resistant MSG-obese rats. Lipids in Health and Disease, 10, 66.

Zhang Z., Wang H., Jiao R., Peng C., Wong Y.M., Yeung V.S.Y., Huang Y., Chen Z.-Y. (2009): Choosing hamsters but not rats as a model for studying plasma cholesterol-lowering activity of functional foods. Molecular Nutrition and Food Research, 53, 921–930.

Corresponding Author

Prof. MVDr. Ing. Tomáš Komprda, CSc., Mendel University in Brno, Department of Food Technology, Zemědělská 1, 613 00 Brno, Czech Republic

Phone: +420 545 133 261, e-mail: komprda@mendelu.cz