Dietary α-Linolenic Acid Alters the Fatty Acid Composition of Lipid Classes in Swine Tissues

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The effects of dietary 18:3$n-3$ on the fatty acid composition of total lipids and lipid classes of pig tissues were investigated. Pigs ($n = 25$) were fed diets containing 18:3$n-3$ at 0% (control), 1.5% (LNA1), 2.5% (LNA2), and 3.6% (LNA3). The total lipid content of the tissues was not affected by dietary treatment. Feeding LNA1, LNA2, and LNA3 diets increased ($P < 0.05$) the 18:3$n-3$ and 20:5$n-3$ content in the total lipids, triglyceride, phosphatidylethanolamine, and phosphatidylcholine of all tissues except brain. The enrichment of 18:3$n-3$ was mainly in the triglycerides. Long-chain $n-6$ and $n-3$ fatty acids were incorporated in the phosphatidylethanolamine. Arachidonic acid and monounsaturated fatty acids were reduced in the phosphatidylethanolamine and phosphatidylcholine of all tissues except for brain by dietary 18:3$n-3$. The 22:6$n-3$ content of brain was not altered by dietary 18:3$n-3$, indicating a resistance of brain tissue to dietary manipulation.

Keywords: *Pig; 18:3n−3; tissues; n−3 polyunsaturated fatty acid*

INTRODUCTION

Long-chain $n-3$ polyunsaturated fatty acids ($n-3$ PUFA) from marine sources have been a source of much discussion, due to their protective effects on cardiovascular diseases, their function as modulators of arachidonic acid metabolism to eicosanoids, and their role in the development and function of brain and retina (Simopoulos, 1991; Fernades and Venkatraman, 1993). α-Linolenic acid (18:3$n-3$) from plant oils represents the parent fatty acid of longer chain $n-3$ PUFA such as eicosapentaenoic acid (20:5$n-3$), docosapentaenoic acid (22:5$n-3$), and docosahexaenoic acid (22:6$n-3$), via *de novo* synthesis through desaturation and elongation (Sprecher, 1981). These long-chain $n-3$ PUFA are an important component of the membrane phospholipid, where it is reported to be essential for maintaining membrane physicochemical properties (Stubbs and Smith, 1984).

In most Western countries the level of $n-3$ PUFA in the diet is low relative to the $n-6$ PUFA (Lands, 1992), and this is reflected in the balance of these fatty acids in the plasma and other tissues (Hodge et al., 1993). Because of the competition between $n-6$ and $n-3$ PUFAs for enzymes involved in fatty acid elongation and desaturation, eicosanoid production, and incorporation into tissue lipids, it has been suggested that the ratio of $n-6$ to $n-3$ PUFA is important and the current high $n-6$:$n-3$ ratio should be decreased (Health and Welfare Canada, 1990). In an attempt to redress the balance of $n-6$ and $n-3$ fatty acids in our current diet, feeding strategies have been adopted by the agrifood industry to alter the $n-3$ fatty acid content of animal products through dietary manipulation (Sim and Cherian, 1994; Van Elswyk, 1993). Manipulation of the $n-3$ PUFA content of pig muscle and fat tissue by addition of fish oil as a dietary source of $n-3$ PUFA has been documented (Morgan et al., 1992; Otten et al., 1993; Irie and Sakimoto, 1992). In contrast to the studies conducted with feeding marine oils, limited studies have been conducted to examine the effects of $n-3$ fatty acids from plant origin on the pattern of incorporation of $n-3$ fatty acids in tissue lipid classes. It has been reported that in laying hens (Cherian and Sim, 1992) or in humans (Holub, 1990) fed diets high in 18:3$n-3$, $n-3$ PUFAs were not distributed evenly among lipid classes but were concentrated in the phosphatidylethanolamine (PE) fraction. In this context, the present research was undertaken to provide information on the manner in which increase in dietary 18:3$n-3$ affects the fatty acid composition of total lipids, lipid classes [triglyceride (TG)], phosphatidylcholine (PC), and PE in tissues most commonly consumed as food (muscle, backfat, heart, liver) or in brain due to the important role of $n-3$ fatty acids in neural development (Wainwright, 1992). As pigs are used as a model for lipid metabolism study in humans, these results may provide insight into where $n-3$ fatty acids will be incorporated in human organs following diet supplementation and may have implications in vegetarians or in patients fed diets devoid of long-chain $n-3$ PUFA for extended periods as in enteral or parenteral nutrition.

MATERIALS AND METHODS

The current experiment was reviewed by the University of Alberta Animal Care Committee to ensure adherence to Canadian Council on Animal Care Guidelines.

Animals and Diets. Twenty-five pigs (Landrace × Yorkshire) of an average initial body weight of 24.5 kg were fed isocaloric and isonitrogenous diets containing wheat, barley, and soy bean meal based diet with added ground flax seeds at 0% (control), 10% (LNA1), 17% (LNA2), and 25% (LNA3). Ground flax seeds were used as a dietary source of 18:3$n-3$. The diets provided 18:3$n-3$ at 0, 1.5, 2.5, or 3.6%. All of the diets were formulated to contain sufficient vitamins and minerals to meet the pig's requirement for growth (NRC, 1988). The composition of the experimental diet is presented in Table 1. Room temperature was maintained at 20–22 °C during the first 4 weeks and at 17–20 °C thereafter. Feed and water were provided *ad libitum*.

Sample Collection. When pigs reached an average of 99–105 kg, they were delivered to a commercial slaughterhouse where they were stunned by electrical shock, bled, scalded,

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0021-8561/95/1443-2911$09.00/0

Table 1. Composition (Percent) of Experimental Rations for Pigs

ingredient	dietary treatment			
	control[a]	LNA1[a]	LNA2[a]	LNA3[a]
wheat	56.5	52.5	45.5	38.7
barley	20.8	20.0	23.0	25.5
soy meal	15.3	11.3	8.6	6.2
flax seed	0.0	10.0	17.5	25.0
animal tallow	3.3	2.0	1.2	0.4
limestone	1.2	1.1	1.1	1.1
calcium phosphate	1.2	1.4	1.4	1.4
salt	0.5	0.5	0.5	0.5
vitamin mix[b]	1.0	1.0	1.0	1.0
lysine	0.16	0.18	0.19	0.18
calcd analysis				
DE (kcal/kg)	3382.0	3381.0	3381.0	3382.0
CP[c]	17.0	17.0	17.0	17.0
CF[d]	4.4	4.5	4.5	4.5
calcium	0.75	0.76	0.76	0.77
lysine	0.85	0.85	0.85	0.85

[a] Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds as source of α-linolenic acid at 0, 10, 17, or 25%, respectively. [b] Vitamin mineral premix supplied the following per kilogram of diet: 5000 IU of vitamin D, 40 mg of vitamin E, 30 μg of vitamin B_{12}, 12 mg of riboflavin, 45 mg of niacin, 25 mg of pantothenic acid, 300 mg of choline, 5 mg of ethoxyquine, 150 mg of Fe, 20 mg of Mn, 120 mg of Zn, 20 mg of I, 20 mg of Cu, 0.3 mg of Se. [c] CP, crude protein. [d] CF, crude fat.

and dressed following normal commercial procedures. Tissue samples such as muscle (longissimus dorsi), backfat, liver (anterior lobe), heart, and brain were collected and placed on ice. All samples were kept frozen at −30 °C to await determination of fatty acid analyses.

Lipid Analyses. Total lipid was extracted from feed, liver, muscle, backfat, heart, and brain tissue according to the method of Folch et al. (1957). Two grams of sample was weighed into a test tube with 20 mL of (chloroform/methanol = 2:1, v/v) and homogenized with a polytron for 5−10 s at high speed. BHA dissolved in 98% ethanol was added prior to homogenization. The homogenate was filtered through a Whatman No. 1 filter paper into a 100 mL graduated cylinder, 5 mL of 0.88% sodium chloride solution was added, the cylinder was stoppered, and the contents were mixed. After phase separation, the volume of the lipid layer was recorded, and the top layer was completely siphoned off. Lipid contents were determined gravimetrically for muscle, heart, liver, brain, and adipose tissue. Five milliliters of the lipid extracts was dried in a block heater under nitrogen atmosphere and used for fatty acid analyses. The dried lipids were redissolved with an appropriate amount of chloroform to obtain a sample with 50 mg of lipid/mL. Fifty microliters of the lipid solution and 1 mL of boron trifluoride/methanol methylation solution were added into screw-capped tubes and incubated in a boiling water bath for 1 h (Metcalfe et al., 1961). After cooling to room temperature, the fatty acid methyl esters were separated as described earlier (Cherian and Sim, 1992a).

The rest of the organic layer from the Folch preparation was dried under nitrogen at 40 °C to obtain a solution with approximately 150 mg of lipid/mL. Two hundred microliters was and as applied on precoated silica gel G plates (20 × 20 cm) that had been previously activated by heating at 100 °C for 30 min. Lipid standards such as TG, PC, and PE were mixed and applied aside the lipid extract. The plates were developed as reported earlier (Cherian and Sim, 1992b). The spots corresponding to TG, PC, and PE were identified under ultraviolet light, were scraped off into screw-capped tubes, and were converted to fatty acid methyl esters as described above. The fatty acid methyl esters of triglyceride, PC, and PE fractions were separated by an automated gas chromatography (Model 3600, Varian Associates, Inc., Sunnyvale, CA) equipped with an on-column injector using a DB-23 fused silica capillary column (Supelco Canada Ltd., Oakville, ON; 30 m × 0.25 mm i.d.). The conditions of the gas chromatograph were described earlier (Cherian and Sim, 1992a). Fatty acid methyl esters were identified by comparison with retention times of authentic standards (Nu-Check-Prep). A Shimadzu EZChrom (Shimadzu Scientific Instruments, Inc., Columbia, MD) chromatography data system was used to integrate peak areas. Fatty acid values are expressed as weight percentages.

Table 2. Fatty Acid Composition (Percent) of the Experimental Diets

fatty acid	dietary treatment			
	control[a]	LNA1[a]	LNA2[a]	LNA3[a]
16:0	13.4	10.4	8.6	7.4
18:0	6.5	4.3	3.7	3.4
20:0	0.5	0.6	0.2	0.2
16:1	0.8	0.4	0.2	0.1
18:1	45.5	30.6	26.3	20.3
20:1	0.5	0.6	0.2	0.2
18:2n−6	28.2	27.2	23.4	21.9
18:3n−3	4.1	26.2	36.	45.7
ΣSFA[b]	20.4	15.3	12.3	11.1
ΣMUFA[c]	46.4	31.	26.5	20.5
n−6:n−3	6.9	1.0	0.6	0.5

[a] Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds as source of α-linolenic acid at 0, 10, 17, or 25%, respectively. [b] SFA, saturated fatty acid. [c] MUFA, monounsaturated fatty acid.

Lipid class separation and percent compositional determination were done by an Iatroscan TH-10 Mark II analyzer (TMA Scientific Supply, Mississagua, ON) in combination with precoated silica gel Chromarods SII. Each sample (1−2 μL) was spotted on the rods using a 10 μL Hamilton syringe and developed for 70 min in chloroform/benzene/formic acid (20:60:0.5 v/v/v). Rods were scanned as described earlier (Cherian and Sim, 1992b). Peak areas were calculated with Shimadzu EZChrom laboratory automation system. Response factors for each lipid class were determined and applied accordingly. Lipid standards (Sigma) were used for identification.

Statistical Analyses. All data are presented as the mean ± standard error. The effect of dietary 18:3n−3 supplementation was analyzed by ANOVA using the GLM procedure (SAS, 1985). Means of each treatment were compared for statistical significance ($P < 0.05$) using a Student−Newman−Keul test (Steel and Torrie, 1980).

RESULTS

The diet composition and fatty acid composition of the diet are shown in Tables 1 and 2. Generally the fatty acid composition of the diet reflected the dietary source. Control diet had the highest level of total saturates and 18:1 as expected from the composition of animal tallow. α-Linolenic (18:3n−3) acid was the only source of n−3 fatty acid in the diet. The addition of extra α-linolenic acid in the LNA1, LNA2, and LNA3 diets increased the 18:3n−3 content of the diet with a concomitant decrease in the n−6:n−3 ratio.

No significant differences in body weight or feed consumption were found among pigs fed control, LNA1, LNA2, or LNA3 diet. Feeding diets of increasing 18:3n−3 altered the n−3 fatty acid composition of muscle, backfat, liver, and heart tissue (Figure 1). The content of 18:3n−3 in the backfat, heart, and liver tissue progressively increased in a dose−response fashion ($P < 0.05$) (Figure 1). The incorporation of 18:3n−3 in the muscle tissue reached a plateau with LNA2 diet. Consequently, the ratio of n−6 to n−3 PUFA in the muscle, backfat, liver, and heart tissue was reduced ($P < 0.05$) by feeding LNA-enriched diets to pigs (Table 3). Dietary 18:3n−3 also resulted in a reduction ($P < 0.05$) in monounsaturated fatty acids and an increase ($P < 0.05$) in PUFA to saturated fatty acid (P:S) ratio in the muscle and adipose tissue (Table 3). A significant ($P < 0.05$) increase in 20:5n−3 was observed in the

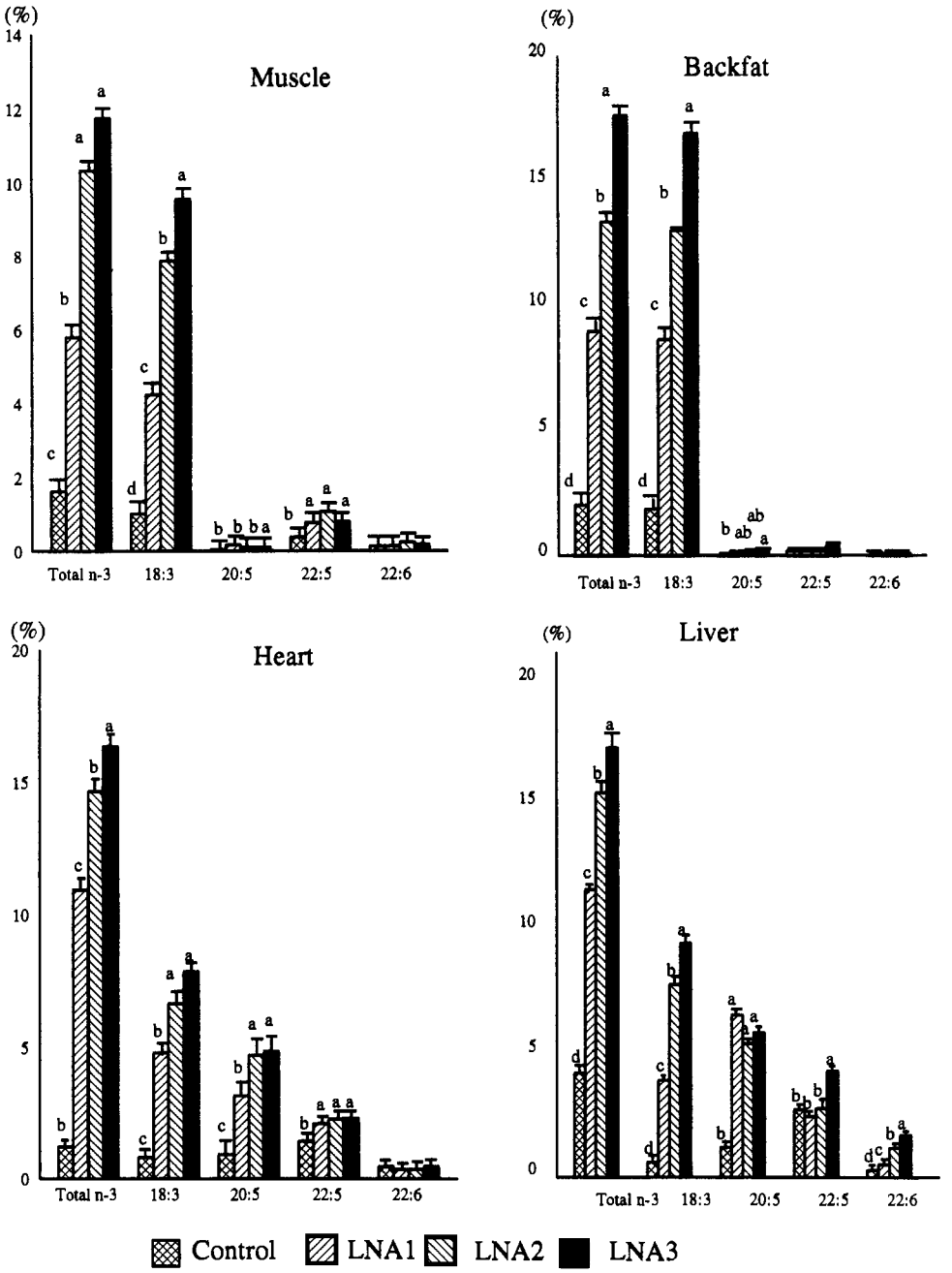

Figure 1. $n-3$ fatty acid composition of muscle, backfat, heart, and liver tissue of pigs fed diets containing different levels of 18:3$n-3$. Data are presented as mean. $n = 5$. a–d: means with no common superscripts within the same group of bars differ significantly ($P < 0.05$). Control, LNA1, LNA2, and LNA3 represent diets containing 0, 10, 17.5, or 25%, respectively, ground flax seed along with wheat, soybean meal based control diet.

backfat, heart and liver tissue total lipids of pigs from LNA1, LNA2, and LNA3 diets compared with that in pigs fed the control diet. However, additional 18:3$n-3$ from LNA1, LNA2, and LNA3 diets did not result in any increase in 22:5$n-3$ in the heart and muscle tissue (Figure 1). No difference was observed in the 22:6$n-3$ content of muscle, backfat, and heart tissue. However, levels of 22:6$n-3$ were ($P < 0.05$) higher in the liver total lipids of pigs receiving LNA1, LNA2, and LNA3 diets compared with those receiving the control diets.

There was no significant dietary effect on the total lipid or lipid composition of any of the tissue examined. The total lipid content of muscle tissue varied, being 3.3. 2.9, 3.1, and 3.8% for control, LNA1, LNA2, and LNA3 diets, respectively. In the muscle total lipids, TG constituted 62.4, 60.7, 63.7 and 61.7% and phospholipids constituted 37.1, 40.0, 37.9, and 38.5% for control, LNA1, LNA2, and LNA3 diets, respectively. The heart tissue total lipids were 2.3, 2.5, 2.4, and 2.3% for control, LNA1, LNA2, and LNA3 diets, respectively. Phospholipids constituted 83.2, 84.4, 86.3, and 86.2% of total lipids. The liver tissue total lipids were 3.9, 3.8, 3.8, and 3.8% for control, LNA1, LNA2, and LNA3 diets, respectively. Phospholipids constituted 88–90% compared with TG at 5–7% of the liver tissue total lipids.

The magnitudes of changes in the fatty acid composition of the tissue lipid classes were different (Tables 4–6). The incorporation of 18:3$n-3$ was predominantly in the TG when compared with PC and PE of muscle, heart, and liver. In contrast, the longer chain $n-3$ fatty acids such as 20:5$n-3$ and 22:5$n-3$ were exclusively in the PE of muscle and liver. Dietary 18:3$n-3$ resulted in a significant ($P < 0.05$) incorporation of 20:5$n-3$ in the heart TG (Table 5). No difference was observed in the 22:6$n-3$ content of muscle and heart tissue TG and PC. The, contents 22:6$n-3$ were increased ($P < 0.05$)

Table 3. Tissue Fatty Acid Composition (Percent) of Pigs Fed Diets Containing Different Levels of 18:3$n-3$

fatty acid	dietary treatment				
	control[a]	LNA1[a]	LNA2[a]	LNA3[a]	SEM[b]
			Muscle		
ΣSFA[c]	35.9	37.3	35.9	35.5	0.84
ΣMUFA[d]	47.4[a]	44.6[b]	38.6[c]	41.2b[c]	1.4
Σ$n-6$	14.0	14.4	15.2	11.6	1.14
Σ$n-3$	1.6[c]	5.9[b]	10.3[a]	11.8[a]	0.52
$n-6$:$n-3$	8.8[a]	2.4[b]	1.5[b]	1.0[b]	0.34
PUFA:SFA[e]	0.4[b]	0.5[b]	0.7[a]	0.7[a]	0.34
			Adipose tissue		
ΣSFA	41.1[a]	35.8[b]	34.9[b]	33.0[b]	1.11
ΣMUFA	45.6[a]	43.1[a]	38.3[b]	35.8[b]	1.19
Σ$n-6$	9.9[b]	12.1[a]	13.2[b]	17.7[a]	0.38
Σ$n-3$	2.0[d]	8.9[c]	13.4[b]	17.7[a]	0.38
$n-6$:$n-3$	4.9[a]	1.4[b]	1.5[b]	1.0[b]	0.34
PUFA:SFA	0.3[b]	0.6[a]	0.8[a]	0.8[a]	0.03
			Heart		
ΣSFA	35.9[a]	36.6[ab]	37.3[a]	36.4[ab]	0.30
ΣMUFA	17.1	17.3	16.1	17.6	1.06
Σ$n-6$	39.0[a]	33.8[b]	31.9[b]	31.3[b]	0.89
Σ$n-3$	4.0[d]	10.9[c]	14.6[b]	16.3[a]	0.51
$n-6$:$n-3$	9.8[a]	3.1[b]	2.2[c]	1.9[c]	0.42
PUFA:SFA	1.2	1.2	1.2	1.3	0.05
			Liver		
ΣSFA	42.8[a]	39.6[a]	30.9[b]	30.7[b]	1.94
ΣMUFA	16.2	16.5	17.3	17.3	1.29
Σ$n-6$	34.8[a]	28.1[b]	35.1[a]	35.8[a]	0.68
Σ$n-3$	4.0[d]	10.9[c]	14.6[b]	16.3[a]	0.39
$n-6$:$n-3$	7.4[a]	3.5[b]	3.2[b]	3.0[b]	0.63
PUFA:SFA	0.9[b]	0.9[b]	1.5[a]	1.5[a]	0.19
			Brain		
ΣSFA	42.9[ab]	45.7[a]	41.9[ab]	40.1[b]	0.71
ΣMUFA	30.4	28.6	29.5	29.8	0.31
Σ$n-6$	12.7	12.2	11.9	11.8	0.36
Σ$n-3$	12.3	13.5	12.9	14.8	0.42
$n-6$:$n-3$	1.0	0.9	0.9	0.8	0.21
PUFA:SFA	0.6[b]	0.6[b]	0.6[b]	0.7[a]	0.01

[a] Control, LNA1, LNA2, and LNA3 represent diets with added ground flax seeds as source of 18:3$n-3$ at 0, 10, 17.5, or 25%, respectively. Data are presented as mean, $n = 5$. Means with no common superscripts within the same rows differ significantly ($P < 0.05$). [b] SEM, standard error of the mean. [c] SFA, saturated fatty acid. [d] MUFA, monounsaturated fatty acids. [e] PUFA, polyunsaturated fatty acid.

in the heart and liver PE of pigs receiving LNA2 and LNA3 diets compared to the pigs receiving control diet. However, doubling the dietary 18:3$n-3$ (LNA1 vs LNA3) did not result in any significant change in liver PE 22:6$n-3$ content (Table 6). Arachidonic acid was significantly lowered ($P < 0.05$) in the muscle, heart, and liver TG, PC, and PE of pigs receiving LNA1, LNA2, and LNA3 diets compared with the control pigs (Tables 4–6). This reduction reached a plateau with LNA2 diet in the lipid classes of muscle and heart tissue.

Dietary 18:3$n-3$ did not result in any change in the total lipid or phospholipid content of brain tissue. The total lipid contents of brain tissue were 9.9, 10.3, 10.9, and 9.9% total lipids. In the brain total lipids, phospholipids constituted 78.6, 79.0, 78.9, and 80% for control, LNA1, LNA2, and LNA3 diets, respectively. Compared with other tissues examined, dietary treatment did not change the $n-6$ or $n-3$ fatty acid composition of brain tissue. Arachidonic acid and DHA constituted the major long-chain $n-6$ and $n-3$ PUFAs of brain tissue. As observed in the other tissue, the longer chain $n-6$ and $n-3$ PUFA were concentrated in the brain PE fraction. In the phospholipid fraction, arachidonic acid constituted over 9% compared with 4% in the PC fraction, and DHA constituted over 10% in the PE fraction compared to 3.5% in the PC fraction. Dietary treatment did not change the monounsaturated fatty acid content in the brain tissue. However, the total saturated fatty acids were significantly lower in pigs fed LNA1, LNA2, and LNA3 diets (Table 3).

Table 4. $n-6$ and $n-3$ Fatty Acid Composition (Percent) of Muscle Triglyceride, Phosphatidylcholine, or Phosphatidylethanolamine in Pigs Fed Diets Containing Different Levels of 18:3$n-3$

fatty acid	dietary treatment				
	control[a]	LNA1[a]	LNA2[a]	LNA4[a]	SEM[b]
			TG		
18:2$n-6$	11.2	10.8	11.7	11.7	0.22
20:4$n-6$	0.2[a]	0.1[b]	0.1[b]	0.1[b]	0.01
18:3$n-3$	2.1[c]	10.2[b]	14.1[a]	15.0[a]	0.49
20:5$n-3$	0.0[c]	0.1[b]	0.2[a]	0.2[a]	0.06
22:5$n-3$	0.1[b]	0.2[a]	0.3[a]	0.3[a]	0.01
			PC		
18:2$n-6$	32.5	30.2	31.0	31.9	0.47
20:4$n-6$	4.1[a]	1.8[b]	1.8[b]	1.9[b]	0.11
18:3$n-3$	0.8[c]	5.0[b]	6.6[a]	7.0[a]	0.23
20:5$n-3$	0.4[c]	1.3[b]	1.8[ab]	2.3[a]	0.07
22:5$n-3$	0.6[b]	0.8[a]	0.9[a]	0.9[a]	0.03
22:6$n-3$	0.4[a]	0.2[b]	0.2[b]	0.2[b]	0.01
			PE		
18:2$n-6$	22.5	23.2	21.9	21.9	0.45
20:4$n-6$	20.2[a]	13.8[b]	9.7[c]	9.8[c]	0.31
18:3$n-3$	0.7[c]	4.6[b]	5.5[c]	5.9[c]	0.18
20:5$n-3$	1.8[c]	7.2[b]	10.2[a]	12.1[a]	0.25
22:5$n-3$	3.1[b]	5.2[a]	5.7[a]	5.9[a]	0.15
22:6$n-3$	0.2	0.1	0.1	0.1	0.02

[a] Control, LNA1, LNA2, and LNA3 represent diets containing 0, 1.5, 2.5, or 3.5% α-linolenic acid along with wheat, soybean meal based control diet. Data are presented as mean, $n = 5$. Means with no common superscripts within the same rows differ significantly ($P < 0.05$). [b] SEM, standard error of the mean.

Table 5. $n-6$ and $n-3$ Fatty Acid Composition (Percent) of Heart Triglyceride, Phosphatidylcholine, or Phosphatidylethanolamine in Pigs Fed Diets Containing Different Levels of 18:3$n-3$

fatty acid	dietary treatment				
	control[a]	LNA1[a]	LNA2[a]	LNA3[a]	SEM[b]
			TG		
18:2$n-6$	38.5	41.1	39.0	40.2	1.78
20:4$n-6$	15.9[a]	6.0[b]	4.4[b]	4.2[b]	0.51
18:3$n-3$	1.7[d]	8.2[c]	10.7[b]	13.0[a]	0.39
20:5$n-3$	1.9[c]	5.6[b]	7.9[a]	8.3[a]	0.63
22:6$n-3$	1.1[a]	0.7[b]	0.6[b]	0.8[b]	0.07
			PC		
18:2$n-6$	16.9	17.0	17.6	16.0	0.89
20:4$n-6$	4.5[a]	1.8[b]	1.4[b]	1.3[b]	0.19
18:3$n-3$	0.5[d]	2.7[c]	3.5[b]	4.4[a]	0.24
20:5$n-3$	0.6[b]	0.9[b]	2.7[a]	1.5[b]	0.33
22:5$n-3$	0.1	0.4	0.3	0.2	0.78
22:6$n-3$	0.4	0.4	0.2	0.3	0.09
			PE		
18:2$n-6$	14.9[b]	18.4[a]	15.3[b]	15.3[b]	0.82
20:4$n-6$	9.9[a]	7.1[ab]	4.2[b]	4.4[b]	1.14
18:3$n-3$	0.9[c]	2.6[b]	3.1[b]	3.8[a]	0.15
20:5$n-3$	1.0[c]	3.8[b]	4.9[a]	5.8[a]	0.31
22:5$n-3$	1.0[b]	1.7[a]	0.9[b]	1.8[a]	0.19
22:6$n-3$	0.4[b]	0.5[b]	0.7[a]	0.7[a]	0.14

[a] Control, LNA1, LNA2, and LNA3represent diets containing 0, 1.5, 2.5, 3.5% α-linolenic acid along with wheat, soybean meal based control diet. Data are presented as mean, $n = 5$. Means with no common superscripts within the same rows differ significantly ($P < 0.05$). [b] SEM, standard error of the mean.

DISCUSSION

Results from the present study indicate that dietary 18:3$n-3$ produced a marked effect on the $n-6$ and $n-3$

Table 6. n−6 and n−3 Fatty Acid Composition (Percent) of Liver Triglyceride, Phosphatidylcholine, or Phosphatidylethanolamine in Pigs Fed Diets Containing Different Levels of 18:3n−3

fatty acid	dietary treatment				
	control[a]	LNA1[a]	LNA2[a]	LNA[a]	SEM[b]
		TG			
18:2n−6	12.2	14.3	14.4	14.6	0.60
20:4n−6	4.8[b]	3.7[b]	2.5[c]	2.2[a]	0.38
18:3n−3	1.3[c]	5.3[b]	6.8[b]	12.5[a]	0.63
20:5n−3	0.6[d]	2.4[c]	4.9[b]	11.8[a]	0.45
22:5n−3	1.2[c]	2.5[b]	3.2[ab]	3.8[a]	0.26
22:6n−3	0.3[b]	0.4[b]	0.3[b]	1.1[a]	0.08
		PC			
18:2n−6	12.9	13.6	11.5	13.1	0.79
20:4n−6	11.8[a]	6.2[b]	4.5[b]	4.6[b]	0.57
18:3n−3	0.9[c]	2.6[b]	3.6[ab]	4.7[a]	0.42
20:5n−3	1.9[c]	5.0[b]	6.3[ab]	8.1[a]	0.64
22:5n−3	2.7	2.8	3.2	3.2	0.28
22:6n−3	1.2[a]	1.1[a]	0.7[b]	0.7[b]	0.11
		PE			
18:2n−6	8.4[b]	11.0[b]	12.1[a]	10.8	0.32
20:4n−6	24.3[a]	12.6[b]	9.1[c]	3.7[d]	0.63
18:3n−3	0.2[d]	2.6[c]	8.9[a]	5.6[b]	0.45
20:5n−3	1.1[d]	7.9[b]	5.8[c]	14.8[a]	0.64
22:5n−3	1.9[b]	2.9[b]	5.0[a]	4.0[a]	0.52
22:6n−3	0.4[b]	1.8[a]	2.3[a]	2.5[a]	0.41

[a] Control, LNA1, LNA2, and LNA3 represent diets containing 0,1.5, 2.5, or 3.5% α-linolenic acid along with wheat, soybean meal based control diet. Data are presented as mean, $n = 5$. Means with no common superscripts within the same rows differ significantly ($P < 0.05$). [b] SEM, standard error of the mean.

PUFA composition of pig tissue total lipids, TG, PC, and PE. However, there appeared to be an anatomical and a lipid class preference among fatty acids for tissue deposition. For example, the increase in n−3 polyunsaturation observed in muscle, backfat, heart, and liver was not evident in the brain tissue. Among the lipid classes, the contents of long-chain PUFA (20:5n−3, 22:6n−3, and 20:4n−6) were 2−5 times higher concentration in the PE as opposed to PC. The biochemical mechanisms for this lipid class-specific incorporation of long-chain PUFA remain to be elucidated. A similar PE-specific increase in long-chain n−3 fatty acids was reported in yolk lipids (Jiang et al., 1991) when flax seeds were fed to laying hens. Human subjects fed diets containing flax seed or oil had marked enrichment of platelet alkenylacyl PE, a subclass of PE, with long-chain n−3 fatty acids (Aukema and Holub, 1989), indicating that this PE-specific incorporation of long-chain n−3 and n−6 fatty acids might not be species-specific for pigs.

The progressive increase in 20:5n−3 in the heart and liver muscle PC and PE fractions through dietary supplementation of 18:3n−3 indicated that pigs efficiently elongated and desaturated 18:3n−3 to 20:5n−3. One of the putative major benefits of increased levels of 20:5n−3 after fish oil consumption is a reduction of 20:4n−6 and a down regulation of eicosanoid formation by n−3 PUFAs (Dyerberg, 1980). In this regard, the present observation of raised 20:5n−3 and lowered 20:4n−6 concentrations in the tissue PC and PE is potentially beneficial since 20:4n−6 has been reported as a standard agonist for inducing platelet aggregation (Lands et al., 1973; Kristensen et al., 1989). The increase in 20:5n−3 in the muscle and heart reached its maximum saturation with LNA2, suggesting a dietary threshold or saturation for the incorporation of 20:5n−3. Beyond that threshold, uptake and/or synthesis of dietary 18:3n−3 to longer chain n−3 fatty acid may be inhibited or limited in these tissues. However, this was not true for the liver or brain PE. This difference in long-chain PUFA and anatomical location may arise mainly from the interactions among the different metabolic pathways for n−3 and n−6 fatty acid groups.

An important interaction between 18:3n−3 and 20:4n−6 is observed in the muscle, backfat, heart, and liver. The decline in arachidonic acid content (predominantly in the PC and PE) suggests a competitive inhibition of 20:4n−6 synthesis or acylation when 18:3n−3 was fed. It has been known that the enzymatic pathway for the synthesis of 20:4n−6 from 18:2n−6 is shared by n−3 fatty acids (Brenner, 1971) and 18:3n−3 inhibits the Δ-6 desaturase and thereby reduces the conversion of 18:2n−6 to 20:4n−6 (Iritani and Narita, 1984; Garg et al., 1988). However, the results from the present study suggest a dietary threshold exists in muscle, backfat, heart, and liver for the competition between 18:2n−6 and 18:3n−3 for desaturation and elongation to form 20:4n−6 and 20:5n−3. Arachidonic acid is an important component of cell membrane phospholipid. In addition to its involvement in maintaining membrane physiochemical properties, 20:4n−6 is a substrate for biosynthesis of eicosanoids. Therefore, insurance of normal concentrations of 20:4n−6 and 20:5n−3 may be a protective mechanism by the tissue for proper tisssue-related functions.

The demonstration that brain percentage of 18:3n−3, 20:4n−6, 20:5n−3, and 22:6n−3 did not change, despite the marked diet-induced changes observed in other tissues examined, is important. These results are consistent with the known resistance of brain tissue relative to other organs to dietary manipulation (Innis, 1991). Recent studies have reported a significant increase in brain tissue DHA in piglets receiving formula rich in 18:3n−3 (Arbuckle et al., 1994) or sow milk high in 18:3n−3 (Cherian and Sim, 1994). The pigs in the present study were at 21 weeks of age when slaughtered, suggesting the vulnerability to dietary manipulation of brain fatty acids may not be as pronounced as in piglets. Whether this effect is specific to brain or reflects a difference in metabolic handling of dietary 18:3n−3 when compared to other tissues studied is unknown.

It is clear from the present study that a high level of 18:3n−3 resulted in an enrichment of 18:3n−3 and 20:5n−3 with a concomitant reduction in 20:4n−6 in the muscle, backfat, heart, and liver lipids. The tissue enhancement of n−3 fatty acids was not uniform, with muscle tissue incorporating the highest level of total n−3 fatty acids (Figure 1). As n−3 fatty acids have been reported to exert beneficial effects on plasma lipids, blood pressure (Simopoulos, 1991; Fernades and Venkatraman, 1993), and platelet function (Tremoli et al., 1995), the change in 20:5n−3 to 20:4n−6 content in these tissue lipid classes (Tables 4−6) would enhance the nutritional value of pork products. Recently (Ahn et al., 1995) reported no quality deterioration or off-flavor in sausages prepared from pigs fed 18:3n−3. As the lipid composition of the food products has been a primary area of consumer concern, the increase in n−3 fatty acids in the muscle, backfat, heart, and liver would make pork or pork products a viable alternate food source of n−3 fatty acids. The nonuniformity in fatty acid incorporation may be a potential consequence of tissue-specific pathways for metabolic turnover of fatty acids. These results may also provide insight into where

$n-3$ fatty acids will be incorporated in human tissues following diet supplementation or may have implications in vegetarians whose dietary source of long-chain $n-3$ PUFA is from 18:3 $n-3$ and in patients fed for extended periods on enteral or parenteral nutrition.

ACKNOWLEDGMENT

The assistance of C. Antoniuk, Q. Feng, M. Fenton, and R. Weingardt in technical and statistical analysis is gratefully acknowledged.

LITERATURE CITED

Ahn, D. U.; Lutz, S.; Cherian, G.; Wolfe, F. H.; Sim, J. S. Lipid oxidation and sensory characteristics of fresh and cured sausages from α-linolenic acid enriched pork. *J. Food Qual.* **1995**, in press.

Arbuckle, L. D.; MacKinnon, M. J.; Innis, S. M. Formula 18:2 (n-6) and 18:3 (n-3) content and ratio influence long-chain polyunsaturated fatty acids in the developing piglet liver and central nervous system. *J. Nutr.* **1994**, *124*, 289–298.

Aukema, H. M.; Holub, B. J. Effect of dietary supplementation of fish oil concentrate on the alkenylacyl class of ethanolamine phospholipids in human platelets. *J. Lipid Res.* **1989**, *30*, 59–64.

Brenner, R. R. The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. *Lipids* **1971**, *6*, 567–575.

Cherian, G.; Sim, J. S. Omega-3 fatty acid composition of the newly hatched chicks from the α-linolenic acid enriched eggs. *Lipids* **1992a**, *27*, 706–710.

Cherian, G.; Sim, J. S. Preferential accumulation of n-3 fatty acids in the brain of chicks from eggs enriched with n-3 fatty acids. *Poult. Sci.* **1992b**, *71*, 1658–1668.

Cherian, G.; Aherne, F. X.; Sim, J. S. Maternal dietary alpha-linolenic acid (18:3 n-3) influences the long chain n-3 fatty acids of sow milk and tissues of milk-fed piglets. *Int. News Fats, Oils Relat. Mater.* **1994**, *5* (4), 472–473.

Dyerberg, J.; Bang, H. O. All cis-5,8,11,14,17-eicosapentaenoic acid and triene prostaglandins: potential anti-thrombotic agents. *Scand. J. Clin. Lab. Invest.* **1980**, *40*, 589–593.

Fernades, G.; Venkatraman, J. T. Role of omega-3 fatty acids in health and disease. *Nutr Res.* **1993**, *13*, S19–S45.

Folch, J.; Lees, M.; Stanley, G. H. S. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957** *226*, 497–507.

Garg, M. L.; Sebokova, E.; Thompson, A. B. R.; Clandinin, M. T. δ^6-Desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω3 fatty acids. *Biochem J.* **1988**, *249*, 351–356

Health and Welfare Canada. *Nutrition Recommendations*; Canadian Government Publishing Centre: Ottawa, 1990; Vol. 24, p 58.

Hodge, J.; Sanders, K.; Sinclair, A. J. Differential utilization of eicosapentaenoic acid and docosahexaenoic acid in human plasma. *Lipids* **1993**, *28* (6), 525–531.

Holub, B. J. Effects of flax and dietary alpha-linolenic acid on blood platelets in human subjects. In *Proceedings of the 53rd Flax Institute of the United States*; Flax Institute: Fargo, ND; 1990; pp 12–15.

Innis, S. M. Essential fatty acids in growth and development. *Prog. Lipid Res.* **1991**, *30*, 1173–1184.

Irie, M.; Sakimoto, S. Fat characteristics of pigs fed fish oil containing eicosapentaenoic and docosahexaenoic acids. *J. Anim. Sci.* **1991**, *70*, 470–477.

Iritani, N.; Narita, R. Changes of arachidonic acid and n-3 polyunsaturated fatty acids of phospholipid classes in liver, plasma and platelets during dietary fat manipulation. *Biochim. Biophys. Acta* **1984**, *793*, 441–448.

Jiang, Z.; Ahn, D. U.; Sim, J. S. Effects of feeding flax and two types of sunflower seeds on fatty acid composition of yolk lipid classes. *Poult. Sci.* **1991**, *70*, 2467–2475.

Kristensen, S. D.; Schmidt, E. B.; Dyerberg, J. Dietary supplementation with n-3 polyunsaturated fatty acids and human platelet function: a review with particular emphasis on implications for cardiovascular disease. *J. Intern. Med.* **1989**, *225*, 141–150.

Lands, W. E. M. Biochemistry and physiology of n-3 fatty acids. *FASEB J.* **1992**, *6*, 2530–2536.

Lands, W. E. M.; LeTellier, P. E.; Rome, L. H.; Vanderhoe, J. Y. Inhibition of prostaglandin biosynthesis. *Adv. Biosci.* **1973**, *9*, 15–28.

Metcalfe, L. D.; Pelka, J. B. A rapid preparation of fatty acids ester for gas chromatography. *Anal Chem.* **1961**, *33*, 363–364.

Morgan, C. A.; Noble, R. C.; Cocchi, M.; McCartney, R. Manipulation of the fatty acid composition of pig meat lipid by dietary means. *J. Agric. Food Sci.* **1992**, *58*, 357–368.

NRC. *Nutrient Requirements of Swine;* National Academy Press: Washington, DC, 1988.

Otten, W.; Wirth, C.; Iaizzo, P. A.; Eichinger, H. M. A high omega-3 fatty acid diet alters fatty acid composition of heart, liver, kidney, adipose tissue and skeletal muscle in swine. *Ann. Nutr. Metab.* **1993**, *37*, 134–141.

SAS. *User's Guide: Statistics*, version 5 ed.; SAS Institute: Cary, NC, 1985.

Sim, J. S.; Cherian, G. Alpha-linolenic acid for designing poultry products for human consumption: Canadian designer eggs and nutritional significance. *Proceedings of the 55th Flax Institute of the Unites States*: Flax Institute: Fargo, ND, 1994

Simopoulos, A. P. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* **1991**, *54*, 438–463.

Sprecher, H. Biochemistry of essential fatty acids. *Prog. Lipid Res.* **1981**, *20*, 13–22.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*, 2nd ed.; McGraw-Hill Book: Toronto, 1980; pp 186–187.

Stubbs, C. D.; Smith, A. D. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **1984**, *779*, 89–137.

Tremoli, E.; Maderna, P.; Marangoni, F.; Colli, S.; Eligini, S.; Catalano, I.; Angeli, M. T.; Pazzucconi, F.; Gianfranceschi, G.; Davi, G.; Stragliotto, E.; Sirtori, C. R.; Galli, C. Prolonged inhibition of platelet aggregation after n-3 fatty acid ethyl ester ingestion by healthy volunteers. *Am. J. Clin. Nutr.* **1995**, *61*, 607–613.

Van Elswyk, M. E. Designer foods: manipulating the fatty acid composition of meat and eggs for the health conscious consumer. *Nutr. Today* **1993**, 21–28.

Wainwright, P. E. Do essential fatty acids play a role in brain and behavioral development. *Neurosci. Behav. Rev.* **1992**, *16*, 193–205.

Received for review February 14, 1995. Accepted August 14, 1995.[⊗] This work was supported by Natural Sciences and Engineering Research Council of Canada, Agriculture Canada, and Western Grain Research Foundation.

JF950096U

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.