

Effect of different dietary fats on the content of fatty acids in the the blood serum and in the *longissimus dorsi* muscle of rats

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ABSTRACT

Fourty eight rats divided into 6 groups were fed semi-purified mixtures with different kind of fats (6%); C – control group, T – tallow, L – lard, RO – rape seed oil, LO – linseed oil, FO – fish oil. The percentage content of fatty acids in the serum and in the *longissimus dorsi* muscle (l.d.m.) of rats was measured.

The dietary fat did not influence the percentage content of saturated fatty acids (SFA) in the rat's serum. Increasing the percentage content of polyunsaturated fatty acids (PUFA) in serum and muscle at the expense of monounsaturated fatty acids (MUFA) was observed in rats receiving linseed oil. Feeding linseed oil and fish oil increased the content of PUFA_{n-3} in serum and muscle. In contrast with serum, in the l.d.m. of rats a negative correlation between PUFA_{n-3} and _{n-6} was not found.

KEY WORDS: fatty acids, fats, linseed oil, fish oil, serum, *longissimus dorsi* muscle, rats

INTRODUCTION

Polyunsaturated fatty acids (PUFA), especially the n-3 series, belong to one of the most deficient compounds of human and animal diet (Keys et al., 1986; Dolecek et al., 1992). Recent studies have demonstrated that increased intake of PUFA leads to an increased incorporation of these fatty acids into the plasma lipids and tissues (Broughton et al., 1991; Vidgren et al., 1997). Dietary fats, however, are not only the source of beneficial fatty acids, but also the source of undesirable

saturated fatty acids (Mattson and Grundy, 1985). The accumulation of optimal fatty acids mainly depends on the dietary intake of these acids and the synthesis of endogenous fatty acids, as well as the relationships between incorporating particular groups or series of fatty acids into particular tissues. Thus, quality and quantity, as well as the combination of supplemented fatty acids seem to be important. The present study was carried out on rats to examine the effects of diets containing different fats on the serum and the *longissimus dorsi* muscle groups and series of fatty acids.

MATERIAL AND METHODS

Animals

Fourty eight male rats Wistar were divided into 6 groups of 8. The average body weight of rats was 87 g (82-95.5 g) at the beginning and 178 g (166.4-215.5 g) at the end of the experiment. Rats were kept in individual standard cages for 36 days. For blood collection rats were anaesthetized using ketamine at a dose of 50 mg/kg body weight intramuscularly after 12 h fasting. After opening the abdominal cavity the blood was collected from *vena cava abdominalis* and serum prepared by centrifugation at 1000 x g. Rats were euthanasized by an overdose of ketamine and samples of *longissimus dorsi musculus* (l.d.m.) were taken for analysis.

Feeding

Rats received semi-purified mixtures, balanced according to the NRC (1978); the chemical composition of the mixtures is shown in Table 1. The diet consisted of (in %): casein, 17; sucrose, 10; wheat starch, 57.86 (62.86, in the control group C); cellulose, 4; DL - methionine, 0.14; vitamins, 2; minerals, 3 supplemented with 6% tallow, group T; lard, group L; rape seed oil, group RO; linseed oil, group LO; fish oil, group FO, supplemented with vitamin and mineral mixtures. The vitamin mixture contained (mg in kg): vit.A, 20000 i.u.; vit. D₃, 2000 i.u.; vit.E, 100 i.u.; vit. K, 5; choline, 200; p-amino benzoic acid, 100; inositol, 100; niacin, 40; riboflavin, 8; thiamine, 5; pyridoxine, 5; folic acid, 2; biotin, 0.4; cyanocobalamin, 0.03; the mineral mixtures contained (g in kg): CaHPO₄, 735.0; K₂HPO₄, 81.0; K₂SO₄, 68; NaCl, 30.6; CaCO₃, 21.0; Na₂HPO₄, 21.4; MgO, 25.0; ferric citrate, 5.58; ZnCO₃, 30.81; MnCO₃, 4.21; CuCO₃, 0.23; KJ, 0.01; citric acid, 7.06.

The percentage content of fatty acids in diets is shown in Table 2. The diet for the control group was supplemented with 1% of soyabean oil to prevent deficiency of essential fatty acids in rats.

TABLE 1

Chemical composition of diets, %

Items	Groups					
	control	tallow	lard	rape seed oil	linseed oil	fish oil
Dry matter	86.71	87.24	87.70	86.30	87.02	87.51
Crude protein	15.49	15.20	15.45	15.56	15.51	15.33
Ether extract	1.05	5.55	5.76	5.71	5.90	6.00
Crude fibre	2.75	3.02	2.4	2.79	3.12	2.69
N-free extractives	64.38	60.88	61.1	60.02	59.75	60.75
Ash	3.04	2.59	2.99	2.22	2.74	2.74

TABLE 2

Content of fatty acids in fat of diet, %

Fatty acid	Groups					
	soyabean oil (control)	tallow	lard	rape seed oil	linseed oil	fish oil
14:0	1.2	3.3	1.4	0.1	0.1	5.3
15:0		0.7	0.1			0.5
16:0	11.8	28.1	23.7	5.0	5.6	14.2
16:1 _{n-7}	0.1	3.7	2.9	0.3	0.1	6.9
17:0		1.5	0.4			
17:1 _{n-8}		0.4	0.4			
18:0	4.2	27.4	12.4	1.7	5.9	3.3
18:1 _{n-7}	0.6	1.2	3.2	3.4	0.6	3.8
18:1 _{n-9}	22.8	29.9	44.6	53.3	22.0	23.2
18:2 _{n-6}	52.6	1.9	7.5	21.4	16.4	7.2
18:3 _{n-3}	6.6	0.5	0.7	9.4	48.8	3.3
18:3 _{n-6}						0.2
20:0		0.4	0.2	0.6	0.2	0.3
20:1 _{n-9}	0.1		1.2	2.1	0.2	1.5
20:2 _{n-6}			0.4	0.1		0.2
20:4 _{n-6}			0.2			
20:5 _{n-3}				0.3		14.2
22:0						1.0
22:1 _{n-9}				2.0		0.8
22:5 _{n-3}			0.1			2.4
22:6 _{n-3}			0.1			9.5
24:0				0.1	0.1	0.1
24:1 _{n-9}				0.2		1.6

Analytical methods

Fatty acids: a sample (1g) of tissue from *longissimus dorsi* was extracted with methanol and chloroform (2:1) according to Folsh et al. (1957). Methyl esters were prepared by estrification with 12-% boron-trifluoride and extraction with *iso*-octane. The methyl esters were analysed in a HP 5890 gas chromatograph (SGE Inc. Austin), using a BPX 70-50 m x 0.22 mm x 0.25 mm column with FID detector, injection temperatures of 220°C, column programmable temperature (140-212°C). Helium was applied to determine fatty acids. Individual fatty acid peaks were identified in comparison with known reference esters.

Fatty acids were divided into groups: SFA = 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0; MUFA = 16:1_{n-7} + 17:1_{n-8} + 18:1_{n-7} + 18:1_{n-9} + 20:1_{n-9} + 22:1_{n-9} + 24:1_{n-9}; PUFA = PUFA_{n-6} + PUFA_{n-3} and series: MUFA_{n-9} = 18:1_{n-9} + 20:1_{n-9} + 22:1_{n-9} + 24:1_{n-9}; PUFA_{n-6} = 18:2_{n-6} + 18:3_{n-6} + 20:2_{n-6} + 20:4_{n-6} + 22:4_{n-6}; PUFA_{n-3} = 18:3_{n-3} + 20:5_{n-3} + 22:5_{n-3} + 22:6_{n-3}.

Dry matter, crude protein (N x 6.25), ether extract and crude fibre of the diets were determined as described by AOAC (1990) using Tecator equipment.

Statistical analysis

The data were analysed by one-way analysis of variance ANOVA and correlation analysis using Statgraphics 6.0 Plus software.

RESULTS

The percentage of SFA in rat serum (Table 3) did not change in response to the highly diverse supply of these acids in the diets (from 0.0187 g/day per animal in the control group to 0.6309 g/day per animal in the group receiving tallow). In the l.d.m., however, a significantly smaller $P < 0.05$ content of these acids was found in rats fed a diet containing rape seed and linseed oils. These fats contain a smaller amount of SFA than the other sources of fat used in this study.

The fats used in this experiment led to differences in the percentage content of MUFA in rat serum (Table 3). A significantly lower $P < 0.01$ percentage of MUFA in the serum of rats receiving linseed and fish oils in their diets was found than, in particular, those fed tallow, lard or rape seed oil. The MUFA content in the l.d.m. was proportional to the amount of these fats in the diets. The lowest percentage of MUFA was characteristic of the l.d.m. of rats fed a diet with linseed oil.

The addition of linseed oil and fish oil led to a rise in the percentage content of PUFA in the serum of the experimental animals (Table 3), whereas in the muscle tissue, the highest level of PUFA was observed in the animals receiving linseed

TABLE 3

Content of fatty acids in fat of diet, blood serum and *longissimus dorsi* muscle of rats, %

Fatty acids	Samples	Feeding groups						ANOVA
		I 0	II Tal.	III Lard	IV R.O.	V L.O.	VI F.O.	
SFA	Feed		61.5	38.1	7.4	11.9	24.7	
	Serum	40.5	37.1	38.3	37.0	38.0	40.2	NS
	Muscle	35.1 ^{ab}	37.0 ^b	37.3 ^b	33.6 ^a	32.3 ^a	36.4 ^{ab}	P=0.0127
MUFA	Feed		35.2	52.3	61.1	22.9	37.1	
	Serum	26.3 ^{ab}	33.3 ^b	35.4 ^b	32.4 ^b	21.7 ^a	21.2 ^a	P=0.0000
	Muscle	39.7 ^{ab}	40.3 ^{ab}	44.5 ^b	45.0 ^b	37.4 ^a	39.2 ^{ab}	P=0.0127
PUFA	Feed		2.4	9.1	31.2	65.2	37.1	
	Serum	33.2 ^d	29.6 ^a	25.9 ^{ac}	30.6 ^a	40.3 ^{bc}	38.5 ^{bc}	P=0.0044
	Muscle	25.2 ^{ab}	22.7 ^a	18.2 ^a	21.2 ^a	29.2 ^b	24.4 ^{ab}	P=0.0015
P/S	Feed		0.1	0.2	4.2	5.5	1.5	
	Serum	0.9	0.8	0.7	0.8	1.09	1.01	NS
	Muscle	0.7 ^{ab}	0.6 ^a	0.5 ^a	0.6 ^a	0.9 ^b	0.7 ^{ab}	P=0.0002

a, b, c, d - P<0.05

oil, while the addition of tallow, lard as well as rape seed oil significantly ($P<0.01$) decreased the PUFA content of the l.d.m. The large difference between the PUFA content of rape seed oil and tallow did not lead to any differences in the PUFA content in the tissues of rats in both of these groups.

The highest percentage content of n-6 fatty acids (Table 4) was found in the serum and muscles of rats in the control group, but it should be stated that the fats used in this study were not a rich source of PUFA_{n-6}. The fish oil used in this experiment and, to a lesser degree, the linseed oil, lowered the percentage content of PUFA_{n-6} in the l.d.m. and serum.

A markedly increased percentage content of PUFA_{n-3} in the serum and l.d.m. of rats receiving linseed and fish oils in the experiment was noted. Similar tendencies were observed for the PUFA_{n-3}/PUFA_{n-6} ratio. It may be worth noting that linseed oil had higher than fish oil PUFA_{n-3} content, while the PUFA_{n-3} content in the serum of rats receiving fish oil was significantly higher in comparison with the content of these acids in the serum of rats fed linseed oil. In muscle tissue, however, the opposite tendency was noted.

DISCUSSION

In the reported experiment a stable percentage content of SFA in the serum and differentiated SFA content in the l.d.m. in response to different sources of fat in the diets of rats were observed.

TABLE 4

Content of fatty acids in fat of diet, blood serum and *longissimus dorsi* muscle of rats, %

Fatty acids	Samples	Feeding groups						ANOVA
		I 0	II Tal.	III Lard	IV R.O.	V L.O.	VI F.O.	
MUFA _{n-9}	Feed		29.9	45.8	57.4	22.2	27.1	
	Serum	22.8 ^a	29.1 ^b	31.5 ^b	28.8 ^b	19.4 ^a	21.0 ^a	P=0.0000
	Muscle	34.9 ^a	36.3 ^a	35.9 ^a	40.9 ^b	34.8 ^a	34.8 ^a	P=0.0188
PUFA _{n-6}	Feed		1.9	8.1	21.5	16.4	7.7	
	Serum	30.6 ^{bc}	28.1 ^b	26.3 ^{ab}	24.9 ^{ab}	23.8 ^{ab}	18.3 ^a	P=0.0008
	Muscle	21.0 ^b	16.4 ^{ab}	17.4 ^{ab}	15.8 ^c	14.2 ^a	12.2 ^a	P=0.0006
PUFA _{n-3}	Feed		0.5	1	9.7	48.8	29.4	
	Serum	1.2 ^a	2.6 ^a	2.0 ^a	5.8 ^{ab}	16.1 ^b	20.3 ^{bc}	P=0.0000
	Muscle	4.3 ^a	6.3 ^a	3.6 ^a	6.0 ^a	14.6 ^b	10.9 ^b	P=0.0000
n-3/n-6	Feed		0.3	0.1	0.5	3.0	3.8	
	Serum	0.1 ^a	0.1 ^a	0.1 ^a	0.2 ^{ab}	0.7 ^b	1.1 ^{bc}	P=0.0000
	Muscle	0.2 ^a	0.3 ^a	0.2 ^a	0.4 ^a	1.1 ^b	1.0 ^b	P=0.0000

a, b, c, d – P<0.05

The effects of quantitative and qualitative modifications of the fatty acid contents in tissues are undoubtedly related to the content of these acids in the diet. On the other hand, the incorporation of fatty acids into the body's lipids may be limited by the structure of the tissue being modified or the composition of the body fluids. It seems that the percentage share of phospholipids, cholesterol esters, free fatty acids, particularly triacylglycerols, in the given lipid structure may be decisive about its susceptibility to modification. Phospholipids show a considerable tendency towards binding saturated fatty acids at the sn-1 glycerol carbon, and unsaturated fatty acids (MUFA and PUFA) at the sn-2 carbon (Brockerhoff, 1967; Edwards-Webb and Gur, 1988; Clamp et al., 1997). It may be expected that those serum lipids that contain more than 35% phospholipids (Ziemlański and Topolowska, 1991) may show considerable resistance to attempts to decrease the percentage content of SFA. In the studies of some authors on various species, the SFA content of phospholipids was not found to decrease under the influence of diets enriched with PUFA (Ahn et al., 1996; Gachet, 1996; Vidrgen et al., 1997). According to others (Arbuckle et al., 1991; Calviello et al., 1997), the addition of fish oil lowered the percentage of SFA in phospholipids and cell membranes. In studies on pigs, Warnants et al. (1996) observed that the fatty acid content of feeds had a greater effect on changing the content of fatty acids in nonpolar lipids than in polar species.

In addition to lipids that are part of membrane structures and contain predominantly phospholipids, muscle tissue mainly accumulates triacylglycerols as the

energy source for the body. Triacylglycerols that are deposited in intramuscular fat in the form of droplets in myocytes as well as adipocytes (Brouns and van der Vusse, 1998) are able to rather freely (in a less restricted manner than phospholipids) accumulate fatty acids. To a certain extent, this may explain the greater effect of dietary fatty acids on the percentage of these acids in muscles, as has already been observed by other authors (Cara et al., 1997; Lauridsen et al., 1997; Onibi et al., 1998). Triacylglycerols, however, also have certain tendencies to bind fatty acids from the SFA, MUFA, and PUFA groups at successive carbon atoms of the glycerol moiety, a trait that will hinder the process of modifying tissues in the direction of a considerable decrease in their SFA content.

In the present experiment, the observed tendency towards enriching serum and l.d.m. lipids in PUFA in response to the inclusion of linseed oil and, to a lesser extent, fish oil, was most likely a result of replacing MUFA by PUFA. Similarly as in this paper (Table 5), Fremont (1995) also observed a negative correlation between PUFA and MUFA. The synthesis of endogenous monounsaturated fatty acids classified as MUFA increases under a deficit of PUFA. The activity of Δ -9 desaturase, an enzyme that controls the synthesis of MUFA, may be regulated through the level of PUFA. Therefore, the inclusion of linseed oil in the diet, as well as fish oil, may have limited the synthesis of MUFA and created favourable conditions for the enrichment of tissues in PUFA. In studies on pigs, Morgan (1991) and Irie and Sakimoto (1992) observed similar tendencies in response to feeding fish oil.

In comparing the MUFA and MUFA_{n-9} contents in serum, it can be seen that including linseed oil and fish oil in the diets of rats decreased the MUFA content of the acids that were not in the n-9 series. The authors' unpublished results indicate that this process most likely entails a reduction in the synthesis of C18:1_{n-7}, and to a lesser extent, also of C18:1_{n-9}.

The similar percentage content of PUFA in the serum and l.d.m. of rats receiving tallow and rape seed oil may point to the fact that decreasing the degree of enriching tissues in MUFA as the result of the presence of PUFA is accomplished rather through inhibition of Δ -9 desaturase, not through the preferential accumulation of PUFA over MUFA resulting from dietary supply.

Polyunsaturated fatty acids may belong to two series: PUFA_{n-6} and PUFA_{n-3}. These acids may compete for sites in specific lipid compounds of particular tissues or body fluids, with different acids showing greater affinity to particular structures. In this experiment, linseed oil and fish oil had a significant effect on enriching lipids with PUFA_{n-3}; these observations corroborate the results obtained in various species (Ruiter et al., 1978; Froyland, 1992; Calviello et al., 1997; Lechowski et al., 1998). However, the incorporation of PUFA_{n-3} into blood lipids was significantly higher when fish oil was used in comparison with linseed oil. Several reasons for this can be envisaged. Limiting the enrichment of serum lipids in PUFA_{n-3} may be

TABLE 5

Correlations between content (%) of groups and series fatty acids in serum and in *longissimus dorsi* muscle in rats (n = 48)

In serum		In muscle	
fatty acids	coefficient of correlation	fatty acids	coefficient of correlation
SFA:MUFA	NS	SFA:MUFA	-0.283*
SFA:PUFA	-0.526**	SFA:PUFA	NS
MUFA:PUFA	-0.846**	MUFA:PUFA	-0.914**
n-9:n-6	-0.398**	n-9:n-6	-0.721**
n-9:n-3	-0.422**	n-9:n-3	-0.295**
n-3:n-6	-0.399**	n-3:n-6	NS

related to the greater supply of PUFA_{n-6} in linseed oil than in fish oil. The negative correlation between PUFA_{n-6} and PUFA_{n-3} in serum (Table 5), also observed by other authors (Foote et al., 1990; Hrboticky et al., 1991), would point to the dominant position of the feed-derived PUFA_{n-6} over PUFA_{n-3} in the process of creating some lipid structures in serum. Another important reason for this effect may be content of long-chain PUFA in fish oil. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids show specific affinity towards specific lipid structures. EPA easily forms esters with cholesterol, while DHA is incorporated into the phospholipids of membranes (Bauer et al., 1997) which, albeit to a small degree, can lead to a rise in serum levels of PUFA_{n-3} (EPA). Moreover, long-chain PUFA_{n-3} may limit the synthesis of arachidonic acid, which potentially could increase the pool of PUFA_{n-6} (Philbrick et al., 1987; Swanson et al., 1988) by inhibiting the activity of Δ -6 desaturase (Raz et al., 1998) and probably Δ -5 desaturase (Horrobin, 1993). PUFA_{n-3} inhibit the desaturation of PUFA_{n-6} more effectively than the converse relationship (Luostarinen, 1995).

In this experiment it was found that only in the case of rats receiving linseed oil was the PUFA_{n-3}/PUFA_{n-6} ratio higher in the l.d.m. than in serum. At the same time, no significant correlation was observed between tissue levels of PUFA_{n-3} and PUFA_{n-6}. Also, a tendency was observed in the direction of lower PUFA, PUFA_{n-3}, PUFA_{n-6} contents in the l.d.m. of rats receiving fish oil in comparison with linseed oil. There exist the chance, then, that by inhibition the activity of desaturases, long chain PUFA_{n-3} limit the synthesis of arachidonic acid (PUFA_{n-6}), as well as the over-eighteen carbon fatty acids from the n-3 series to which they belong.

CONCLUSIONS

It may be stated that the addition of 6% of various fats to the diets of rats did not affect percentage content of SFA in the lipid fraction of serum. The addition of

linseed oil and fish oil favoured the enrichment of serum lipids in PUFA, as well as limited the synthesis of monounsaturated fatty acids belonging probably to the n-7 series. The addition of fish oil to the diet very effectively enriched serum lipids in PUFA_{n-3}. However, tendencies were found that pointed to the fact that dietary linseed oil facilitates the incorporation of PUFA_{n-3} into the lipids of the l.d.m. to a greater degree than does fish oil, particularly, that a negative correlation was not found between the percentage content of PUFA_{n-3} and PUFA_{n-6} in muscle tissue.

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STRESZCZENIE

Wpływ różnych tłuszczów w diecie na zawartość kwasów tłuszczowych w surowicy krwi i mięśniu najdłuższym grzbietu szczurów

Czterdzieści osiem szczurów (Wistar), podzielonych na 6 grup, żywiono półsyntetyczną mieszanką z udziałem (6%) różnych rodzajów tłuszczu; I – grupa kontrolna, II – łój wołowy, III – smalec, IV – olej rzepakowy, V – olej lniany, VI – olej rybny. W doświadczeniu określono procentową zawartość kwasów tłuszczowych w surowicy i mięśniu *longissimus dorsi* metodą chromatografii gazowej.

Tłuszcz diety nie wpłynął na zawartość procentową nasyconych kwasów tłuszczowych (SFA) w surowicy krwi szczurów. W surowicy i tkance mięśniowej zwiększyła się procentowa zawartość wielonienasyconych kwasów tłuszczowych (PUFA), zmniejszyła zawartość jednonienasyconych kwasów tłuszczowych (MUFA). Olej lniany oraz rybny wpłynął na zwiększenie zawartości PUFA_{n-3} w surowicy i mięśniu. W tkance mięśniowej w przeciwieństwie do surowicy nie stwierdzono ujemnej korelacji pomiędzy PUFA_{n-3} i PUFA_{n-6}.