Modification of fatty acid profile of liver, cardiac and skeletal muscle of pigs by varying dietary n-6 and n-3 ratio

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Background

Reduced prevalence of cardiovascular disease and death rate from this disease have been associated with diets containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are rich in the lipid of marine animals. This has led to a recommendation to increase the intake of long chain n-3 fatty acid and reduce the ratio of n-6/n-3 fatty acid in the diet of the British public (Department of Health, 1994). Several attempts have been made to increase the n-3 fatty acid content of pork meat by dietary means (Hertzman et al., 1988; Morgan et al., 1992; Irie & Sakimoto, 1992; Leskanich et al., 1997). Rapeseed oil, linseed oil and fish oil have been widely used as dietary n-3 sources to enrich n-3 fatty acid in pork meat and body fat. However, oils rich in linolenic acid produced only small changes in long chain n-3 PUFA of adipose tissue (Romans et al., 1995) while inclusion of fish meal or fish oil is necessary to provide significant increase in long chain n-3 fatty acids (Valaja et al., 1992; Morgan et al., 1992). So far studies with pigs have concentrated mainly on fatty acid profile of fat tissue and skeletal muscle and the range of test oils compared within one trial is limited. There is little information available in the literature about oils with widely different fatty acid profiles and their effect on the fatty acid composition of other tissues, especially liver and heart. Such data may indicate the health of pigs as well as providing insight into where n-3 fatty acid may be incorporated in human organs following diet supplementation.

Objectives

As part of an investigation of the effect of different dietary fats and different dietary ratios of n-6:n-3 fatty acids, without or with supranutritional amounts of vitamin E, on cardiac function of the pig, the effect of the dietary treatments on the fatty acid compostion of different tissues was investigated.

Methods

Sixteen large White x Landrace male pigs weighing 13.8±0.84kg were divided into one of eight treatment diets (30 g/kg of beef dripping (BD), corn oil (CO), fish oil (FO) or rapeseed oil (RO) with 50 mg/kg or 200 mg/kg dietary vitamin E). Liver, *L. dorsi* and heart muscle were taken when the pig weighing approximately 40 kg was killed by an overdose of anaesthetic after cardiac function tests. Fatty acid methyl esters (FAME) of dry diets were prepared according to Sukhija and Palmquist (1988). A modified Folch method (Hamilton and Hamilton, 1992) was used to extract the lipid of tissues. Extracted lipids were converted to methyl esters according to Joseph and Ackman (1992) and analysed for individual FAME using a Hewlett Packard 5890A Gas Chromatograph equiped with HP 7673 Auto Injector (on column) and Omegawax™ 250 Capillary column (0.25mm x 30m, Supel Co. Bellefonte, PA). The initial temperature was set at 170 °C, raised to 185 °C by 1 °C/minute, and to 220 °C by 2 °C/minute, and finally 30 °C/minute to 240 °C where it was held for 3 minutes.

Results and Discussion

The analysed fatty acid composition of the experimental diets (Table 1) indicated a wide range of the major fatty acids and in the ratio of n-6/n-3. The four oils used in the present study each had its own special fatty acid profile, which were reflected in the final diets: beef dripping rich in saturated fatty acid and oleic acid; corn oil rich in linoleic acid; fish oil a good source of long chain n-3 PUFA; rapeseed oil rich in oleic acid and linoleic acid and moderate in linolenic acid.

Table 1 Fatty acid composition of grower diets

	BD	CO	FO	RO
C16:0	26.20	15.76	20.31	12.85
C18:0	15.77	3.41	4.77	3.48
C18:1	30.18	23.95	19.06	42.71
C18:3n-3	1.92	2.43	2.50	7.00
C20:5n-3			4.35	0.07
C22:5n-3	0.07		0.92	
C22:6n-3			7.01	
C18:2n-6	18.09	50.81	17.75	28.53
C20:4n-6	0.05		0.56	
Saturated	45.48	21.05	30.40	18.62
Mono	32.95	24.91	32.60	44.81
n-3	2.39	2.60	17.04	7.42
n-6	18.59	51.08	19.46	28.80
n-6/n-3	7.78	19.66	1.14	3.88
Total FA mg/g	46.3	43.6	37.0	43.5

Dietary vitamin E had no statistically significant effect on any fatty acid in all the tissues analysed. Data presented in Table 2 are the main effect for each dietary oil treatment (4 pigs).

The content of total saturated fatty acid (TSFA) in all three tissues did not show a positive response to dietary manipulation. BD treatment resulted in a higher TSFA in *L dorsi* muscle lipid, but differences were not statistically significant between any of the four treatments. Significant differences observed in TSFA of liver and heart lipid were due to small variation among the samples since the actual differences between treatments were very small. Corn oil significantly decreased oleic acid deposition in all three tissues when compared with BD and RO treatments; comparing with FO treatment, the difference was significant ($P<0.001$) in heart lipid but not in liver and muscle. Although oleic acid in the RO diet was 1.4 times that in the BO treatment, the amount deposited in tissue from RO treatment was slightly less than in the BD treatment.

n-3 Fatty acid content in the tissues were in the order of FO>RO>BD>CO, the same order as in the diets, and significantly different ($P<0.001$) from each other in the liver and heart lipid, and significantly different between FO treatment and the other treatments in the *L dorsi* muscle lipid. The effect of dietary fatty acid on tissue n-6 fatty acid profile was exactly opposite to the pattern of n-3 fatty acid described above. CO significantly increased linoleic in all three tissues and arachidonic acid in heart and liver, while FO brought about the opposite result. Arachidonic acid of liver and

Table 2 Fatty acid composition (% of total detectable fatty acid) of heart, liver and *L. dorsi* muscle from pig fed beef dripping (BD), corn oil (CO), fish oil (FO) and rapeseed oil (RO)

	heart					liver					muscle				
	BD	CO	FO	RO	SEM	BD	CO	FO	RO	SEM	BD	CO	FO	RO	SEM
C16:0	14.9β	14.7β	16.3α	14.8β	0.23	16.2α	13.9β	14.0β	13.5β	0.41	21.8	20.9	17.5	20.6	1.74
C18:0	15.1a	15.6a	12.8b	14.5a	0.27	25.7β	27.1α	27.8α	26.5αβ	0.29	12.8	12.5	14.0	11.8	0.37
C18:1	20.0a	14.7b	19.9a	19.3a	0.30	17.3a	11.3b	11.4b	16.1a	0.36	40.2α	30.5γ	33.1βγ	37.8αβ	1.45
C18:3n-3	0.6b	0.4c	0.7b	1.4a	0.03	0.2c	0.2c	0.4b	0.7a	0.02	0.7b	0.5c	0.8b	1.5a	0.04
C20:5n-3	1.1b	0.3c	9.7a	1.6b	0.14	0.8c	0.3c	11.2a	2.0b	0.16	0.4b	0.2b	3.6a	0.6b	0.18
C22:5n-3	1.6c	1.1d	2.8a	2.1b	0.03	2.4c	1.8d	4.2a	3.0b	0.11	0.7b	0.6b	2.1a	1.0b	0.14
C22:6n-3	0.9b	0.6b	5.1a	0.9b	0.13	3.3b	2.2b	9.8a	3.1b	0.35	0.5b	0.4b	3.7a	0.5b	0.15
C18:2n-6	30.5b	34.0a	22.5c	31.0b	0.38	12.5c	17.7a	9.8d	15.2b	0.28	12.5b	22.6a	13.8b	15.7b	1.13
C20:4n-6	11.0b	14.0a	6.0c	10.6b	0.21	16.2b	20.0a	7.0c	15.8b	0.33	3.0	4.5	2.7	3.5	0.42
saturated	30.4α	30.7α	29.6β	29.8β	0.11	43.0α	41.9αβ	42.7α	40.8β	0.35	36.2	35.0	33.3	33.8	1.46
mono	21.4a	15.5b	21.9a	20.5a	0.33	18.9a	12.3b	13.2b	17.5a	0.41	44.2α	33.4β	37.8αβ	41.3α	1.55
n-3	4.2c	2.3d	18.5a	6.0b	0.29	6.9c	4.5d	26.0a	8.9b	0.28	2.5b	1.9b	11.0a	3.9b	0.47
n-6	44.0b	51.5a	30.1c	43.9b	0.41	31.2c	41.3a	18.0d	32.9b	0.28	17.1β	29.7α	18.1β	21.0β	1.61
n-6/n-3	10.4	22.0	1.6	7.3		4.6	9.1	0.7	3.7		6.8	15.9	1.7	5.4	

Within the same tissue and the same row, values with different letters differ at $p<0.01$ to 0.0001, value with different symbols (α,β,γ) differ at $p<0.05$

heart from RO treatment was slightly less than BD treatment although the RO diet contained much more of the precursor linoleic acid (28.5 % vs 18.1%).

The fatty acid pattern of different tissues varied significantly. Liver lipid contained over 40% of TSFA, muscle about 34% while heart lipid only about 30%. This difference mainly was due to much greater stearic acid found in liver lipid compared (14.8 %) accounted for almost half of the total n-6 PUFA. This acid in heart was 10.3 %, but in muscle only 3.4 %.

Total n-3 PUFA in muscle lipid from the FO treatment was more than 2.5 times that from RO, 4 times that from BD and 5 times that from CO. This increase was even greater in liver and heart. n-3 PUFA content in liver from the FO treatment reached the same level as in fish oil, whereas the level reached in the heart was about the same as the diet. This resulted in a n-6/n-3 ratio in heart and muscle of less than 2, and in liver of less than 1, well below the ratio of n-6/n3 of 4 suggested by British Nutrition Foundation (1992).

An amount of 200 mg of the EPA plus DHA has been recommended as the daily human intake (Department of Health, 1994). This target can be easily reached by consumption of liver, or meat from animals fed fish oil (Table 3). Compared with meat from BD, meat from RO does not contribute any appreciable extra EPA and DHA, while even a 100g serving of liver would only provide a small increase.

Table 3 Long chain n-3 PUFA (mg) in 100g tissue

	Total FA	Dietary oil			
	mg/g	BD	CO	FO	RO
Heart	11.5	41.4	23.0	202.4	52.9
Liver	21.9	142.4	94.2	551.9	177.4
Muscle	10.4	16.6	12.5	97.8	21.8

Conclusions

The response of liver, heart and skeletal muscle to dietary n-3 fatty acid composition differed markedly and followed the order of liver>heart muscle>skeletal muscle. Only sources rich in preformed long chain n-3 such as fish oil can substantially increase long chain n-3 PUFA deposition. The effect of rapeseed oil is relatively moderate despite its content of linolenic acid. It is difficult to reduce the total saturated fatty acid content of the animal tissues by dietary means.

with heart and muscle lipid; in contrast, muscle lipid contained more palmitic acid. *L dorsi* muscle lipid contained more than twice as much monounsaturated fatty acid as in liver lipid, and twice or less as in heart lipid. The n-3 PUFA content in the three tissues were in the order of liver (11.6)>heart (10.3) >muscle (4.8), while the n-6 PUFA were in the order of heart (42.4)>liver (32.9) > muscle (21.5). Arachidonic acid in liver

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