Effect of Dietary α-Linolenic Acid and Its Ratio to Linoleic Acid on Platelet and Plasma Fatty Acids and Thrombogenesis

Joanna K. Chan[a,1], Bruce E. McDonald[a,*], Jon M. Gerrard[b], Vivian M. Bruce[a], Bonnie J. Weaver[c] and Bruce J. Holub[c]

[a]Department of Foods and Nutrition, University of Manitoba, Winnipeg, R3T 2N2 Canada, [b]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, R3A 1S1 Canada and [c]Department of Nutritional Sciences, University of Guelph, Guelph, N1G 2W1 Canada

The effect of dietary α-linolenic acid (18:3n-3) and its ratio to linoleic acid (18:2n-6) on platelet and plasma phospholipid (PL) fatty acid patterns and prostanoid production were studied in normolipidemic men. The study consisted of two 42-d phases. Each was divided into a 6-d pre-experimental period, during which a mixed fat diet was fed, and two 18-d experimental periods, during which a mixture of sunflower and olive oil [low 18:3n-3 content, high 18:2/18:3 ratio (LO-HI diet)], soybean oil (intermediate 18:3n-3 content, intermediate 18:2/18:3 ratio), canola oil (intermediate 18:3n-3 content, low 18:2/18:3 ratio) and a mixture of sunflower, olive and flax oil [high 18:3n-3 content, low 18:2/18:3 ratio (HI-LO diet)] provided 77% of the fat (26% of the energy) in the diet. The 18:3n-3 content and the 18:2/18:3 ratio of the experimental diets were: 0.8%, 27.4; 6.5%, 6.9; 6.6%, 3.0; and 13.4%, 2.7, respectively. There were appreciable differences in the fatty acid composition of platelet and plasma PLs. Nevertheless, 18:1n-9, 18:2n-6 and 18:3n-3 levels in PL reflected the fatty acid composition of the diets, although very little 18:3n-3 was incorporated into PL. Both the level of 18:3n-3 in the diet and the 18:2/18:3 ratio were important in influencing the levels of longer chain n-3 fatty acid, especially 20:5n-3, in platelet and plasma PL. Production of 6-keto-$PGF_{1\alpha}$ was significantly ($P < 0.05$) higher following the HI-LO diet than the LO-HI diet although dietary fat source had no effect on bleeding time or thromboxane B_2 production. The present study showed that both the level of 18:3n-3 in the diet and its ratio to 18:2n-6 were important in influencing long-chain n-3 fatty acid levels in platelet and plasma PL and that prostanoid production coincided with the diet-induced differences in PL fatty acid patterns.

Lipids 28, 811-817 (1993).

Coronary heart disease is a degenerative process involving atherosclerosis and thrombogenesis (1,2). Dietary fat affects both of these phenomena (3). Polyunsaturated fatty acids (PUFA) of the n-3 and n-6 families may influence thrombus formation through their effects on the production of prostanoids in the endothelial wall and in the circulating platelets (4-6).

Dietary linoleic acid (18:2n-6) can be desaturated and elongated to arachidonic acid (20:4n-6), which is the precursor of thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) (6,7). Similarly, dietary α-linolenic acid (18:3n-3) can be desaturated and elongated to eicosapentaenoic acid (20:5n-3), the precursor of TXA_3 and PGI_3 (6,7), but the efficiency of conversion has been reported to be relatively low in humans (8,9). TXA_2 is a potent prothrombogenic agent whereas TXA_3 is a weak platelet aggregating agent. By contrast, both PGI_2 and PGI_3 are antithrombogenic (2).

Most of the research on the antithrombogenic effect of dietary fatty acids has focused on dietary 20:5n-3, mainly because it is the direct precursor of antithrombogenic prostanoids and a powerful inhibitor of n-6 PUFA metabolism (9). However, 20:5n-3 and docosahexaenoic acid (22:6n-3) are not effective in lowering blood cholesterol levels (10). Thus, interest has turned to the possible antiatherogenic and antithrombogenic role of dietary 18:3n-3. Both 18:2n-6 and 18:3n-3 share the same enzyme system for desaturation and elongation (2,6,7); hence, there is the possibility for competition between them. Thus, the relative and absolute amounts of these fatty acids in the diet could have an effect on the metabolism of n-6 and n-3 fatty acids which, in turn, could alter prostanoid metabolism and thrombogenesis. The importance of the 18:2/18:3 ratio in the diet has been studied by several investigators (8,11,12). However, a comparison of the effect of the absolute amounts of 18:2n-6 and 18:3n-3 and their ratio in the diet on plasma fatty acid patterns and thrombogenic tendency in the human has not been reported.

The present study was designed to investigate the effect of dietary 18:2n-6 and 18:3n-3 and their ratio on n-3 and n-6 PUFA patterns in platelet and plasma phospholipids (PL) and on bleeding time and on bleeding-time prostanoid formation. Fatty acid patterns of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and alkenylacyl glycerophosphoethanolamines (PPE) are reported. PPE has been shown to be the major storage site for 20:5n-3, although an appreciable amount also is found in the diacyl PC and PE fractions of platelet PL (13,14).

MATERIALS AND METHODS

Experimental design. The study consisted of two 42-d phases with a 2-mon break between phases. Each phase was divided into three periods: a 6-d pre-experimental period and two 18-d experimental periods. Eight male subjects participated in the study. All subjects were fed a mixed fat diet during the pre-experimental period of each phase. In the first experimental period of phase I, two subjects were randomly assigned to one of four experimental diets: sunflower and olive oil [low 18:3n-3 content, high 18:2/18:3 ratio (LO-HI)]; soybean oil [intermediate 18:3n-3 content, intermediate 18:2/18:3 ratio (IN-IN)]; canola oil [intermediate 18:3n-3 content, low 18:2/18:3 ratio (IN-LO)]; and sunflower, olive and flax oil [high 18:3n-3 content, low 18:2/18:3 ratio (HI-LO)]. In the

[1]Present address: Department of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Canada.

*To whom correspondence should be addressed at Department of Foods & Nutrition, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada.

Abbreviations: EDTA, ethylenediaminetetraacetic acid; GLC, gas-liquid chromatography; HI-LO, high 18:3n-3 content, low 18:2/18:3 ratio; IN-IN, intermediate 18:3n-3 content, intermediate 18:2/18:3 ratio; IN-LO, intermediate 18:3n-3 content, low 18:2/18:3 ratio; LO-HI, low 18:3n-3 content, high 18:2/18:3 ratio; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGI, prostacyclin; PL, phospholipid(s); PPE, alkenylacyl glycerophosphoethanolamines; PPP, platelet-poor plasma; PUFA, polyunsaturated fatty acids; TXA, thromboxane.

subsequent experimental periods of phases I and II, each pair of subjects was switched to a different diet according to the following order: LO-HI to IN-IN; IN-IN to IN-LO; IN-LO to HI-LO; and HI-LO to LO-HI.

Subjects. The subjects, who ranged in age from 20 to 34 yr, were students at the University of Manitoba. They were normolipidemic with plasma total cholesterol levels between 3.80 and 5.86 mmol/L at the beginning of the study. The subjects engaged in their normal activities and resided in their own residences throughout the study. They were instructed not to use aspirin during the study and to consult the directors before taking any medication. One subject left after the completion of phase I, and a replacement was recruited for phase II. All meals were served in the metabolic unit located in the Faculty of Human Ecology. The study protocol was approved by the Faculty of Human Ecology Ethics Committee, and written consent was obtained from each subject.

Diets. A two-day cyclic menu of conventional foods was used during the study. Diets used in the study were identical in all respects except sources of added fat which accounted for approximately 26% of total energy (77% of the 120 g/d of total dietary fat). The fatty acid composition of the diets is presented in Table 1. The LO-HI and the IN-LO diets had similar amounts of oleic acid (18:1n-9) and 18:2n-6, but the latter contained about eight times more 18:3n-3. The IN-IN diet was similar in 18:3n-3 content to the IN-LO diet but contained twice the level of 18:2n-6. The levels of 18:2n-6 and 18:3n-3 in the HI-LO diet were approximately twice those in the IN-LO diet, but the 18:2/18:3 ratio was similar to that of the IN-LO diet (3.0 *vs.* 2.7). A one-day composite of each diet was collected during each phase of the study. The composites were homogenized and aliquots lyophilized for chemical analysis. Fat content of the diets was determined by the method of Bligh and Dyer (15). Fatty acid methyl esters were prepared using sodium methoxide in methanol (16), and the fatty acid composition was determined by gas-liquid chromatography (GLC).

TABLE 1

Fatty Acid Composition of the Diets[a]

	Diets[b]				
Fatty acid	Mixed fat	LO-HI	IN-IN	IN-LO	HI-LO
	Percent of total fatty acids				
14:0	4.5	1.5	1.5	1.5	1.6
16:0	23.8	13.0	14.5	9.8	11.7
16:1n-7	1.8	0.8	0.6	0.7	0.6
18:0	12.4	4.7	5.2	3.8	5.6
18:1n-9	38.5	56.0	24.8	54.2	29.6
18:2n-6	12.0	21.9	44.9	19.5	36.0
18:3n-3	1.0	0.8	6.5	6.6	13.4
20:0	0.3	0.4	0.3	0.6	0.3
20:1n-9	0.5	0.4	0.2	1.3	0.1
22:0	trace[c]	0.3	0.3	0.4	0.4
18:2/18:3 Ratio	12.0	27.4	6.9	3.0	2.7

[a]Means of four duplicates (two of each menu).
[b]Source of added fat: Mixed fat, 11% corn oil, 22% lard, 22% tallow, and 22% shortening; low 18:3n-3 content, high 18:2/18:3 ratio (LO-HI), 20% sunflower oil and 80% olive oil; intermediate 18:3n-3 content, intermediate 18:2/18:3 ratio (IN-IN), 100% soybean oil; intermediate 18:3n-3 content, low 18:2/18:3 ratio (IN-LO), 100% canola oil; and high 18:3n-3 content, low 18:2/18:3 ratio (HI-LO), 47% sunflower oil, 20% olive oil and 33% flax oil.
[c]Less than 0.05%.

Fatty acid analysis of platelet and plasma PL. Blood samples were collected from the subjects into ethylenediaminetetraacetic acid (EDTA)-containing tubes following a 12-h overnight fast at the end of each experimental period. The samples were centrifuged at 250 × *g*, 18°C for 15 min to remove the red blood cells. Platelets were separated from the resulting plasma by centrifuging at 1400 × *g*, 4°C for 15 min. Platelet-poor plasma (PPP) was removed from the samples and stored under nitrogen at −10°C. Washed suspensions of the platelets were prepared by the method of McKean *et al.* (17). The final suspension was in 1 mL of cold buffer (2 mmol Na_2EDTA/L, 0.15 mol NaCl/L, 0.02 mol Tris hydroxyaminomethane/L; pH 7.4). The tubes containing the final suspensions were flushed with nitrogen, stored at −22°C and then shipped on dry ice to the University of Guelph. The platelet suspensions were thawed on ice and the lipids extracted by the method of Bligh and Dyer (15). The PPP samples were thawed in the refrigerator and the lipids extracted immediately by the method of Folch *et al.* (18). The PL in the platelets and plasma were separated using two-directional thin-layer chromatography on pre-coated plates (Merck silica gel 60 HR, Terochem Laboratories Ltd., Toronto, Canada). The spotted plates were first developed in chloroform/methanol/ammonium hydroxide (65:35:5.5, by vol) and then in a second direction in chloroform/methanol/formic acid (55:25:5, by vol). Following the first run, the plates were dried under nitrogen and placed in a chamber containing HCl fumes for 10 min to hydrolyze the alkenyl group from the PPE fraction (19). Following development, the plates were sprayed with dichlorofluorescein dye, exposed to ammonia fumes and the lipid factors were localized under ultraviolet light. The PC, PE and PPE fractions were scraped into test tubes and transmethylated with 6% sulfuric acid in methanol in the presence of known amounts of monopentadecanoin as internal standard (Nu-Chek-Prep, Elysian, MN) in the case of platelet PL and with sodium methoxide in methanol (16) in the presence of internal standard in the case of the plasma PL. Similar areas of gel were scraped from blank plates and processed as for the samples. The fatty acid methyl esters were separated by GLC as described previously for platelets (20) and plasma (21) PL fractions. The amounts of fatty acids in each sample were adjusted by subtracting the amounts present in the spots scraped from the blank plates, relative to the amount of monopentadecanoin in each.

Bleeding time and prostanoid production. Bleeding time and the bleeding-time production of TXB_2, the stable metabolite of TXA_2, and of 6-keto-$PGF_{1\alpha}$, the stable metabolite of PGI_2, were measured at the end of each experimental period. Duplicate bleeding times were measured by making incisions 5 mm long and 1 mm deep on the volar surface of the arm using a sterile, disposable, standardized device (Simplate, General Diagnostics, Warner Lambert, Morris-Plains, NJ). A constant pressure was maintained with a sphygmomanometer cuff on the upper arm inflated to 44 mm Hg. Bleeding time was measured with a stopwatch. Blood from the edge of the incision was collected into heparinized microhematocrit tubes containing 10 μL of 1 g indomethacin/L for the determination

of TXB_2 and 6-keto-$PGF_{1\alpha}$. Immediately following collection of the blood, 3000 dpm of [^{3}H]PGE_2 (sp. act., 185 Ci/mmol) was added to each sample in order to correct for recovery of TXB_2 and 6-keto-$PGF_{1\alpha}$. The tubes were centrifuged, the plasma volume measured and the plasma removed for prostanoid measurement. Plasma samples were loaded onto a C_{18} Sepak cartridge (Waters Scientific, Mississauga, Canada). Water (20 mL), 10% (by vol) ethanol (20 mL) and petroleum ether (10 mL) were passed through the cartridges, and the prostanoids were then extracted with 7.5 mL methyl formate. This fraction was evaporated under a stream of nitrogen and the sample reconstituted in 0.5 mL bovine serum albumin in phosphate-buffered saline. The amounts of 6-keto-$PGF_{1\alpha}$, and TXB_2 were assayed by using highly specific anti-6-keto-$PGF_{1\alpha}$ and anti-TXB_2 in an enzyme-linked immunosorbent assay (22,23). Bleeding-time values for prostanoid production refer to blood collected in the first 4 min after incision. TXB_2 is expressed as a concentration (μg/L) whereas 6-keto-$PGF_{1\alpha}$ is expressed in terms of rate of production (pg/min) (23,24).

Statistical analysis. The experimental design of the study was a modified Latin square (25). F tests were performed on the estimated diet effects obtained from regression analysis of the data for plasma PL fatty acids, bleeding times and bleeding-time prostanoid production (25). Analyses were performed using the SAS computer program (1984, 1986 SAS Institute Inc., Cary, NC). Diet effects on the platelet fatty acid composition were determined for adjusted treatment means compared by protected least significant differences (26). All means were adjusted for the withdrawal of a subject after the completion of phase I.

RESULTS

Fatty acid patterns of platelet and plasma PL. Fatty acid patterns of platelet (Tables 2–4) and plasma (Tables 5–7) PL reflected the fatty acid composition of the diets even though there were some major differences in fatty acid composition between the platelet and plasma fractions, *e.g.*, appreciably higher 20:4n-6 and lower 18:2n-6 and 22:6n-3 levels in platelet than plasma PL. The 18:1n-9 levels were significantly higher in all PL fractions following the LO–HI and IN–LO diets, which were appreciably

TABLE 2

Mean Platelet Phosphatidylcholine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
16:0	30.4 ± 3.5[a]	31.1 ± 3.3[a]	31.4 ± 3.3[a]	30.0 ± 3.7[a]
18:0	12.5 ± 1.5[a]	14.2 ± 1.7[b]	12.5 ± 1.4[b]	14.6 ± 1.9[c]
18:1	26.0 ± 0.7[a]	20.6 ± 0.9[b]	26.0 ± 1.5[a]	22.2 ± 1.9[c]
18:2n-6	8.9 ± 1.1[a]	13.1 ± 1.6[b]	8.6 ± 0.9[a]	12.0 ± 1.0[c]
18:3n-3	0.2 ± 0.1[a]	0.3 ± 0.1[b]	0.3 ± 0.1[b]	0.4 ± 0.1[b]
20:3n-6	1.5 ± 0.1[a]	1.4 ± 0.2[a]	1.3 ± 0.2[b]	1.4 ± 0.2[a]
20:4n-6	11.3 ± 1.6[a]	11.2 ± 1.5[a]	10.4 ± 0.7[a]	11.0 ± 1.4[a]
20:5n-3	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.1[b]	0.2 ± 0.1[b]
22:4n-6	0.8 ± 0.2[a]	0.8 ± 0.2[a]	0.7 ± 0.1[b]	0.7 ± 0.1[b]
22:5n-3	0.4 ± 0.1[a]	0.5 ± 0.1[a,b]	0.5 ± 0.1[b]	0.6 ± 0.1[c]
22:6n-3	0.6 ± 0.1[a]	0.5 ± 0.1[a]	0.5 ± 0.2[a]	0.5 ± 0.2[a]

[a]Values in the same row with the same superscript letter (a–c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.

TABLE 3

Mean Platelet Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
16:0	6.4 ± 1.0[a]	6.9 ± 0.8[b]	6.6 ± 1.1[a,b]	6.2 ± 1.2[a]
18:0	27.2 ± 2.4[a]	29.2 ± 2.9[b]	26.9 ± 1.4[a]	30.4 ± 1.7[b]
18:1	17.5 ± 1.0[a]	11.4 ± 1.3[b]	20.2 ± 1.6[c]	12.3 ± 1.3[b]
18:2n-6[b]	4.9 ± 0.7	7.4 ± 1.2	4.9 ± 0.6	6.7 ± 0.3
18:3n-3	0.1 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[c]	0.2 ± 0.0[c]
20:3n-6	1.1 ± 0.2[a,b]	1.0 ± 0.2[c]	1.1 ± 0.3[b]	1.0 ± 0.4[a,c]
20:4n-6	31.8 ± 1.8[a]	32.6 ± 2.0[a]	27.9 ± 1.6[b]	32.9 ± 1.7[a]
20:5n-3	0.2 ± 0.1[a]	0.2 ± 0.0[a]	0.3 ± 0.1[b]	0.4 ± 0.1[b]
22:4n-6	2.2 ± 0.5[a]	2.3 ± 0.3[a]	1.9 ± 0.3[a]	1.8 ± 0.5[a]
22:5n-3	1.4 ± 0.4[a]	1.5 ± 0.2[b]	1.5 ± 0.3[b]	1.8 ± 0.6[c]
22:6n-3	1.6 ± 0.6[a,b]	1.5 ± 0.4[a,c]	1.7 ± 0.4[b]	1.1 ± 0.2[c]

[a]Values in the same row with the same superscript letter (a–c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.
[b]Not subjected to statistical analysis due to treatment covariate interaction.

TABLE 4

Mean Platelet Alkenylacyl Glycerophosphoethanolamine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
18:1	3.6 ± 0.6[a]	2.4 ± 0.7[b]	4.1 ± 0.6[c]	2.7 ± 0.5[b]
18:2n-6[b]	1.9 ± 0.6	2.5 ± 0.7	1.7 ± 0.5	2.3 ± 0.5
20:3n-6	0.7 ± 0.3[a]	0.6 ± 0.2[a]	0.7 ± 0.2[a]	0.7 ± 0.2[a]
20:4n-6	65.7 ± 3.7[a]	65.3 ± 2.4[a]	64.8 ± 3.2[a]	64.2 ± 3.6[a]
20:5n-3	0.5 ± 0.2[a]	0.6 ± 0.1[a]	1.1 ± 0.3[b]	1.2 ± 0.3[b]
22:4n-6	11.9 ± 2.0[a]	12.4 ± 1.7[a]	9.9 ± 1.8[b]	10.4 ± 1.8[b]
22:5n-3	6.1 ± 1.0[a]	7.4 ± 1.5[b]	7.2 ± 1.5[b]	9.6 ± 2.0[c]
22:6n-3	4.0 ± 1.3[a]	3.8 ± 1.0[a]	4.7 ± 1.2[a]	3.8 ± 1.1[a]

[a]Values in the same row with the same superscript letter (a-c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.
[b]Not subjected to statistical analysis due to treatment covariate interaction.

higher in 18:1n-9 content (Table 1), than following the IN-IN and HI-LO diets. Similarly, the 18:2n-6 levels were significantly higher following the IN-IN and HI-LO diets than following the LO-HI and IN-LO diets. Although only small amounts of 18:3n-3 were incorporated into PL, the LO-HI diet led to significantly lower levels of 18:3n-3 than the other diets while the levels following the HI-LO diet were significantly higher for the plasma PL (Tables 5-7). Levels of long-chain PUFA in platelet and plasma PL also were affected by the fatty acid composition of the diets although dietary fatty acid composition had no effect on the 20:4n-6 content, the principal PUFA in the PL fraction, except for the lower level in plasma PC following the HI-LO diet (Table 5) and in platelet PE following the IN-LO diet (Table 3). However, differences in PUFA content among the diet groups were less pronounced than for 18:1n-9, 18:2n-6 and 18:3n-3. In general, the PL tended to be higher in long-chain n-3 PUFA following the HI-LO diet and lower following the LO-HI diet although dietary fat source had little effect on 22:6n-3, the major n-3 fatty acid in the PL fractions. The ratio of 18:2/18:3 in the diet also had an effect on PUFA levels in the PL fractions. The levels of 20:5n-3 were significantly higher following the IN-LO and HI-LO diets than following the LO-HI and IN-IN diets in all fractions for both tissues (Tables 2-7). By contrast, the level of 22:4n-6 in the platelet PPE fraction (Table 4) was significantly lower following the IN-LO and HI-LO diets than the LO-HI and IN-IN diets.

Thrombogenic effect of the experimental diets. No differences in bleeding time and in bleeding-time TXB_2 production were observed among the experimental diets, although levels of TXB_2 tended to be lower following the IN-LO and HI-LO diets than following the LO-HI and IN-IN diets (Table 8). Production of 6-keto-$PGF_{1\alpha}$ in blood collected from the bleeding time wounds was significantly higher following the HI-LO diet than the LO-HI diet. 6-Keto-$PGF_{1\alpha}$ production following the IN-LO and IN-IN diets fell midway between the values on the HI-LO and LO-HI regimen. As a result, the 6-keto-$PGF_{1\alpha}$ to TXB_2 ratio tended to be higher following the HI-LO and IN-LO diets than following the LO-HI and

TABLE 5

Mean Plasma Phosphatidylcholine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
16:0	32.4 ± 3.0[a]	33.7 ± 2.7[a]	31.8 ± 2.7[a]	33.7 ± 3.0[a]
18:0	14.3 ± 0.8[a]	15.3 ± 0.8[b]	14.7 ± 0.8[a,b]	16.3 ± 0.8[c]
18:1	14.4 ± 1.1[a]	8.8 ± 0.8[b]	14.9 ± 0.8[a]	9.4 ± 1.1[b]
18:2n-6	22.3 ± 1.6[a]	26.6 ± 1.3[b]	20.9 ± 1.3[a]	25.7 ± 1.6[b]
18:3n-3	0.2 ± 0.1[a]	0.3 ± 0.1[b]	0.4 ± 0.1[b]	0.7 ± 0.1[c]
20:3n-6	2.3 ± 0.5[a]	1.4 ± 0.3[b]	1.9 ± 0.3[a]	1.4 ± 0.5[b]
20:4n-6	7.2 ± 1.0[a]	7.0 ± 0.8[a]	7.0 ± 0.8[a]	5.8 ± 1.0[b]
20:5n-3	0.3 ± 0.2[a]	0.3 ± 0.2[a]	0.8 ± 0.2[b]	0.6 ± 0.2[c]
22:4n-6	0.2 ± 0.1[a]	0.1 ± 0.1[b]	0.1 ± 0.1[b]	0.1 ± 0.1[b]
22:5n-3	0.4 ± 0.2[a]	0.4 ± 0.2[a]	0.6 ± 0.2[b]	0.5 ± 0.2[a,b]
22:6n-3	2.0 ± 0.5[a]	1.7 ± 0.5[a]	2.0 ± 0.5[a]	1.6 ± 0.5[a]

[a]Values in the same row with the same superscript letter (a-c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.

TABLE 6

Mean Plasma Phosphatidylethanolamine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
16:0	11.9 ± 1.9[a]	12.1 ± 1.6[a]	11.1 ± 1.6[a]	11.1 ± 1.9[a]
18:0	28.3 ± 2.2[a]	31.1 ± 1.9[b]	28.2 ± 1.9[a]	33.0 ± 2.2[b]
18:1	18.0 ± 1.9[a]	11.6 ± 1.9[b]	18.2 ± 1.9[a]	13.0 ± 1.9[b]
18:2n-6	11.9 ± 2.4[a]	15.8 ± 2.2[b]	11.5 ± 2.2[a]	14.9 ± 2.4[b]
18:3n-3	0.3 ± 0.2[a]	0.5 ± 0.2[b]	0.6 ± 0.2[b]	0.9 ± 0.2[c]
20:3n-6	1.2 ± 0.2[a]	0.9 ± 0.2[b]	1.2 ± 0.2[a]	0.9 ± 0.2[b]
20:4n-6	17.3 ± 2.7[a]	16.7 ± 2.4[a]	16.2 ± 2.4[a]	15.1 ± 2.7[a]
20:5n-3	0.4 ± 0.2[a]	0.3 ± 0.2[a]	0.9 ± 0.2[b]	0.8 ± 0.2[b]
22:4n-6	0.5 ± 0.2[a]	0.4 ± 0.2[a,b]	0.3 ± 0.2[b]	0.4 ± 0.2[a,b]
22:5n-3	0.9 ± 0.3[a]	1.0 ± 0.3[a,b]	1.1 ± 0.3[a,b]	1.3 ± 0.3[b]
22:6n-3	4.5 ± 1.3[a]	4.4 ± 1.3[a]	5.0 ± 1.3[a]	4.0 ± 1.3[a]

[a]Values in the same row with the same superscript letter (a-c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.

TABLE 7

Mean Plasma Alkenkylacyl Glycerophosphoethanolamine Fatty Acid Levels Following Different Experimental Diets[a]

Fatty acid	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
	mol% of total fatty acids			
18:1	8.9 ± 1.6[a]	5.5 ± 1.3[b]	8.1 ± 1.3[a]	5.0 ± 1.6[b]
18:2n-6	14.8 ± 2.9[a,b]	17.7 ± 2.6[b,c]	12.4 ± 2.6[a]	18.9 ± 2.9[c]
18:3n-3	0.3 ± 0.2[a]	0.5 ± 0.2[b]	0.7 ± 0.2[b,c]	0.9 ± 0.2[c]
20:3n-6	2.0 ± 0.3[a]	1.3 ± 0.3[c]	1.8 ± 0.3[a,b]	1.5 ± 0.3[b,c]
20:4n-6	45.1 ± 3.1[a]	44.2 ± 2.9[a]	44.3 ± 2.9[a]	42.7 ± 3.1[a]
20:5n-3	1.4 ± 0.5[a]	1.5 ± 0.5[a]	3.4 ± 0.5[b]	2.9 ± 0.5[b]
22:4n-6	2.5 ± 0.3[a]	2.8 ± 0.3[b]	2.2 ± 0.3[a]	2.4 ± 0.3[a]
22:5n-3	4.5 ± 0.8[a]	5.5 ± 0.8[b]	5.3 ± 0.8[b]	6.1 ± 0.8[b]
22:6n-3	12.7 ± 1.3[a]	13.9 ± 1.3[a]	13.6 ± 1.3[a]	12.9 ± 1.3[a]

[a]Values in the same row with the same superscript letter (a-c) do not differ significantly ($P > 0.05$). All values are mean ± SD; n = 8; see Table 1 for abbreviations.

TABLE 8

Bleeding Times and Bleeding-Time Prostanoid Production Following the Experimental Diets[a]

	Experimental diets			
	LO-HI	IN-IN	IN-LO	HI-LO
Bleeding time (min)	5.00 ± 0.26[a]	4.94 ± 0.23[a]	5.27 ± 0.23[a]	5.07 ± 0.26[a]
Bleeding-time production of:				
TXB_2 (μg/L)	5.01 ± 0.60[a]	5.15 ± 0.52[a]	3.81 ± 0.52[a]	4.11 ± 0.60[a]
6-Keto-$PGF_{1\alpha}$ (pg/min)	2.90 ± 0.51[a]	3.64 ± 0.44[a,b]	3.84 ± 0.44[a,b]	4.70 ± 0.51[b]
6-Keto-$PGF_{1\alpha}$/TBX_2 ratio	0.81 ± 0.17[a,b]	0.74 ± 0.12[a]	1.08 ± 0.16[a,b]	1.52 ± 0.31[b]

[a]Values in the same row with the same superscript letter (a-b) do not differ significantly ($P > 0.05$). All values are mean ± SEM; n = 8; see Table 1 for abbreviations.

IN-IN diets; the ratio following the HI-LO diet was significantly ($P < 0.05$) higher than the ratio following the IN-IN diet.

DISCUSSION

Levels of 18:1n-9, 18:2n-6 and 18:3n-3 in platelet and plasma PL following the consumption of the experimental diets reflected the difference in the levels of these fatty acids in the diets. Similar effects of dietary fat sources on the fatty acid composition of platelet and plasma PL have been reported (8,21,27-29). The magnitude of the differences in the fatty acid composition of the platelet and plasma PL, however, was small compared to the differences in dietary fatty acid composition, especially for 18:3n-3. The small amount of 18:3n-3 incorporated into

the PL could be due to its preferential oxidation for energy purposes. Leyton *et al.* (30) found that 18:1n-9 and 18:3n-3 were oxidized more rapidly than other long-chain fatty acids. Restricted incorporation of 18:3n-3 into PL or competition for the elongase and desaturase enzymes present in the liver and other tissues also could be responsible for the limited changes observed in platelet and plasma fatty acid composition (12, 31).

The IN-LO and HI-LO diets, which had relatively low 18:2/18:3 ratios, were associated with significantly higher levels of platelet and plasma long-chain n-3 PUFA, in particular the level of 20:5n-3, which was double that following the LO-HI and IN-IN diets. These results confirm previous studies (9,12,21,27-29) which indicated that humans can desaturate and elongate 18:3n-3 to higher homologs and that desaturation-elongation varies with the level of 18:3n-3 in the diet (12,21,27-29,31,32) and its ratio to 18:2n-6 (12,33,34). However, the *in vivo* conversion of dietary 18:3n-3 to 20:5n-3 is not as effective in raising n-3 levels in platelet and plasma PL as is direct supplementation with 20:5n-3 (8,9). The fact that the IN-LO diet, which had a lower dietary 18:2/18:3 ratio than the IN-IN diet, resulted in significantly higher levels of platelet and plasma 20:5n-3 suggests that the higher 20:5n-3 levels associated with the lower 18:2/18:3 ratio could be due to the effective competition of 18:3n-3 for the Δ6 desaturase enzyme (35). Results of the present study also suggest that the 18:2/18:3 ratio has to be less than 6.9 to have a significant effect on the level of long-chain n-3 PUFA.

Neither the lower level of 18:3n-3 in the diet nor its ratio to 18:2n-6 had any consistent effect on the longer chain n-6 homologs in platelet or plasma PL. Lasserre *et al.* (34) also found that dietary 18:3n-3 at a level of 1.5% of total energy (slightly less than the levels in the IN-IN and IN-LO diets in the present study) had no effect on serum levels of n-6 PUFA. By contrast, supplementing subjects with 30 mL linseed oil per day (approximately 16 g/d of 18:3n-3, which was similar to the amount present in the HI-LO diet) (31) or 60 mL/d (32) led to significant decreases in plasma 20:4n-6 levels.

The dietary fat source also had no effect on plasma 22:6n-3 levels in the present study. Sanders and Younger (8), Renaud *et al.* (27) and Lasserre *et al.* (34) also found that dietary 18:3n-3 had no effect on plasma 22:6n-3 levels. Mest *et al.* (31), on the other hand, reported an increase in 22:6n-3 level following linseed oil supplementation.

Individual PL species were analyzed in the present study so as to avoid the possibility that a large amount of one fraction might conceal changes in the fatty acid composition of other fractions. The apparent rates of incorporation of individual fatty acids differed among the three PL species reported here. For instance, appreciably higher levels of 20:4n-6, 22:4n-6, 22:5n-3 and 22:6n-3 were incorporated into the PPE than into the PC or PE fractions. However, the pattern of change was similar for the three PL species. Likewise, changes in the fatty acid composition of platelet and plasma PL responded in a similar way to differences in dietary fatty acid composition in spite of major differences in the fatty acid composition of the platelet and plasma PL, *e.g.*, higher levels of 20:4n-6 and lower levels of 18:2n-6 and 22:6n-3 in platelets.

Renaud *et al.* (27) and Budowski *et al.* (32) reported significant decreases in platelet aggregability and platelet coagulant activity following an increase in 18:3n-3 intake. Borchgrevink *et al.* (36), on the other hand, failed to find any effect of a linseed oil supplement on bleeding time. A study similar to the present (37), however, reported longer bleeding times and higher bleeding-time prostacyclin production following a canola oil diet, which provided 2.7% of total energy as 18:3n-3, than following a mixed fat diet (0.4% of energy as 18:3n-3). However, sunflower oil (0.3% of energy as 18:3n-3) produced a similar response to that of canola oil. In the present study, the experimental diets did not affect bleeding times even though bleeding-time production of 6-keto-$PGF_{1\alpha}$ was significantly higher following the HI-LO diet, in which 18:3n-3 provided 4.6% of total energy, than following the LO-HI diet, in which 18:3n-3 provided only 0.3% of total energy. There is no obvious explanation for the higher production of 6-keto-$PGF_{1\alpha}$ on the HI-LO diet. In fact, the n-6 fatty acid level in plasma PC was significantly lower following the HI-LO diet; however, plasma PL fatty acid levels may not reflect vascular tissue PL composition, the assumed source of PGI in the bleeding-time blood analyzed in the present study. Although there were no differences in bleeding-time TXB_2 production due to diet, TXB_2 production tended to be lower following the HI-LO and IN-LO diets, both of which resulted in higher 20:5n-3 levels in platelet and plasma PL. Morita *et al.* (38) reported a strong inhibition of *in vitro* TXA_2 synthesis by human platelets as a result of adding 20:5n-3 to the medium. It has been suggested (6,39) that higher tissue levels of 20:5n-3 may inhibit prostanoid formation from 20:4n-6 through an effect on cyclooxygenase activity or the availability of 20:4n-6. However, Mest *et al.* (31) found no relation between 20:5n-3 levels in serum PL and *in vitro* TXA_2 production by platelets. The ratio of 6-keto-$PGF_{1\alpha}$/TXB_2 was highest following the HI-LO diet and lowest following the IN-IN diet; the ratio following the IN-LO diet was intermediate between the HI-LO and the IN-IN regimens. Since TXA_2 promotes and PGI_2 inhibits platelet aggregation, the balance between *in vivo* production of TXA and PGI is important (39). It is interesting to note that the levels of 20:5n-3 in the PL fractions were significantly higher following the HI-LO and IN-LO diets than following the LO-HI and IN-IN diets but that the level of 20:4n-6 was significantly lower only for the platelet PE and the plasma PC fractions following the IN-LO and HI-LO diets, respectively.

A significant effect of diet on prostacyclin production and on the ratio of prostacyclin to thromboxane production under conditions where there was no significant change in the bleeding time requires comment. It is possible that the HI-LO diet may have an antithrombotic effect (on the basis of altered prostanoid production) without producing an increased tendency to bleed, a situation that would be very desirable from the point-of-view of coronary heart disease. On the other hand, it is possible that the apparent beneficial effect of the HI-LO diet, in terms of prostanoid production, could be balanced by changes in other factors, not measured in this study, so that the overall antithrombotic effect may not be as significant as the changes in prostanoid production would suggest. Which of these alternative interpretations of our results is more pertinent will still require further investigation.

The results of the present study indicate that dietary 18:3n-3 intake has an appreciable effect on the fatty acid

composition of platelet and plasma PL. The ratio of 18:2/18:3 in the diet appears especially important in influencing n-3 PUFA patterns. The changes in the production of TXB_2 and 6-keto-$PGF_{1\alpha}$ were found to coincide with the differences in the n-3 PUFA patterns of platelet and plasma PL.

ACKNOWLEDGMENTS

This research was supported by grants from the Canola Council of Canada and the Flax Council of Canada. We gratefully acknowledge the competent technical assistance of Stacey Johnson, Sandra Singhroy and Elena Duta, the participation and cooperation of the subjects, and the helpful advice of Dr. Bruce Johnston with the statistical analysis. Canola oil and sunflower oil were kindly supplied by CSP Foods Ltd. (Winnipeg, Canada) and soybean oil by Canada Packers Ltd. (Toronto, Canada).

REFERENCES

1. Ross, R. (1986) *New Eng. J. Med. 314*, 488-500.
2. Leaf, A., and Weber, P.C. (1988) *New Engl. J. Med. 318*, 549-557.
3. Ulbricht, T.L.V., and Southgate, D.A.T. (1991) *Lancet 338*, 985-992.
4. Smith, W.L., and Borgeat, P. (1985) in *Biochemistry of Lipids and Membranes* (Vance, D.E., and Vance, J.E., eds.) pp. 325-360, Benjamin/Cummings Publishing Co. Inc., Menlo Park.
5. Kinsella, J.E. (1988) *Food Technol. 42*, 126-142.
6. Kinsella, J.E., Lokesh, B.E., and Stone, R.A. (1990) *Am. J. Clin. Nutr. 52*, 1-28.
7. Mead, J.F., Alfin-Slater, R.B., Howton, D.R., and Popjak, G. (1986) *Lipids: Chemistry, Biochemistry and Nutrition*, pp. 273-283, Plenum Press, New York.
8. Sanders, T.A.B., and Younger, K.M. (1981) *Br. J. Nutr. 45*, 613-616.
9. Sanders, T.A.B., and Roshanai, F. (1983) *Clin. Sci. 64*, 91-99.
10. Harris, W.S. (1989) *J. Lipid Res. 30*, 785-805.
11. Marshall, L.A., and Johnston, P.V. (1982) *Lipids 17*, 905-913.
12. Adam, O., Wolfram, G., and Zollner, N. (1986) *J. Lipid Res. 27*, 421-426.
13. Mahadevappa, V.G., and Holub, B.J. (1987) *J. Lipid Res. 28*, 1275-1280.
14. Holub, B.J., Celi, B., and Skeaff, C.M. (1988) *Thromb. Res. 50*, 135-143.
15. Bligh, E.G., and Dyer, W.J. (1959) *Can. J. Biochem. Physiol. 37*, 911-918.
16. Bannon, C.D., Craske, J.D., and Hilliker, A.E. (1985) *J. Am. Oil Chem. Soc. 62*, 1501-1507.
17. McKean, M.L., Smith, J.B., and Silver, M.J. (1981) *J. Biol. Chem. 256*, 1522-1524.
18. Folch, J., Lees, M., and Sloane Stanley, G.H. (1957) *J. Biol. Chem. 226*, 497-509.
19. Aukema, H.M., and Holub, B.J. (1988) in *Dietary ω3 and ω6 Fatty Acids: Biological Effects and Nutritional Essentiality* (Galli, C., and Simopoulos, A.P., eds.), pp. 81-90, Plenum Press, New York.
20. Holub, B.J., and Skeaff, C.M. (1987) *Methods Enzymol. 41*, 234-244.
21. Corner, E.J., Bruce, V.M., and McDonald, B.E., (1990) *Lipids 25*, 598-601.
22. Docherty, J.C., and Gerrard, J.M. (1986) *Prostglandins 31*, 375-383.
23. Gerrard, J.M., Taback, S., Singhroy, S., Docherty, J.C., Kostolansky, I., McNicol, A., Kobrinsky, N.L., McKenzie, J.K., and Rowe, R. (1989) *Circulation 79*, 29-38.
24. Gerrard, J.M. (1989) *Can. J. Physiol. Pharmacol. 67*, 922-928.
25. Steel, R.G.D., and Torrie, J.H. (1980) *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd edn., pp. 311-333, McGraw-Hill Book Company, New York.
26. Snedecor, G.W., and Cochran, W.G. (1980) *Statistical Methods*, 7th edn., pp. 234-240, Iowa State University Press, Ames.
27. Renaud, S., Godsey, F., Dumont, E., Thevenon, C., Ortchanian, E., and Martin, J.L. (1986) *Am. J. Clin. Nutr. 43*, 136-150.
28. Weaver, B.J., Corner, E.J., Bruce, V.M., McDonald, B.E., and Holub, B.J. (1990) *Am. J. Clin. Nutr. 51*, 594-598.
29. Kwon, J-S., Snook, J.T., Wardlaw, G.M., and Hwang, D.H. (1991) *Am. J. Clin. Nutr. 54*, 351-358.
30. Leyton, J., Drury, P.J., and Crawford, M.A. (1987) *Br. J. Nutr. 57*, 383-393.
31. Mest, H.J., Beitz, J., Heinroth, I., Block, H.U., and Forster, W. (1983) *Klin. Wochenschr. 61*, 187-191.
32. Budowski, P., Trostler, N., Lupo, M., Vaisman, N., and Eldor, A. (1984) *Nutr. Res. 4*, 343-346.
33. Budowski, P., and Crawford, M.A. (1985) *Proc. Nutr. Soc. 44*, 221-229.
34. Lasserre, M., Mendy, F., Spielmann, D., and Jacotot, B. (1985) *Lipids 20*, 227-233.
35. Ziboh, V.A., and Chapkin, R.S. (1988) *Prog. Lipid Res. 27*, 81-107.
36. Borchgrevink, C.F., Berg, K.J., Skaga, E., Skjeggestad, O., and Stormorken, H. (1965) *Lancet ii*, 980-982.
37. McDonald, B.E., Gerrard, J.M., Bruce, V.M., and Corner, E.J. (1989) *Am. J. Clin. Nutr. 50*, 1382-1388.
38. Morita, I., Takahashi, R., Saito, Y., and Murota, S. (1983) *Thromb. Res. 31*, 211-217.
39. ten Hoor, F., de Deckere, E.A.M., Haddeman, E., Hornstra, G., and Quadt, J.F.A. (1980) *Adv. Prostaglandin Thromboxane Res. 8*, 1771-1781.

[Received October 6, 1992, and in final revised form May 19, 1993; Revision accepted June 23, 1993]