Effects of n-3 fatty acid supplementation on lipid composition in muscle, subcutaneous fat, liver and kidney of swine

W. OTTEN, C. WIRTH and H. M. Eichinger
Versuchsstation Thalhausen, Techn. Univ. München, W-8051 Kranzberg, Germany

SUMMARY

N-3 fatty acids are important precursors of prostanoids and leucotrienes. Once incorporated into biomembranes these fatty acids with increased numbers of double bonds are thought to alter membrane fluidity and influence cellular structures and functions. Higher content of n-3 fatty acids in membranes are also considered to help prevent cardiovascular diseases in humans. We investigated the fatty acid patterns in various tissues of 6 pigs fed a diet supplemented with 5% fish oil (i.e., rich in n-3 fatty acids). The tissues studied were two skeletal muscles, adipose tissue, heart, liver and kidney. These patterns were compared to those of 6 pigs fed an equicaloric diet which was supplemented with 5% coconut fat. All animals were slaughtered at 100 kg BM, and subsequently the tissues were removed, lipids extracted by chloroform-methanol, and after transesterification, fatty acid methyl esters (FAMES) were analysed by capillary gas chromatography (GC). We observed that n-3 fatty acid supplementation enhanced significantly the relative amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in all examined tissues. In the heart, liver and kidney tissues the increases in n-3 fatty acids were compensated by decreases primarily in arachidonic acid, but in some cases also in lauric acid (C 12:0) and myristic acid (C 14:0). The highest n-3 fatty acid contents following supplementation were found in liver and heart tissue (EPA: 12.9 and 13.6%, DHA: 13.0 and 6.2% respectively), whereas the lowest levels were in adipose tissue (EPA: 1.8%, DHA: 3.1%). On the average, in the control group, the n-3 fatty acid content in the different tissues was 20-60% lower.

Our results indicate that supplementation of n-3 fatty acids in swine significantly alters the fatty acid pattern of skeletal muscle, adipose tissue, heart, liver and kidney. However, the extent of incorporation was significantly different between these samples which may be indicative of tissue specific metabolic pathways.

INTRODUCTION

There is presently an increased awareness of the possible association between cardiovascular disease and nutrition. Several studies have implicated a direct correlation between the levels of n-3 fatty acids in diet with a reduced incidence of atherosclerosis, hypertention, and/or other cardiovascular diseases (Knapp, 1990; Wolfram, 1989).

From a nutritionalist point of view, these studies have primarily focussed on increasing the human consumption of foods naturally rich in these fatty acids, namely fish (Beare-Rogers, 1988). Few have taken the approach to increase the incorporation of such fatty acids in blood, heart and liver of swine (Ruiter, 1988; Hartog et al., 1987). Also little is reported on the incorporation of n-3 fatty acids in pig muscle and adipose tissues as commonly consumed food. Hence in the present study, one of our aims was to determine to which extend swine fed a supplemental diet rich in n-3 fatty acids would incorporate n-3 fatty acids in organs and tissues. In addition, using the swine as an animal model these studies could also provide new insights as to where and how these n-3 fatty acids might be incorporated into human tissues.

MATERIALS AND METHODS

Twelve German Landrace swine were studied. Six of these animals were fed a diet supplemented with 5% fish oil rich in n-3 fatty acids (including eicosapentaenoic acid, C 20:5n-3, EPA; and docosahexaenoic acid, C 22:6n-3, DHA). The other six animals were fed a diet counterbalanced with 5% coconut oil. The supplementations were given over a 13 week period which was initiated when the animals had an average weight of 29 kg (at approximately 12 weeks of age). When the average body weight was 100 kg the animals were slaughtered. Tissues samples were immediately removed and lipid extraction began thereafter according to Blight and Dyer (1959). Samples from the longissimus dorsi muscle, the supraspinatus muscle, subcutaneous back fat, the heart, the liver and kidney were investigated.

The fatty acids in these samples were transesterificated to fatty acid methyl esters and analyzed by gas chromatographic analysis (Shehata, 1970).

RESULTS AND DISCUSSION

In general, the total lipid content was similar between the two feeding groups; only in the liver there was a slightly lower total lipid content in the swine fed fish oil. However, n-3 fatty acid supplementation significantly enhanced the amounts of EPA and DHA found within all examined tissues (Tab. 1, 2 and 3). The increased amounts of these highly unsaturated fatty acids might be related to the reduced levels of arachidonic acid (C 20:4) found in the heart, liver, and kidney samples of these animals (Tab 2 and 3). It was also interesting that the relative amounts of lauric acid (C 12:0) and myristic acid (C14:0) were generally reduced in the animals fed the fish oil (unpublished data).

The highest concentrations of EPA and DHA (i.e. total n-3 levels) were found in the samples of the liver of the animals fed fish oil (Tab. 1, 2, and 3). This level was 40% greater than that found in the coconut oil fed group (Tab. 2). Although the liver had the highest concentration of the n-3 fatty acids it was not the tissue with the largest incorporation due to the supplementations. The values for the other are as follows: kidney, 60%; heart, 62%; longissimus dorsi, 92%; supraspinatus, 151%, and adipose tissue, 165%. However, it should be noted that even though the adipose tissue had the highest difference in n-3 fatty acids between the groups, it was the sample with very low absolute concentrations. This is most likely due to the facts that the metabolic turnover of fatty acids is low in fat, and that adipose tissues do not have adequate membranal densities.

CONCLUSIONS

We conclude, that n-3 supplementation of monogastric animals produced for human consumption is a potentially useful way to increase the level of these fatty acids in our daily diet, while at the same time perhaps lowering the levels of saturated fats. In addition, these data indicate that the incorporation of n-3 fatty acids into the various tissues is not uniform, which relates to different mechanisms or degrees of metabolic turnover of fatty acids.

Tab.1: Relative amounts of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA) and further pooled fatty acids in two skeletal muscles (musc. long. dorsi, musc. supraspinatus) of swine after dietary supplementation with either 5% n-3 rich fish oil (FO) or 5% coconut oil (CO).

tissue	treatments FO n=6	treatments CO n=6	differences +/-	differences sign.
m.long.dorsi				
EPA, C 20:5 n-3	3.4 (1.1)	1.6 (0.4)	+ 1.8	**
DHA, C 22:6 n-3	2.6 (0.8)	1.4 (0.3)	+ 1.2	**
AA, C 20:4	1.9 (0.6)	2.2 (0.5)	- 0.3	n.s.
total n-3 FA	6.9 (2.1)	3.6 (0.6)	+ 3.3	**
monoenic FA	43.7 (3.7)	46.7 (4.0)	- 3.0	n.s.
polyenic FA	19.4 (3.3)	15.8 (3.0)	+ 3.6	n.s.
saturated FA	36.9 (1.3)	37.5 (2.1)	- 0.6	n.s.
P/S	0.53 (0.09)	0.42 (0.08)	+ 0.11	*
m.suprasp.				
EPA, C 20:5 n-3	4.0 (1.4)	1.4 (0.4)	+ 2.6	***
DHA, C 20:6 n-3	3.3 (0.8)	1.2 (0.2)	+ 2.1	***
AA, C 20:4	1.6 (0.4)	2.1 (0.8)	- 0.5	n.s.
total n-3 FA	8.8 (2.3)	3.5 (0.7)	+ 5.3	***
monoenic FA	39.0 (4.8)	43.9 (2.8)	- 4.9	*
polyenic FA	23.9 (5.8)	17.5 (3.0)	+ 6.4	*
saturated FA	37.1 (1.4)	38.6 (1.5)	- 1.5	n.s.
P/S	0.65 (0.18)	0.46 (0.09)	+ 0.19	*

FA = fatty acids; P/S = polyenic FA / saturated FA
* = signif. diff. p<0.05 ** = signif. diff. p<0.01 *** = signif. diff. p<0.001
n.s. = no significant difference between the feeding groups

Tab.2: Relative amounts of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA) and further pooled fatty acids in heart and liver of swine after dietary supplementation with either 5% n-3 rich fish oil (FO) or 5% coconut oil (CO)

tissue	treatments FO n=6	treatments CO n=6	differences +/-	differences sign.
heart				
EPA, C 20:5 n-3	13.6 (2.0)	8.4 (0.7)	+ 5.2	***
DHA, C 22:6 n-3	6.2 (1.6)	3.8 (0.5)	+ 2.4	**
AA, C 20:4	5.6 (1.2)	9.3 (1.5)	- 3.7	***
total n-3 FA	20.7 (3.7)	12.8 (1.1)	+ 7.9	***
monoenic FA	17.8 (1.3)	17.6 (1.9)	+ 0.2	n.s.
polyenic FA	53.6 (2.7)	52.4 (3.8)	+ 1.2	n.s.
saturated FA	28.5 (1.6)	30.0 (2.2)	- 1.5	n.s.
P/S	1.89 (0.20)	1.76 (0.24)	+ 0.13	n.s.
liver				
C 20:5 n-3	12.9 (1.7)	8.4 (2.7)	+ 4.5	**
C 22:6 n-3	13.0 (1.5)	10.4 (1.5)	+ 2.6	*
C 20:4	6.7 (1.3)	11.4 (1.5)	- 4.7	***
total n-3 FA	27.1 (2.8)	19.4 (3.6)	+ 7.7	**
monoenic FA	14.2 (2.2)	13.3 (2.4)	+ 0.9	n.s.
polyenic FA	44.8 (1.9)	43.6 (2.3)	+ 1.2	n.s.
saturated FA	41.0 (2.3)	43.2 (0.9)	- 2.2	n.s.
P/S	1.09 (0.09)	1.01 (0.06)	+ 0.08	n.s.

FA = fatty acids; P/S = polyenic FA / saturated FA
* = signif. diff. p<0.05 ** = signif. diff. p<0.01 *** = signif. diff. p<0.001
n.s. = no significant difference between the feeding groups

ab.3: Relative amounts of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic cid (AA) and further pooled fatty acids in adipose tissue (lard) and kidney of swine after etary supplementation with either 5% n-3 rich fish oil (FO) or 5% coconut oil (CO)

tissue	treatments		differences	
	FO	CO		
	n=6	n=6	+/-	sign.
adipose tis.				
EPA, C 20:5 n-3	1.8 (0.1)	0.5 (0.1)	+ 1.3	***
DHA, C 22:6 n-3	3.1 (0.3)	0.9 (0.1)	+ 2.2	***
AA, C 20:4	0.3 (0.1)	2.5 (0.1)	- 2.2	n.s.
total n-3 FA	6.9 (0.3)	2.6 (0.1)	+ 4.3	***
monoenic FA	41.6 (1.2)	42.6 (2.3)	- 1.0	n.s.
polyenic FA	17.7 (0.9)	12.5 (0.8)	+ 5.2	***
saturated FA	40.8 (0.7)	44.9 (2.3)	- 4.1	***
P/S	0.43 (0.02)	0.28 (0.02)	+ 0.15	***
kidney				
EPA, C 20:5 n-3	16.1 (1.4)	9.0 (3.6)	+ 7.1	***
DHA, C 22:6 n-3	4.5 (0.8)	3.8 (0.9)	+ 0.7	n.s.
AA, C 20:4	5.6 (0.8)	9.9 (2.6)	- 4.3	**
total n-3 FA	21.7 (1.7)	13.6 (4.2)	+ 8.1	***
monoenic FA	20.1 (2.9)	20.2 (5.4)	- 0.1	n.s.
polyenic FA	40.4 (4.3)	39.8 (8.4)	+ 0.6	n.s.
saturated FA	39.5 (1.7)	40.0 (3.3)	- 0.5	n.s.
P/S	1.03 (0.14)	1.02 (0.27)	+ 0.01	n.s.

FA = fatty acids; P/S = polyenic FA / saturated FA
* = signif. diff. $p<0.05$ ** = signif. diff. $p<0.01$ *** = signif. diff. $p<0.001$
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EPA, C 20:5 n-3	3.4 (1.1)	1.6 (0.4)	+ 1.8	**
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AA, C 20:4	1.9 (0.6)	2.2 (0.5)	- 0.3	n.s.
total n-3 FA	6.9 (2.1)	3.6 (0.6)	+ 3.3	**
monoenic FA	43.7 (3.7)	46.7 (4.0)	- 3.0	n.s.
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P/S	0.53 (0.09)	0.42 (0.08)	+ 0.11	*
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DHA, C 20:6 n-3	3.3 (0.8)	1.2 (0.2)	+ 2.1	***
AA, C 20:4	1.6 (0.4)	2.1 (0.8)	- 0.5	n.s.
total n-3 FA	8.8 (2.3)	3.5 (0.7)	+ 5.3	***
monoenic FA	39.0 (4.8)	43.9 (2.8)	- 4.9	*
polyenic FA	23.9 (5.8)	17.5 (3.0)	+ 6.4	*
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heart				
EPA, C 20:5 n-3	13.6 (2.0)	8.4 (0.7)	+ 5.2	***
DHA, C 22:6 n-3	6.2 (1.6)	3.8 (0.5)	+ 2.4	**
AA, C 20:4	5.6 (1.2)	9.3 (1.5)	- 3.7	***
total n-3 FA	20.7 (3.7)	12.8 (1.1)	+ 7.9	***
monoenic FA	17.8 (1.3)	17.6 (1.9)	+ 0.2	n.s.
polyenic FA	53.6 (2.7)	52.4 (3.8)	+ 1.2	n.s.
saturated FA	28.5 (1.6)	30.0 (2.2)	- 1.5	n.s.
P/S	1.89 (0.20)	1.76 (0.24)	+ 0.13	n.s.
liver				
C 20:5 n-3	12.9 (1.7)	8.4 (2.7)	+ 4.5	**
C 22:6 n-3	13.0 (1.5)	10.4 (1.5)	+ 2.6	*
C 20:4	6.7 (1.3)	11.4 (1.5)	- 4.7	***
total n-3 FA	27.1 (2.8)	19.4 (3.6)	+ 7.7	**
monoenic FA	14.2 (2.2)	13.3 (2.4)	+ 0.9	n.s.
polyenic FA	44.8 (1.9)	43.6 (2.3)	+ 1.2	n.s.
saturated FA	41.0 (2.3)	43.2 (0.9)	- 2.2	n.s.
P/S	1.09 (0.09)	1.01 (0.06)	+ 0.08	n.s.

FA = fatty acids; P/S = polyenic FA / saturated FA
* = signif. diff. $p<0.05$ ** = signif. diff. $p<0.01$ *** = signif. diff. $p<0.001$
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b.3: Relative amounts of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic id (AA) and further pooled fatty acids in adipose tissue (lard) and kidney of swine after etary supplementation with either 5% n-3 rich fish oil (FO) or 5% coconut oil (CO)

	treatments		differences	
	FO	CO		
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DHA, C 22:6 n-3	3.1 (0.3)	0.9 (0.1)	+ 2.2	***
AA, C 20:4	0.3 (0.1)	2.5 (0.1)	- 2.2	n.s.
total n-3 FA	6.9 (0.3)	2.6 (0.1)	+ 4.3	***
monoenic FA	41.6 (1.2)	42.6 (2.3)	- 1.0	n.s.
polyenic FA	17.7 (0.9)	12.5 (0.8)	+ 5.2	***
saturated FA	40.8 (0.7)	44.9 (2.3)	- 4.1	***
P/S	0.43 (0.02)	0.28 (0.02)	+ 0.15	***
kidney				
EPA, C 20:5 n-3	16.1 (1.4)	9.0 (3.6)	+ 7.1	***
DHA, C 22:6 n-3	4.5 (0.8)	3.8 (0.9)	+ 0.7	n.s.
AA, C 20:4	5.6 (0.8)	9.9 (2.6)	- 4.3	**
total n-3 FA	21.7 (1.7)	13.6 (4.2)	+ 8.1	***
monoenic FA	20.1 (2.9)	20.2 (5.4)	- 0.1	n.s.
polyenic FA	40.4 (4.3)	39.8 (8.4)	+ 0.6	n.s.
saturated FA	39.5 (1.7)	40.0 (3.3)	- 0.5	n.s.
P/S	1.03 (0.14)	1.02 (0.27)	+ 0.01	n.s.

FA = fatty acids; P/S = polyenic FA / saturated FA
* = signif. diff. p<0.05 ** = signif. diff. p<0.01 *** = signif. diff. p<0.001
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