Praca oryginalna *Original paper*

Effect of dietary plant or animal fats on serum fatty acid profile and cholesterol and triacylglycerol content in rats

PIOTR HANCZAKOWSKI, BEATA SZYMCZYK, MONIKA FRYS-ŻUREK

Department of Animal Nutrition and Feed Science, National Research Institute of Animal Production, 32-083 Balice, Poland

Hanczakowski P., Szymczyk B., Frys-Żurek M.

Effect of dietary plant or animal fats on serum fatty acid profile and cholesterol and triacylglycerol content in rats

Summary

The effect of plant (linseed oil and olive oil) or animal fat (fish oil or beef tallow) on fatty acids, cholesterol and triacylglycerol content of rat blood was investigated. Four experimental groups, each comprised 6 rats weighing about 160 g at the beginning of the experiment were fed standard diets containing 100 per 1 kg of one of these fats. After 6 weeks rats were anaesthetized and blood was collected by cardiac puncture. It was found that differences in fatty acids content in rat blood especially in the case of saturated acids with a shorter chain (myristic and palmitic) were smaller than those in the dietary fats but the differences in unsaturated fatty acids also decreased. The lowest levels of cholesterol and triacylglycerol were found in the blood of rats fed with linseed oil.

Keywords: fatty acids, cholesterol, triacylglycerol, rats

It is known that coronary heart disease is connected with the kind and amount of consumed fat (16). The major risk factors are high serum triacylglycerol and cholesterol levels (2, 14). Both are largely food/feed dependent and fat and its fatty acid pattern seem to be one of the most important factors (3). It is still not exactly known how particular fatty acids affect lipids content of blood.

According to Grimsgaard et al. (7) blood concentrations of fatty acids rather than their dietary intake should be used to examine the relationship between individual fatty acids and serum lipids. According to Ding et al. (5) the fatty acid composition of plasma reflected the composition of the diet to a large extent, but also reflected *de novo* fatty acids synthesis. Also Rioux et al. (13) are of the opinion that saturated fatty acids (mainly myristic acid) may participate in the regulation of highly unsaturated fatty acid biosynthesis and metabolism.

In our earlier experiments (8) the authors found that the total cholesterol content in the blood of rats receiving pure linseed oil was lower than that of rats fed with pure olive oil but the fatty acids content in their blood was not estimated.

The aim of this study was to determine the fatty acid content of blood of rats fed with plant or animal fats and their effect on the cholesterol and triacylglycerol levels in blood.

Material and methods

Diets. Four experimental diets were prepared. In all diets soy protein isolate (200 g kg^{-1}) was the only protein source. Diets also contained (g kg^{-1}): saccharose (200), maize starch (400) cellulose (40), mineral (40) and vitamin (20) mixtures, both according to Eggum (6). Group I received linseed oil, group II virgin olive oil and groups III and IV fish oil or beef tallow, respectively. Experimental diets contained 100 g of each fat for 1 kg of feed.

Rats. Four groups of 50-day-old male albino rats, each weighing about 160 g at the beginning of the experiment, were kept individually in plastic cages and had free access to feed and water. Each group comprised six animals. Body mass was measured at the beginning and the end of the experiment and feed consumption was measured daily.

Blood sampling. After the 6 weeks of the experimental period was completed the rats were fasted overnight (12 h) and anaesthetized with thiopental (Biochemie GmbH), Vienna). Blood was collected by cardiac puncture and serum samples were separated by low-speed centrifugation (1500 g for 15 min).

Chemical analyses. Fatty acids were analyzed as methyl esters in a 25 mm id × 50 m long fused silica SP 2330 capillary column (Supelco Inc., Bellefonte, USA) using

a Hewlett-Packard gas chromatograph model 5890 equipped with a flame ionizator detector. Total cholesterol (TC) content of blood serum was analyzed enzymatically according to Allain et al. (1). The serum triacylglycerol (TAG) content was estimated according to Mc Gowan et al. (12).

Statistical analysis. Statistical analysis of treatment effects was concluded by analysis of variance with comparison of means by Duncan's multiple range test at $P < 0.05$ and $P < 0.01$ levels of significance using the Statistica v 5.1 package.

Results and discussion

Fatty acid profile of fats used in the experiment differed distinctly (tab. 1). Plant oils contained more unsaturated fatty acids, especially linoleic acid and oleic acid. Animal fats contained more saturated acids, especially myristic and palmitic acids. Both fats of animal origin also contained a relatively high amount of monounsaturated oleic acid.

Differences in fatty acids content in rat blood especially in the case of saturated acids with a shorter carbon chain, i.e. myristic and palmitic ones, were smaller than those in the dietary fats (tab. 2). For instance, the content of myristic acid in dietary fats varied considerably, especially in olive and fish oils, but in the blood of rats receiving these fats the difference in its content was small. Differences in polyunsaturated acids content also decreased distinctly. Dietary linseed oil contained much more linoleic acid than fish oil acid and fish oil while its content in rat blood of relevant groups increased even though the difference was still significant.

These results suggest that the content of particular fatty acids in blood is not a plain reflection of fatty acid pattern of dietary fat. Similar results were obtained by Kloareg et al. (10) in an experiment on pigs. Those authors found that average composition of *de novo* synthesized fatty acids corresponded to 30.3 and 45.9 in the case of palmitic and oleic acids, respectively. In the experiment of Ding et al. (5) palmitic acid was the primary product of *de novo* synthesis and the proportion of arachidonic acid ($C_{20:4}$) was also high, probably reflecting chain elongation and desaturation. Also in this experiment, a high proportion of palmitic acid especially in rats fed with plant oils and a high level of arachidonic acid in blood were found. The significantly increased level of palmitic acid in tissues of rats fed with a different amount of myristic acid was also found by Rioux et al. (13). These results suggest that palmitic acid plays a special role in fatty acids metabolism.

According to Legrand (11) Δ6 desaturaze (a key enzyme in the biosynthesis of polyunsaturated fatty acids) is activated by myristic acid, possibly through an acylation mechanism or a gene induction. This shows that saturated fatty acids are implicated in long chain polyunsaturated fatty acids biosynthesis, suggesting that there is a functional complementarity of all dietary fatty acids. He also considers that there is a possibility of biosynthesis of essential fatty acids in animal cells when submitted to a strong lipid deficien-

Tab. 1. Fatty acid composition (% of total FA) of different fats in experimental diets for rats

Fatty acid	Dietary fat			
	Linseed oil	Olive oil	Fish oil	Beef tallow
C8:0	0.20	0.16	0.17	0.02
C12:0	0.04	0.01	0.15	0.07
C14:0	0.23	0.00	10.94	3.25
C16:0	10.59	11.44	33.81	25.89
C16:1	0.10	0.36	4.96	1.35
C18:0	5.76	4.07	4.62	25.18
C18:1	18.04	74.13	35.86	39.47
C18:2	63.25	7.79	1.46	2.63
C18:3	0.59	0.08	0.05	0.19
C20:0	0.19	0.77	0.39	0.39
C20:5	1.63	0.64	4.47	0.19
C22:6	0.36	0.49	5.07	0.20
SFA*	17.01	16.46	46.69	54.80
MUFA	18.14	74.49	40.82	40.82
PUFA	64.20	9.00	11.05	3.02

Explanations: *SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

Tab. 2. Serum fatty acids content in rats (% of total FA) fed diets with different sources of fat

Fatty acid	Dietary fat				SEM
	Linseed oil	Olive oil	Fish oil	Beef tallow	
C10:0	0.71	0.66	0.62	0.64	0.080
C12:0	0.78	0.58	0.83	0.62	0.110
C14:0	2.56	2.15	4.40	3.24	0.350
C16:0	24.44	24.12	28.18	26.14	0.408
C16:1	1.69^{A}	2.07^{A}	8.25^{B}	3.82^{A}	0.460
C18:0	10.14^{B}	8.29^{A}	7.96^{A}	14.68^{B}	0.270
C18:1	10.50^{A}	31.82^{C}	21.05^{C}	22.08^{C}	0.788
C18:2	18.05^{B}	10.27^{A}	11.43^{A}	11.45^{A}	0.539
C18:3	0.78^{C}	0.24^{A}	0.54^{B}	0.68^{C}	0.097
C20:4	27.70^{B}	16.75^{A}	11.30^{A}	12.24^{A}	0.732
C20:5	0.06^{A}	0.10^{A}	0.51^{B}	0.16^{A}	0.087
C22:6	1.50^{A}	1.43^{A}	3.51^{B}	1.32^{A}	0.266
SFA*	39.24	36.67	42.01	45.32	0.581
MUFA	12.33^{A}	34.00^{C}	29.31^{B}	25.90^{B}	0.815
PUFA	48.43^{B}	29.32^{A}	28.67^{A}	25.85^{A}	0.866

Explanations: *SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; A, B, C – Values in rows with different letters differ significantly ($P < 0.01$); NS ($P \geq 0.05$)

Tab. 3. Serum lipid profile in rats fed diets with different sources of fat

Lipid fraction	Dietary fat				SEM
	Linseed oil	Olive oil	Fish oil	Beef tallow	
Total cholesterol - TC (mg/dl)	56.3^{A}	90.2^{B}	91.8^{B}	94.6^{B}	0.849
HDL - C (mg/dl)	36.0^{A}	56.2^{B}	58.3^{B}	58.2^{B}	0.681
LDL - C (mg/dl)	20.3^{A}	38.4^{B}	33.5^{B}	36.5^{B}	0.620
HDL:TC	0.639	0.622	0.634	0.614	0.040
Triacylglycerol (TAG)	42.7^{A}	66.8^{A}	67.4^{A}	145^{B}	1.350

Explanations: A, B – Values in rows with different letters differ significantly ($P < 0.01$); NS ($P \geq 0.05$)

cy. Considering the different fatty acids content of diets used in this experiment and differences in fatty acid patterns and lipid content of rat blood it difficult to say if dietary or blood content of particular fatty acids determined cholesterol and triacylglycerol content in the blood.

Cholesterol content was lowest in the blood of rats fed with linseed oil and there was no difference between the remaining groups (tab. 3). Triacylglycerol level was also lowest in linseed oil group though the difference between this group and groups receiving olive oil or fish oil was insignificant at $P < 0.01$. Its content in the blood of rats receiving beef tallow was high.

According to the early work of Hegsted et al. (9) dietary myristic acid is a most hypercholesterolemic fatty acid. In this experiment fish oil contained the highest amount of this acid but it had no hypercholesterolemic activity. In our earlier experiment on rats (unpublished) we also found cholesterolemic activity of myristic acid, but only pure, saturated fatty acids were used as the only fat sources, so its results can hardly be compared to these obtained with natural oils also containing unsaturated acids. According to Salter et al. (15) palmitic rather than myristic or stearic acids is hypercholesterolemic. However, in this experiment fish oil also contained the highest amount of this acid but cholesterol content in the blood of rats fed with this oil was comparable to that of other groups except those receiving linseed oil.

Linseed oil caused the lowest level of cholesterol and triacyglycerol in rats' blood (though in this last case the difference was insignificant). This was probably due to the high content of polyunsaturated linoleic acid in this oil. The hypocholesterolemic activity of polyunsaturated fatty acid was also found by Cintra et al. (3) and Grimsgaard et al. (7). The extremely high content of triacylglycerol in the blood of rats fed with beef tallow is hard to explain. Though this fat contained a high amount of saturated stearic acid this acid according to Cowles et al. (4) is unique among saturated fatty acids because it had no hyperlipidemic activity. On the other hand Zock and Katan (17) showed that serum trigliceride concentration in humans increased significantly on a stearic acid enriched diet, but they compared it with a diet containing unsaturated linoleic acid which also in our experiment proved to lower the triacylglycerol content in blood.

On the basis of this experiment it can be concluded that the fatty acids content of rat blood is associated with that of dietary fat, though the content of some acids is leveled by the rats metabolism. A high level of monounsaturated oleic acid in dietary fat or in blood does not decrease the cholesterol content in blood.

References

1. *Allain C. C., Poon L. S., Chan C. S., Richmond W., Fu P. C.*: Enzymatic determination of total serum cholesterol. Clin. Chem. 1974, 20, 470-474.
2. *Aznaouridis K., Vlachopoulos C., Dima I., Ioakeimidis N., Stefanadis C.*: Triglyceride level is associated with wave reflections and arterial stiffness in apparently healthy middle-aged men. Heart 2007, 93, 613-614.
3. *Cintra D. E., Costa A. V., Peluzio Mdo C., Matta S. L., Silva M. T., Costa N. M.*: Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout, or chicken skin. Nutrition 2006, 22, 197-205.
4. *Cowles R. L., Lee J.-Y., Gallaher D. D., Stuefer-Powell C. L., Carr T. P.*: Dietary stearic acid alters gallbladder bile acid composition in hamsters fed cereal-based diets. J. Nutr. 2002, 132, 3119-3122.
5. *Ding S. T., Lapillonne A., Heird W. C., Mersmann H. J.*: Dietary fat has minimal effects on fatty acid metabolism transcript concentrations in pigs. J. Anim. Sci. 2003, 81, 423-431.
6. *Eggum B. O.*: A study of certain factors influencing protein utilization in rats and pigs. Beret. Forsoegslab. Statens. Husdyrbrugsudvalg 1973, 406, 17-30.
7. *Grimsgaard S., Břnaa K. H., Bjerve K. S.*: Fatty acid chain length and degree of unsaturation are inversely associated with serum triglicerides. Lipids 2000, 35, 1185-1193.
8. *Hanczakowski P., Szymczyk B.*: The effect of olive or linseed oils supplemented with pure saturated fatty acids on serum cholesterol levels in the rat. J. Anim. Feed Sci. 2006, 15, 287-294.
9. *Hegsted D. M., McGandy R. B., Myers M. L., Stare F. J.*: Quantitative effects of dietary fats on serum cholesterol in man. Am. J. Clin. Nutr. 1965, 17, 281--295.
10. *Kloareg M., Le Bellego L., Mourot J., Noblet J., Milgen J.*: Deposition of dietary fatty acids and de novo synthesized fatty acids in growing pigs: effects of high ambient temperature and feeding restriction. Br. J. Nutr. 2005, 93, 803-811.
11. *Legrand P.*: Recent knowledges on saturated fatty acids; a better understanding. Sciences des Aliments 2002, 22, 351-354.
12. *Mc Gowan M. W., Artiss J. D., Strandbergh D. R., Zak B.*: A peroxidaze--coupled-method for the colorimetric determination of serum triglicerides. Clin. Chem. 1983, 29, 538-542.
13. *Rioux V., Catheline D., Bouriel M., Legrand P.*: Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat. Reprod. Nutr. Dev. 2005, 45, 599-612.
14. *Rudkowska I., Jones P. J.*: Functional foods for the prevention and treatment of cardiovascular disease: cholesterol and beyond. Expert. Rev. Cardiovasc. Ther. 2007, 5, 477-490.
15. *Salter A. M., Mangiapane E. H., Bennett A. J., Bruce J. S., Billett M. A., Anderton K. L., Marenach C. B., Lawson N., White D. A.*: The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. Br. J. Nutr. 1998, 79, 195-202.
16. *Wijendran V., Hayes K. C.*: Dietary n-6 and n-3 fatty acid balance and cardiovascular health. Ann. Rev. Nutr. 2004, 24, 597-615.
17. *Zock P. L., Katan M. B.*: Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J. Lipid Res. 1992, 33, 399-410.

Adres autora: prof. dr hab. Piotr Hanczakowski, ul. Na Polach 27/6, 31-344 Kraków