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Fatty Acid Composition of Porcine Muscle and Adipose Tissue Lipids as Affected by Anatomical Location and Cod Liver Oil Supplementation of the Diet

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Taugbøl, O. and K. Saarem: Fatty acid composition of porcine muscle and adipose tissue lipids as affected by anatomical location and cod liver oil supplementation of the diet. Acta vet. scand. 1995, 36, 93-101. – In pigs fed a standard pig mash the contents of polyunsaturated fatty acids (PUFAs) of both the n-6 and n-3 series were significantly higher in the dark red *mm adductores* compared to the light coloured *m longissimus lumborum*. Perirenal fat had a higher concentration of saturated fatty acids (14:0, 16:0, 18:0) than backfat, and a lower concentration of monounsaturated fatty acids, such as 16:1n-7 and 18:1n-9. Daily supplementation of 50 ml cod liver oil, rich in n-3 PUFAs, during the fourth and third week before slaughter led to a 1.4 to 1.7 times increase in the contents of n-3 PUFAs in muscles and fat depots. There was no difference between the incorporation of n-3 PUFAs in dark and light muscles. Perirenal fat contained more 20:5n-3 (EPA) and 22:6n-3 (DHA), but less 20:1n-9 (eicosenoic acid) than the backfat, after cod liver oil supplementation rich in these 3 fatty acids. Supplementation of cod liver oil reduced the n-6/n-3 fatty acid ratio in all anatomical locations examined.

fish oil; meat quality.

Introduction

Polyunsaturated fatty acids (PUFAs), especially the n-3 PUFA eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are currently of great interest due to their important biological functions (*Simopoulos* 1991). The n-3 PUFAs are found in abundance in sea food like fish oil (cod liver oil), but are scarce in fat from grain fed animals. Pigs supplemented with fish oil incorporate n-3 PUFAs into depot fat after a few days in a dose dependent manner *(Irie & Sakimoto* 1992, *Taugbøl* 1993). Pigs fed a supplementation of fish oil for 4 weeks until the day of slaughter, showed some differences in the distribution of the supplemented fatty acids to perirenal fat and subcutaneous fat *(Irie & Sakimoto* 1992). Intramuscular fat showed a delayed rise in n-3 PUFAs content compared to depot fat *(Taugbøl* 1993). *Malmfors et al.* (1978) reported that the dark muscles, which are associated with aerobic muscle fibres, contained a higher proportion of the PUFAs: 18:2n-6, 18:3n-3 and 20:4n-6, than the light muscles of normal fed pigs. It is not known whether incorporation of n-3 PUFAs differ in dark and light muscles, or if fish oil supplementation will change the fatty acid pattern in muscle lipids.

Table 1. Composition of the standard diet (%).

Component	%
Barley grain	40.2
Oat meal	40.0
Meat and bone meal	6.0
Wheat bran	5.0
Soybean meal, solvent extracted	2.63
Animal fat	1.5
Rapeseed meal (Canola)	1.2
Fish meal, herring	1.0
Molasses	1.0
Limestonemeal	0.5
Salt	0.25
Trace mineral mix*	0.1
Vitaminpremix**	0.4
L-lysine	0.16
L-threonine	0.06
Calculated composition	
Crude protein	15.4
Crude fat	5.4
Calcium	0.8
Phosphorus	0.62
ME, MJ/kg	12.32

* Contained the following trace minerals: Fe 5%, Mn 4%, Zn 7%, Cu 1%, I .05%, Se .02%.

** Provided the following per kg. of diet: vitamin A 3000 IU, vitamin D3 400 IU, vitamin E 40 mg, vitamin B2 3 mg, vitamin B12 0.02 mg, pantothenic acid 10 mg.

The present experiment was undertaken to provide information about the fatty acid pattern, with special reference to the n-3 PUFAs in different muscles and in different fat depots of pigs fed a standard pig mash, and to test if supplementation with cod liver oil will change the fatty acid distribution in these tissues.

Materials and methods

Animals and diets

Two trials involving 30 Norwegian Landrace *(Sus scrofa* f. domestica) barrows and gilts were conducted. The pigs were delivered to a commercial slaughterhouse at approximately 100 kg live weight. In trial I all pigs were fed exclusively a standard pig mash (Table 1). In trial II 6 pigs out of 12 received a supplement of 50 ml cod liver oil (Peter Möller, Norway), from which vitamin A and D have been removed by molecular distillation, every day during the fourth and third week before slaughter. The other 6 pigs, referred to as the control group, were fed only the standard pig mash. The amount of pig mash fed was 3 kg daily the last 4 weeks before slaughter in both trials. Adding 50 ml cod liver oil for 2 weeks led to an about 5% higher daily energy intake in the supplemented group compared to the control group. Although this might have resulted in a slightly higher weight gain, the primary objective of this trial was to study the changes of the fatty acid pattern in muscle and fat tissues after a supplementation with cod liver oil. The fatty acid composition of the basal diet and the cod liver oil is given in Table 2.

Experimental procedure

Two parallel samples were taken from the dark red *mm adductores*, the light *m longissimus lumborum* and the red *m psoas minor* from each pig. In trial II samples from the backfat, including both outer and inner layers, and perirenal fat were also included. The samples, weighing 400-600 mg, were stored in small plastic tubes containing 0.9% saline solution at –80°C until analysed.

A part of each sample (150 mg) was homogenized for 2 min in a 3 ml mixture of chloroform and methanol (2:1). Thereafter, 0.6 ml isotonic saline solution was added and the content homogenized for 30 sec *(Folch et al.* 1957). The homogenate was then filtered through purified cotton wool, and stored at –20°C until the next day. The aqueous phase was removed and the extract was placed in a waterbath at 45°C and evaporated under nitrogen. To the residue 5 ml methanol with 2

vol % sulfuric acid was added, before placing it in a waterbath at 80°C for 3h for methylation.

The methylated fatty acids were extracted with 1 ml heptane and separated on a fused silica wall coated capillary column (Omegawax 320, 30 m×0.32 mm, film thickness 0.25 mm (Supelco Inc, Bellefonte) in a Perkin Elmer 8400 gas chromatograph fitted with a flame-ionization detector and a programmable temperature vaporizer (PTV) injector. The samples were injected at splitless condition by an autosampler (Perkin Elmer AS-3000). Carrier gas was helium at a flow rate of 1.3 ml/min. The injector and detector temperature was 275°C. The column temperature was kept at 70°C for 2 min, then increased to 170°C at a rate of 30°C/min, then up to 250°C at a rate of 4°C/min. This temperature was held for 10 to 15 min. Peak areas were determined by The Omega-2 Integrator System (Perkin Elmer) by an Epson AX2-40 personal computer. Identification of major peaks was made by comparing the retention time with those of standard fatty acid methyl esters obtained from Supelco Inc, Bellefonte, or Sigma Chemical Co, USA.

Muscle samples from 10 other slaughtered pigs were used for fiber typing. The muscle samples from the *mm adductores*, *m longissimus lumborum* and *m psoas minor* were frozen in Freon 22, pre-cooled to about its melting point (i.e. –160°C). The muscle samples (still frozen) were mounted in a mounting medium (Lab-Tek Products, Napersville, III., USA) on a metal plate and stored at –80°C. Serial cross sections (10 mm) were cut transversal to the fibre direction at –20°C. The sections were stained for myofibrillar ATPase after both acid and alkaline preincubation. The first procedure involved only 1 preincubation in an acid buffer (pH 4.25) containing 25 mM. $CaCl_2$. In sections stained with this procedure type I fibres appeared dark in contrast to the light type II fibres. In the second procedure, sections were first preincubated in an alkaline buffer (pH 10.3) containing 75 mM $CaCl_2$, fixed in formaldehyde and then preincubated in an acid buffer (pH 4.6) containing 20 mM $CaCl_2$. In these sections the type I fibres appeared light and the type II fibres appeared dark *(Brook & Kaiser* 1970, *Vøllestad et al.* 1984). The fibre types were identified as type I and II fibres. Between 100 and 500 fibres were identified from each muscle sample.

Table 2. Fatty acid composition (area%) of standard diet and cod liver oil.

Fatty acid	Standard diet	Cod liver oil*
14:0	2.0	3.7
16:0	19.4	10.4
16:1n-7	2.0	8.0
18:0	3.7	2.2
18:1n-9	27.4	23.0
18:2n-6	37.6	1.6
18:3n-3	3.5	1.0
18:4n-3	0.3	2.6
20:1n-9	1.1	13.3
22:1n-11/13	0.5	7.7
20:5n-3 (EPA)	0.3	9.7
22:5n-3 (DPA)	–	1.1
22:6n-3 (DHA)	0.5	12.7
Not accounted for	1.7	3.0

* Vitamin A: < 30 IU/g; Vitamin D: < 1 IU/g

Statistical methods

The results are described as the number of pigs (N), the mean and standard deviation. For the evaluation of the muscle samples, where 2 replicate measurements within each pig were made, a repeated measures ANOVA was performed *(Hand & Taylor* 1991). In case of a statistical significance, Tukey's multiple comparison procedure was performed to rank the responces *(Montgomery* 1984). For the

Table 3. Fatty acid composition (area%) of lipid fraction from *mm adductores, m longissimus lumborum* and *m psoas minor* in pigs fed a standard pig mash (n = 18).

	Mm adductores		*M psoas minor*		*M longissimus lumborum*		
Fatty acid	Mean	SD	Mean	SD	Mean	SD	p<0.05
14:0	1.27	.12	1.33	.15	1.34	.14	
16:0	20.45	.71	21.84	.98	21.73	1.04	
16:1n-7	1.49	.42	1.97	.43	2.62	.79	a
18:0	11.06	.62	12.16	.99	11.22	1.05	
18:1n-9	21.05	4.26	28.54	3.99	33.04	5.87	a
18:1n-7	3.20	.23	2.85	.40	3.02	.47	
18:2n-6	22.02	2.27	17.57	2.41	13.77	3.16	b
18:3n-3	0.60	.06	0.72	.10	0.55	.07	
20:1n-9	0.57	.12	0.74	.07	0.84	.14	c
20:4n-6	6.41	1.08	3.66	1.09	3.43	1.62	d
20:5n-3 (EPA)	0.57	.11	0.33	.09	0.31	.15	d
22:5n-3 (DPA)	0.84	.13	0.61	.16	0.59	.24	d
22:6n-3 (DHA)	0.76	.21	0.49	.15	0.50	.23	d

a: *M longissimus lumborum* > *M psoas minor* > *Mm adductores*
b: *Mm adductores* > *M psoas minor* > *M longissimus lumborum*
c: *M longissimus lumborum* and *M psoas minor* > *Mm adductores*
d: *Mm adductores* > *M psoas minor* and *M longissimus lumborum*

lipid samples, with just 1 observation from each pig, an ANOVA was performed, with the position, the case number and the group as the class variables. The groups were compared with Dunnett's test. A complete model cheque was performed using the Jackknife residuals, Cook's d and Mallow's C_p (*Kleinbaum* 1988). All p-values below 0.05 were considered significant.

Table 4. Muscle fibre composition (%) of *mm adductores, m psoas minor* and *m longissimus lumborum* (n= 10).

	Mm adductores		*M psoas minor*		*M longissimus lumborum*	
Fibre type	Mean	SD	Mean	SD	Mean	SD
I	22	6	16	14	20	3
II	77	6	83	13	79	3
unident.	1		1		1	

Results

Muscles

In pigs fed a standard pig mash the contents of PUFAs (18:2n-6; 20:4n-6; 20:5n-3; 22:5n-3; 22:6n-3) of the dark red *mm adductores* were significantly higher than in both *m psoas minor* and the *m longissimus lumborum*. The contents of 18:2n-6 of the red *m psoas minor* were significantly higher than for the light *m longissimus lumborum*. The contents of the monounsaturated fatty acids (16:1n-7; 18:1n-9; 20:1n-9) were higher in the lipidfraction of *m longissimus lumborum* and *m psoas minor* than of *mm adductores*, and the 16:1n-7 and 18:1n-9 concentrations were higher in *m longissimus lumborum* compared to *m psoas minor* (Table 3).

There was no difference between the 3 muscles with regard to fibre composition (Table 4). After cod liver oil supplementation the n-3

Table 5. Distribution (area%) of n-3 PUFAs in lipid fractions of *mm adductores, m psoas minor* and *m longissimus lumborum* from pigs fed a standard pig mash (control) (n = 6) and pigs supplemented with cod liver oil (CLO) (n = 6).

		Mm adductors		*M psoas minor*		*M longissimus lumborum*		
Fatty acid	Group	Mean	SD	Mean	SD	Mean	SD	p<0.05
20:5n-3	control	0.60	.15	0.42	.16	0.29	.16	a
(EPA)	CLO	1.29	.25	0.72	.31	0.55	.32	a,b
22:5n-3	control	0.85	.17	0.60	.15	0.50	.21	a
(DPA)	CLO	1.06	.16	0.72	.19	0.68	.37	a,b
22:6n-3	control	0.82	.30	0.61	.22	0.40	.17	a
(DHA)	CLO	1.24	.50	0.92	.34	0.82	.50	a,b

a: *Mm adductores* > *M psoas minor* and *M longissimus lumborum.*
b: CLO > Control.

PUFAs (EPA; DPA; DHA), were significantly higher than in the control group for all muscle samples (Table 5). Other fatty acids analyzed in the 3 muscles did not differ between the 2 groups. In both groups the contents of n-3 PUFAs were significantly higher in the *mm adductores* compared to the 2 other muscles.

Fat

In pigs fed the control diet perirenal fat was lower in 16:1n-7 and 18:1n-9 and higher in 16:0 than backfat. For the other 2 saturated fatty acids examined (14:0 and 18:0) statistical borderline values were found, with higher content in perirenal fat than in backfat (Table 6). Cod liver oil supplementation increased the

Table 6. Fatty acid composition (area%) of backfat and perirenal fat in pigs fed a standard pig mash (n = 6).

	Backfat		Perirenal fat		
Fatty acid	Mean	SD	Mean	SD	p<0.05
14:0	1.19	.10	1.28	.06	(p = 0.06)
16:0	21.35	1.22	23.63	1.34	a
16:1n-7	2.16	.27	1.65	.24	b
18:0	18.54	4.12	24.18	5.41	(p = 0.07)
18:1n-9	36.35	3.16	27.26	3.12	b
18:2n-6	14.15	2.07	15.65	2.92	
18:3n-3	0.96	.10	1.03	.15	
20:1n-9	1.22	.08	1.16	.11	
20:4n-6	0.28	.09	0.27	.07	
20:5n-3 (EPA)	0.06	.01	0.06	.01	
22:5n-3 (DPA)	0.17	.02	0.18	.04	
22:6n-3 (DHA)	0.17	.03	0.22	.06	(p = 0.13)

a: Backfat < Perirenal fat.
b: Backfat > Perirenal fat.

Table 7. Distribution (area%) of 20:1n-9 and n-3 PUFAs in backfat and perirenal fat from pigs fed a standard pig mash (control) (n = 6) and pigs supplemented with cod liver oil (CLO) (n = 6).

		Backfat		Perirenal fat		
Fatty acid	Group	Mean	SD	Mean	SD	p<0.05
20:1n-9	Control	1.22	.08	1.16	.11	
	CLO	1.40	.08	1.27	.08	a, b
20:5n-3	Control	0.06	.01	0.06	.01	
(EPA)	CLO	0.12	.02	0.14	.02	a, c
22:5n-3	Control	0.17	.02	0.18	.04	
(DPA)	CLO	0.25	.03	0.26	.02	a
22:6n-3	Control	0.17	.03	0.22	.06	
(DHA)	CLO	0.30	.03	0.37	.03	a, c

a: CLO > Control
b: Backfat > Perirenal fat (CLO)
c: Backfat < Perirenal fat (CLO)

contents of 20:1n-9 (eicosenoic acid) and n-3 PUFAs in both backfat and perirenal fat. The monounsaturated fatty acid 20:1n-9 was found in higher concentration in backfat than in perirenal fat, whereas EPA and DHA were higher in perirenal fat (Table 7).

The ratio of n-6/n-3 fatty acids

The ratio of n-6/n-3 fatty acids was calculated, based on the following fatty acids: 18:2n-6, 20:4n-6, 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3. The ratio decreased in both fat and muscle tissues after cod liver oil supplementation (Table 8).

Discussion

In pigs fed a standard pig mash the contents of PUFAs of both the n-6 and n-3 series were significantly higher in the dark red *mm adductores* compared to the light *m longissimus lumborum*. A similar difference in PUFAs with 18 and 20 carbon atoms was found between the dark *m quadriceps* and the light *m longissimus dorsi (Malmfors et al.* 1978), and between the red *m psoas major* and the light *m longissimus dorsi (Allen et al.* 1967). One would expect these differences to be due to differences in fibre composition in the tested muscles. The 3 muscles did not, however, differ with regard to type I and II fibres. Type I fibre is referred to as slow twitch fibre and type II as fast twitch fibre. Fast twitch fibres have high myofibrillar ATPase activity, and can be further divided into fast twitch fati-

Table 8. Ratio n-6/n-3 fatty acid in different tissues from pigs fed a standard pig mash (control) and pigs supplemented with cod liver oil (CLO). The following fatty acids are considered: 18:2n-6 and 20:4n-6 / 18:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3.

Group	Control	CLO
Muscles		
Mm adductores	10.26	7.62
M psoas minor	9.87	7.52
M longissimus lumborum	8.82	6.47
Fat depots		
Backfat	10.61	8.38
Perirenal fat	10.68	8.07

gable and fast twitch fatigue-resistant. Fast twitch fatigable fibres fatigue rapidly due to, a low capacity for oxidative metabolism. These fibres have a low mitochondrial and myoglobin content. Fast twitch fatigue-resistant fibres, on the other hand, have a high content of both mitochondria and myoglobin, and therefore a high oxidative capacity. Slow twitch and fast twitch fatigue-resistant muscle fibres are both red in colour due to the high myoglobin content. Since the fibre identification in this study was based solely on ATPase stain, the type II fibres could not be subdivided. However, the dark, red colour of *mm adductores* indicates a large proportion of aerobic type fast twitch fatigue-resistant fibres with high mitochondrial content *(Brook & Kaiser* 1970). PUFAs are preferably incorporated in the phospholipid fraction of membranes, rather than being converted by competing pathways, such as fatty acid oxidation and triacylglycerol synthesis *(Iritani & Narita* 1984). Thus, the higher contents of n-3 and n-6 PUFAs in *mm adductores* compared to *m longissimus lumborum* may partly be due to a larger mitochondrial membrane area of the former. Although the contents of the individual n-3 PUFAs of the red *m psoas minor* were not statistically different from those of the light *m longissimus lumborum*, all the mean values of *m psoas minor* were higher.

The fatty acid pattern of pork fat of control diet fed pigs was consistent with those reported by *Villegas et al.* (1973) and *Irie & Sakimoto* (1992). Perirenal fat had a higher concentration of saturated fatty acids (14:0, 16:0, 18:0) than backfat, and a lower concentration of monounsaturated fatty acids, such as 16:1n-7 and 18:1n-9.

The basal daily diet contained about 7.5 g n-3 PUFAs, while 50 ml cod liver oil contained 13.6 g n-3 PUFAs. The daily amount of EPA plus DHA was 1.3 g and 11.2 g, respectively. Daily supplementation of 50 ml cod liver oil during the fourth and third week before slaughter led to a 1.4 to 1.7 times increase in the contents of n-3 PUFAs in muscles and fat depots. There was no difference between the incorporation of n-3 PUFAs in dark and light muscles. Regarding the n-3 PUFAs pattern after fish oil supplementation in perirenal fat, our results agree with those of *Irie & Sakimoto* (1992). Perirenal fat contained more EPA and DHA than backfat after cod liver oil supplementation. Furthermore, a similar trend was noted for DHA in the control group. The contents of the monounsaturated fatty acid 20:1n-9, which made up 13.3% of the fatty acids in cod liver oil, were higher in backfat than in perirenal fat. The distribution mechanisms of dietary fatty acids seemed to be different in the 2 fat depots. In growing rats, fed a high fat diet, fish oil limited the cell hypertrophy of retroperitoneal fat, but did not affect hyperplasia in the same tissue *(Belzung et al. 1993)*. The preferential increase of n-3 PUFAs in the perirenal fat, found in our study, might therefore be due to a larger cell membrane area in this fat depot compared to the subcutaneous fat.

Since the industrial revolution the human consumption of n-3 fatty acids has diminished as the intake of n-6 fatty acids has increased. While the n-6/n-3 fatty acid ratio in the diet was nearly 1 during the evolutionary period of the human genetic constitution *(Leaf & Weber* 1987), it might exceed 9 in the Western societies today *(Galli & Simopoulos* 1989). A recommended n-6/n-3 fatty acid ratio in dietary fats for human consumption today is about 4 *(Galli & Simopoulos* 1989). The n-6/n-3 fatty acid ratio found in muscle and fat tissues of pigs fed a standard pig mash, corresponded to the ratio of the diet, which was about 8. *Koizumi et al.* (1991) showed that wild pigs have a more favourable n-6/n-3 fatty

acid ratio than pen fed pigs. This study has clearly shown that it is possible to lower the n-6/n-3 fatty acid ratio in both muscle and fat tissues from pigs by dietary means. One way to change the n-6/n-3 fatty acid ratio in pig mash, is to supplement with cod liver oil as used in this study, but one must have in mind that this could downgrade the sensoric score of pig meat products, if used in higher doses and until the day of slaughter (*Øverland et al.* 1992, *Taugbøl* 1993).

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Sammendrag

Fettsyresammensetningen i muskulatur og fettvev hos gris i forhold til anatomisk lokalisering og etter tilskudd av tran.

Fra griser som var blitt fôret utelukkende på standard svinefôr, ble fettsyresammensetningen fra 3 ulike muskler, de mørke røde *mm adductores*, den lyse *m longissimus lumborum* og den røde *m psoas minor*, og fett fra nyreregionen og underhudsfett undersøkt. Innholdet av flerumettede fettsyrer fra både n-6 og n-3 seriene var høyere i *mm adductores* sammenlignet med *m longissimus lumborum*. Perirenalt fett hadde et høyere innhold av de mettede fettsyrene (14:0, 16:0 og 18:0), og et lavere innhold av de enumettede fettsyrene (16:1n-7 og 18:1n-9) enn underhudsfett.

Tilskudd av 50 ml tran, rik på n-3 flerumettede fettsyrer, daglig fjerde og tredje uke før slakting førte til at innholdet av n-3 fettsyrer i muskulatur og fett økte rundt 50%. Innholdet av de flerumettede fettsyrene av n-3 serien økte likt i alle 3 typer muskler. Fett fra nyreregionen inneholdt mer 20:5n-3 (EPA) og 22:6n-3 (DHA) enn underhudsfett, men mindre 20:1n-9, selv om alle disse fettsyrene finnes i rikelige mengder i tran.

Forholdet mellom n-6 og n-3 fettsyrer ble redusert i alle anatomiske lokalisasjoner som ble undersøkt.

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