Blood and Aorta Lipid Status and Platelet Function in Swine Modified by Dietary α-Linolenic Acid-Rich Flax Seed

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The effects of dietary 18:3*n* − 3 on the fatty acid composition of plasma, red blood cells, aorta, platelets, plasma cholesterol, triglycerides, and platelet response to collagen-induced aggregation in swine was 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). Dietary 18:3*n* − 3 resulted in a significant ($P < 0.05$) enrichment of 18:3*n* − 3, 20:5*n* − 3, and 22:5*n* − 3 in plasma, red blood cells, aorta, platelet neutral, and phospholipids with a concomitant reduction in 20:4*n* − 6 ($P < 0.05$). Dietary 18:3*n* − 3 did not alter the plasma cholesterol concentration or 22:6*n* − 3 fatty acid in any of the tissues examined. However, plasma triglyceride concentration was reduced significantly ($P < 0.05$) in pigs receiving LNA1, LNA2, and LNA3 diets when compared with the pigs receiving the control diet. Platelet aggregation induced by collagen was significantly higher ($P < 0.05$) in the control group when compared with LNA2 and LNA3 diets, respectively.

Keywords: *Pig; 18:3n − 3; 20:5n − 3; blood; aorta; platelet; platelet aggregation*

INTRODUCTION

Long-chain *n* − 3 polyunsaturated fatty acids (PUFA) such as 20:5*n* − 3 and 22:6*n* − 3 from marine oils has been a source of much research due to their beneficial effects on plasma triglycerides (Surette et al., 1992), blood pressure (Simopoulos, 1991; Singer and Hueve, 1991), platelet function (Tremoli et al., 1995) and thrombosis (Dyerberg, 1982, 1986; Kinsella et al., 1990). The beneficial effects of *n* − 3 PUFA from marine oils on thrombosis are mediated by alterations in the synthesis of eicosanoids (prostaglandins, thromboxanes, prostacyclin, and leukotrienes) derived from 20:5*n* − 3 (Herold and Kinsella, 1986; Lokesh et al.,1986; Leaf and Webber, 1988).

Most of the research on the health benefits and antiaggregatory effects of dietary *n* − 3 fatty acids has focused on fish oil-rich 20:5*n* − 3, mainly because it is the direct precursor of antithrombogenic prostanoids. However, increase in cellular 20:5*n* − 3 concentrations has also resulted from ingestion of diets rich in 18:3*n* − 3, the parent fatty acid of 20:5*n* − 3 (Mantzioris et al., 1994; Lorgeril et al., 1994). Both 18:3*n* − 3 and 18:2*n* − 6 share the same enzyme system for desaturation and elongation (Sprecher, 1981) to form longer chain *n* − 3 and *n* − 6 fatty acids such as 20:5*n* − 3 and 20:4*n* − 6. Due to structural similarities, dietary *n* − 3 fatty acids can displace 20:4*n* − 6 from tissue phospholipids by competing for acylation pathways (Lands, 1992). The extent of *n* − 3 PUFA incorporation into cellular phospholipids may therefore depend on the amounts of 18:3*n* − 3 and 18:2*n* − 6 in the diet. Consequently, the ability of dietary *n* − 3 PUFA to modulate tissue 20:4*n* − 6 levels, eicosanoid synthesis, and thrombogenesis may be limited by the levels of 18:2*n* − 6 and 18:3*n* − 3 (Chan et al., 1993). This is of special interest in the vascular component, where these compounds perform diverse biological activities and may modulate the progression of pathological states.

Blood platelet aggregation response and platelet−blood vessel wall interactions were reported to be reduced by increased 20:5*n* − 3:20:4*n* − 6 ratio in platelet phospholipids (Burri et al., 1991; Chan et al., 1993). Considering the conditions that may optimize the capacity to increase tissue 20:5*n* − 3, it is important to examine different levels of 18:3*n* − 3 in the diet and its effect on the 20:4*n* − 6:20:5*n* − 3 ratio in the blood and tissues. In the present study the effect of feeding different levels of 18:3*n* − 3 on the fatty acid content of plasma, red blood cells (rbc), platelets, and aorta and the plasma triglyceride, cholesterol and platelet response to collagen-induced aggregation in pigs were explored. As pigs are used as a model for lipid metabolism study in humans, these results may provide insight into the pattern of *n* − 3 fatty acid incorporation in human blood lipids 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 soybean meal-based diet with added ground flax seeds at 0%, (control) 10% (LNA1), 17% (LNA2), and 25% (LNA3). The experimental diets also contained animal tallow at 3.3% (control), 2.0% (LNA1), 1.2% (LNA2) and 0.4% (LNA3), respectively. The composition of the diet was reported earlier (Cherian and Sim, 1995). Ground flax seeds were used as a dietary source of 18:3*n* − 3. The LNA1, LNA2, and LNA3 diets

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Table 1. Fatty Acid Composition of the Experimental Diets

fatty acid[a] (%)	control[b]	LNA1[b]	LNA2[b]	LNA3[b]
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
18:2$n-6$	28.2	27.2	23.4	21.9
18:3$n-3$	4.1	26.2	36.6	45.7
∑SFA	20.4	15.3	12.3	11.1
∑MUFA	46.4	31.1	26.5	20.5

[a] SFA = saturated fatty acid; MUFA = monounsturated fatty acid. [b] Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds at 0, 10, 17, or 25%.

provided 18:3$n-3$ at 1.5, 2.5, or 3.6%. All the diets were formulated to contain sufficient vitamins and minerals to meet the pig's requirement for growth (NRC, 1988). The fatty acid composition of the experimental diet is presented in Table 1. Feed and water were provided ad libitum. The pigs were fed the experimental diets for a period of 4 months.

Sample Collection and Preparation. Blood (6 mL) was collected at the end of the trial period from the ear vein using evacuated citrated tubes. When pigs reached an average of 99−105 kg, they were delivered to a commercial slaughterhouse where they were stunned by electrical shock. The aorta was excised washed with saline, and kept frozen till analyses.

Platelet-rich plasma was separated from the blood (3 mL) by centrifugation for 15 min at 120*g* at 4 °C. The platelets were recovered by further centrifugation, 15 min at 1100*g* at 4 °C. The platelets were washed twice with 3 mM ethylenediaminetetraacetic acid in saline (Innis et al., 1993). Plasma and rbc were separated by centrifugation of whole blood (3 mL) at 2500*g* for 25 min at 4 °C.

Lipid Analyses. Total lipid was extracted from feed samples, plasma, rbc, platelets, and aorta according to the method of Folch et al. (1957) with 20 mL of (chloroform: methanol = 2:1, v/v). Platelet neutral and phospholipids were separated on precoated silica gel G plates (20 × 20 cm) according to Christie (1982). The spots corresponding to neutral and phospholipids were identified under ultraviolet light, and were scraped off into screw-capped tubes. Aliquots of the lipid extracts from plasma, rbc, aorta, platelet neutral, and phospholipids were converted to fatty acid methyl esters (FAME) with boron trifluoride, methanol, and hexane (35:45: 20, v/v/v) solution in a boiling water bath for 1 h (Metcalfe and Pelka, 1961). FAME were separated by an automated gas chromatograph (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, Canada, 30 m × 0.25 mm inside diameter). The conditions of the gas chromatograph were described earlier (Cherian and Sim, 1992). FAME were identified by comparison with retention times of authentic standards (Nu-Check-Prep). A Shimadzu EZChrom (Shimadzu Scientific Instruments, Inc., Columbia, MD) laboratory data integration system was used to integrate peak areas. Fatty acid values are expressed as weight percentages. Cholesterol content was determined on plasma samples after saponification with 5-α-cholestane as the internal standard, and the cholesterol content was measured by gas chromatography. The conditions of the gas chromatograph were described earlier (Fenton and Sim, 1990). Plasma triglycerides were determined calorimetrically using a Sigma diagnostic kit (Sigma Chemical, St. Louis, MO).

Platelet Aggregation in Whole Blood. For whole blood platelet aggregation blood samples (1 mL) were mixed with 1 mL of saline (Gerwitz et al., 1985). The diluted blood (1 mL) was transferred to an aggregometer cuvette in the thermostated (37 °C) cuvette holder of a whole blood aggregometer (Model 500, Chrono-log Corp). A Teflon-coated magnetic stir bar was used to stir the sample at 1000 rpm. After standardization of the apparatus, 2 μg of collagen (Chrono-log, Canada) was added to the cuvette using a micropipet and the change in impedance, which reflects platelet aggregation around platelets adherent to the electrodes (Ingerman-Wojenski and Silver, 1984; Cardinal and Flower, 1985), was recorded for 4 min. As collagen is the first aggregating factor platelets encounter during trauma, in vitro study of platelet response to collagen has assumed considerable importance. The platelet aggregation response is reported in ohms.

Table 2. Major $n-6$, $n-3$ Fatty Acid Composition, Total Saturated and Monounsaturated Fatty Acids of Plasma and Red Blood Cells of Pigs Fed Different Levels of 18:3$n-3$[e]

	dietary treatments				
fatty acid (%)	control	LNA1	LNA2	LNA3	SEM
		Plasma			
18:2$n-6$	23.2	25.8	22.7	23.0	1.06
20:4$n-6$	6.0[a]	2.9[b]	3.1[b]	1.8[c]	0.26
18:3$n-3$	1.2[c]	7.4[b]	8.7[b]	14.1[a]	0.53
20:5$n-3$	0.3[c]	3.6[b]	6.3[a]	5.0[a,b]	0.51
22:5$n-3$	1.3[c]	1.7[b]	2.4[a]	1.3[c]	0.38
22:6$n-3$	0.0	0.1	0.1	0.1	0.01
20:4$n-6$: 20:5$n-3$	20.0[a]	0.8[b]	0.5[b]	0.4[b]	0.41
∑SFA	32.4	30.2	31.3	29.4	0.90
∑MUFA	31.0[a]	26.5[a,b]	23.2[b]	22.9[b]	1.74
		Red Blood Cells			
18:2$n-6$	16.6	16.2	17.4	16.0	1.11
20:4$n-6$	5.3[a]	2.9[b]	2.4[c]	1.9[d]	0.14
18:3$n-3$	1.1[d]	4.0[c]	5.7[b]	7.5[a]	0.41
20:5$n-3$	0.7[c]	2.2[b]	3.8[a]	3.4[a]	0.18
22:5$n-3$	1.1[a,b]	1.2[a,b]	1.3[a]	0.9[b]	0.09
22:6$n-3$	1.4[b]	3.0[a]	2.7[a]	1.7[b]	0.28
20:4$n-6$: 20:5$n-3$	7.6[a]	1.3b	0.6[c]	0.5[c]	0.34
∑SFA	38.1	36.9	37.6	40.8	1.23
∑MUFA	34.8[a]	33.1[a]	28.6[b]	27.4[b]	1.34

[a−c] Means with no common superscripts within the same rows differ significantly ($P < 0.05$). SEM = standard error of the mean. Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds at 0, 10, 17, or 25%. [e] Data are presented as mean, $n = 5$. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

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

RESULTS AND DISCUSSION

The fatty acid compositions of the diets are shown in Table 1. Generally the fatty acid composition of the diet reflected the dietary source. The control diet had the highest level of total saturates and 18:1 as expected from the composition of animal tallow. α-Linolenic (18:3$n-3$) 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 18:3$n-3$ content of the diet with a concomitant decrease in $n-6$:$n-3$ ratio.

Plasma and Red Blood Cell Fatty Acids. The changes observed in the fatty acid composition of plasma and rbc reflected the fatty acid composition of the diets. Dietary 18:3$n-3$ resulted in a significant enrichment of 18:3$n-3$, and 20:5$n-3$ in plasma total lipids (Table 2) with a concomitant reduction in 20:4$n-6$:20:5$n-3$ ratio. The most pronounced differences were in the ratio of 20:4$n-6$:20:5$n-3$ in pigs receiving LNA1, LNA2, or LNA3 diets when compared to the pigs receiving the control diet ($P < 0.05$) (Table 2). As the diets were devoid of long-chain $n-3$ fatty acids, these

Table 3. Major $n-6$, $n-3$ Fatty Composition, Total Saturated and Monounsaturated Fatty Acids of Platelet Neutral and Phospholipids of Pigs Fed Diets Containing Different Levels of 18:3$n-3$[e]

	dietary treatments				
fatty acid (%)	control	LNA1	LNA2	LNA3	SEM
	Neutral Lipids				
18:2$n-6$	26.2[a]	15.1[c]	19.7[b]	23.1[a]	1.11
20:4$n-6$	4.6[b]	4.2[a]	1.8[d]	2.8[c]	0.12
18:3$n-3$	2.8[b]	3.9[b]	10.0[a]	12.6[a]	1.48
20:5$n-3$	0.7[a]	2.5[b]	2.9[b]	3.1[b]	0.27
22:5$n-3$	0.1[a]	0.3[a]	0.4[a]	0.8[b]	0.19
22:6$n-3$	0.0	0.1	0.4	0.3	0.11
20:4$n-6$:20:5$n-3$	6.6[a]	1.7[b]	0.6[c]	0.9[c]	0.23
∑SFA	24.9[b]	31.3[a]	28.1[a,b]	23.8[b]	1.83
∑MUFA	38.4[a]	41.5[a]	31.2[b]	30.1[b]	1.09
	Phospholipids				
18:2$n-6$	19.1[a]	12.8[b]	11.9[b]	18.5[a]	0.47
20:4$n-6$	6.6[a]	4.5[b]	3.4[b,c]	2.7[c]	0.43
18:3$n-3$	2.4[b]	2.5[b]	7.0[a]	7.0[a]	0.64
20:5$n-3$	1.7[b]	2.0[c]	2.6[a]	2.6[a]	0.13
22:5$n-3$	0.8	0.7	0.6	1.3	0.22
22:6$n-3$	0.6	0.6	0.6	0.7	0.13
20:4$n-6$:20:5$n-3$	3.9[a]	2.3[b]	1.3[c]	1.0[c]	0.02
∑SFA	40.4[b,c]	46.8[a]	38.7[c]	41.8[b]	0.62
∑MUFA	24.8[b]	27.5[a]	21.6[c]	19.2[d]	0.32

[a–d] Means with no common superscripts within the same rows differ significantly ($P < 0.05$). SEM = standard error of the mean. Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds at 0, 10, 17, or 25%. [e] Data are presented as mean, $n = 5$. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

Table 4. Major $n-6$, $n-3$ Fatty Composition, Total Saturated and Monounsaturated Fatty Acids of Aorta from Pigs Fed Diets Containing Different Levels of 18:3$n-3$[d]

	dietary treatments				
fatty acid (%)	control	LNA1	LNA2	LNA3	SEM
18:2$n-6$	6.2	8.6	7.7	6.9	0.69
20:4$n-6$	3.1[a]	2.5[b]	1.5[c]	1.3[c]	0.51
18:3$n-3$	0.8[b]	5.1[a]	6.3[a]	5.9[a]	0.85
20:5$n-3$	0.2[c]	0.6[b]	1.1[a]	1.0[a]	0.11
22:5$n-3$	0.5[a]	0.6[a]	1.0[b]	1.0[b]	0.09
22:6$n-3$	0.2	0.2	0.3	0.3	0.05
∑SFA	43.3	43.1	41.7	42.8	1.12
∑MUFA	40.7[a]	42.3[a]	39.6[a,b]	36.4[b]	1.01

[a–c] Means with no common superscripts within the same rows differ significantly ($P < 0.05$). SEM = standard error of the mean. Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds at 0, 10, 17 or 25%. [d] Data are presented as mean, $n = 5$. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid.

results suggests that the increase in plasma and rbc content of 20:5$n-3$ is primarily attributable to synthesis from dietary 18:3$n-3$. These results clearly establish the effectiveness of altering the 18:3$n-3$ content in increasing the concentration of 20:5$n-3$ in plasma and rbc total lipids. A leveling effect of 18:3$n-3$ on 20:5$n-3$ content of plasma and rbc fatty acid was observed. The content of longer chain 20:5$n-3$ in plasma and rbc did not alter beyond the LNA2 diet, suggesting that a dietary threshold or saturation for the incorporation of 20:5$n-3$ exists in pigs. Doubling or tripling the 18:3 $n-3$ did not result in any change in plasma 22:6$n-3$ content. These results suggests that the conversion of 18:3$n-3$ to 22:6$n-3$ may be limited in swine or a longer trial period may be needed to increase the plasma and rbc level of 22:5$n-3$ and 22:6$n-3$. The possibility of 22:6$n-3$ being diverted to other tissues or being assimilated to form eicosanoids is not known. These observations corroborate previous reports in humans consuming flax oil rich in 18:3$n-3$ (Mantzioris et al., 1994; Sanders and Yonger, 1981). No effect of 18:3$n-3$ was observed on the 18:2$n-6$ concentrations of plasma and rbc. Additional 18:3$n-3$ in the LNA1, LNA2, and LNA3 diets compared with the control diet did not result in any change in the saturated fatty acid content in the plasma and rbc total lipids (data not shown). However, a reduction in the content of monounsaturated fatty acids (MUFA) were observed in pigs receiving LNA1, LNA2, and LNA3 diets. The content of 18:1 varied from 29.5, 25.4, 22.0, and 21.7 for control, LNA1, LNA2, and LNA3 diets, respectively.

Fatty Acid Composition of Platelet Neutral and Phospholipids. Platelet neutral and phospholipid 18:3$n-3$ fatty acids in the LNA1, LNA2, and LNA3 diets increased ($P < 0.05$), reflecting the dietary fatty acid composition (Table 3). These results are in agreement with previous authors (Sanders and Younger, 1981; Renaud et al., 1986; Weaver et al., 1990). There appeared to be a plateau in incorporation of 18:3$n-3$ and 20:5$n-3$ in the platelet neutral and phospholipids (Table 3). Dietary level of 2.5% (LNA2) 18:3 $n-3$ was sufficient to result in a maximum incorporation of 18:3$n-3$ and 20:5$n-3$ in the platelet lipids. The fatty acid pattern in platelet phospholipids reflects dietary intake coupled with dietary fatty acid metabolism in liver, because platelets do not contain Δ-6 desaturase enzymes responsible for desaturation of 18:3$n-3$ and 18:2$n-6$ (Nordoy and Roset, 1971). In the present study, the content of 20:5$n-3$ in the platelet neutral increased from 0.7% in the control diet to 2.5% in the LNA1 diet. Further increase in dietary 18:3$n-3$ did not result in any change in the 20:5$n-3$ content of platelet neutral lipids. A similar response was observed in the platelet phospholipids. The conversion of 18:3$n-3$ to 20:5$n-3$ in platelets has been reported in humans fed a canola oil-based diet (Weaver et al., 1990). Importantly, the content of 20:4$n-6$ decreased ($P < 0.05$) in the platelet neutral and phospholipids of pigs receiving 18:3$n-3$-supplemented diets. The decrease in 20:4$n-6$ plateaued with the LNA2 diet in the platelet phospholipids. No changes were observed in the 22:6$n-3$ content in platelet neutral and phospholipids. A significant decrease ($P < 0.05$) was observed in the 20:4$n-6$:20:5$n-3$ ratio in the platelet neutral and phospholipids (Table 3). The changes in fatty acyl components of platelet lipids described in this study are of significance since blood platelets play an essential role in arterial thrombosis and atherosclerosis (Chandler, 1982). These changes in the ratio of 20:4$n-6$:20:5$n-3$ in the platelets are seen as desirable because the content of 20:4$n-6$ in the platelet lipids may directly reflect their synthesis of thromboxane A_2 (Lands, 1992), and also because of the association between excess $n-6$ fatty acids in cardiovascular disease and inflammatory conditions (Clelan et al., 1990).

Fatty Acid Composition of Aorta. Pigs fed 18:3$n-3$ rich flax-based diets showed marked increase in 18:3$n-3$ with no differences between LNA1, LNA2, and LNA3 diets (Table 4). Arachidonic acid was decreased ($P < 0.05$) in the aorta of pigs receiving LNA2 and LNA3 diets. The metabolites of 18:3$n-3$ such as 20:5$n-3$ and 22:5$n-3$ were increased to $P < 0.05$ in the LNA2 and LNA3 diets fed pigs. Compared to the pigs fed the control diet, a 50% increase was observed for 22:5$n-3$ in pigs fed the LNA2 diet (Table 4). As observed in plasma, rbc, and platelets, the ingestion of 18:3$n-3$ did not lead to any change in 22:6$n-3$ content in the

Table 5. Total Cholesterol and Triglyceride Content in the Plasma and Platelet Aggregation Due to Collagen in Pigs Fed Diets Containing Different Levels of 18:3*n* − 3[d]

	dietary treatments				
	control	LNA1	LNA2	LNA3	SEM
cholesterol (mg/dL)	100.4	92.8	91.6	91.0	4.98
triglyceride (mg/dL)	52.5[a]	44.6[b]	39.8[c]	38.8[c]	0.72
aggregation (ohm)	19.6[a]	16.0[b]	12.4[c]	11.6[c]	0.17

[a−c] Means with no common superscripts within the same rows differ significantly ($P < 0.05$). SEM = standard error of the mean. Control, LNA1, LNA2, and LNA3 represent diets with added flax seeds at 0, 10, 17, or 25%. [d] Data are presented as mean, $n = 5$.

aorta. The increase in $n - 3$ fatty acids in the LNA2 and LNA3 diets appeared to be at the expense of monounsaturated fatty acids (MUFA). The total MUFA (16:1, 18:1) were reduced ($P < 0.05$) in the LNA2 and LNA3, when compared to control and LNA1 diets, respectively. (Table 4). The saturated fatty acids in the aorta were not altered by dietary treatments. Although the duration on experimental diets was of short period, the increase in 18:3$n - 3$, 20:5$n - 3$, and 22:5$n - 3$ with a concomitant decrease in 20:4$n - 6$ in the aortic tissue fatty acids may be of significance in the vascular compartment where eicosanoids derived from 20-carbon $n - 6$ and $n - 3$ fatty acids are involved in cardiovascular and inflammatory conditions.

Plasma Cholesterol and Triglycerides. The concentrations of plasma cholesterol and triglycerides are presented in Table 5. Although dietary 18:3$n - 3$ produced a favorable effect on the plasma fatty acids, their effect on plasma cholesterol was minimal. There was no significant dietary effect on plasma cholesterol concentration. However, plasma triglyceride concentrations were reduced ($P < 0.05$) in pigs receiving LNA1, LNA2, and LNA3 diets when compared with pigs receiving the control diet. Additional dietary 18:3$n - 3$ in the LNA2 and LNA3 diets when compared to LNA1 diet did not result in any change in the plasma triglyceride levels, suggesting that pigs respond to a moderate level of dietary 18:3$n - 3$ with a lowering of plasma triglyceride levels. A recurring response to dietary $n - 3$ PUFA from fish oils in human and animal trials is a decrease in circulating plasma triglycerides (Surette et al., 1995). Consumption of flax-based bread (Bierenbaum et al., 1993), flax seed oil (Mantzioris et al., 1994), and eggs enriched with 18:3$n - 3$ (Ferrier et al., 1992; Jiang et al., 1993; Sim and Cherian, 1994) has been reported to reduce plasma triglycerides in human volunteers. It has been reported that $n - 3$ fatty acids affect the enzymes involved in hepatic triglyceride synthesis, thus reducing the pool of fatty acids available for triglyceride synthesis and very low density lipoprotein−triglyceride secretion, and/or enhances the β-oxidation of fatty acids in liver (Surette et al., 1992). With the evidence implicating the circulating plasma triglyceride level as an independent risk factor for coronary heart disease (Austin, 1991), the results presented here suggest that ingestion of 18:3$n - 3$ offers some protection against the risk factor for heart disease.

Platelet Aggregation. The extent of platelet aggregation was significantly higher ($P < 0.05$) in the control group when compared with LNA2 and LNA3, indicating enhanced platelet response to collagen. The lowered collagen-induced platelet aggregation in the flax-based diets rich in 18:3$n - 3$ suggests a beneficial effect of 18:3$n - 3$ on platelet function. The changes in platelet function as observed in the present study may be due to altered ratio of 20:4$n - 6$:20:5$n - 3$ observed in the platelet lipids. Significant decrease in platelet aggregability following 18:3$n - 3$ intake has been reported in humans (Renaud et al., 1986; Freese et al., 1994). Canola oil containing moderate levels of 18:3$n - 3$ has been reported to decrease platelet aggregation more than sunflower oil containing $n - 3$ fatty acids (McDonald et al., 1989; Kwon et al., 1991). The influence of diets enriched with 18:3$n - 3$ on degree of platelet aggregation is thought to be mediated through prostaglandins and thromboxane metabolism. Dietary 18:3$n - 3$ inhibits not only the metabolism of arachidonic acid via cyclooxygenase but also the conversion of 18:2$n - 6$ to 20:4$n - 6$ (Kinsella, 1990). Suppression of eicosanoids derived from 20:4$n - 6$ by dietary 18:3$n - 3$ has been demonstrated (Hwang, 1980; Marshall and Johnson, 1982) in plasma, rbc, and platelet lipids. Because of this precursor−product relation, enhancement of the dietary intake of 18:3$n - 3$ may affect eicosanoid production and platelet function in the body. In the light of the present results it appears that an increase in 18:3$n - 3$ content and a decrease in the ratio of 20:4$n - 6$ to 20:5$n - 3$ decrease platelet response due to collagen.

In the present study, the diets high in 18:3$n - 3$ also were low in saturated fatty acids and polyunsaturated: saturated fatty acid (P:S) ratio. Therefore, the differences in intakes of saturated fatty acid and reduced P:S ratio may be taken into consideration. Over the past century, the Western diet has been predominated by higher saturated fats and a high dietary ratio of 18:2$n - 6$:18:3$n - 3$ (Simopoulos, 1991; Lands, 1992). This dietary imbalance is reflected in the low levels of 20:5$n - 3$ and 22:6$n - 3$ in the plasma, erythrocytes, platelets, and atherosclerotic plaques of people on a typical Western diet (Hodge et al., 1993; Sassen et al., 1993). While feeding 18:3$n - 3$-rich flax seed resulted in dramatic changes in 20:5$n - 3$ and 20:4$n - 6$ in blood, aorta, and platelets as observed in the present study, it is important to consider vegetable sources of $n - 3$ fatty acids, such as 18:3$n - 3$, particularly under conditions that may optimize their capacity to increase tissue concentration of 20:5$n - 3$. The potential exists for the long-term use of moderate amounts of linolenic acid-rich vegetable oils such as canola oil in place of other cooking oils or linolenic acid-enriched agri-food products such as breads, eggs, and meat could lead to significant changes in blood and aortic tissue 20:5$n - 3$ and 20:4$n - 6$ concentrations and reduce the risk of cardiovascular diseases.

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