The Effect of Short-Term Diets Rich in Fish, Red Meat, or White Meat on Thromboxane and Prostacyclin Synthesis in Humans

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ABSTRACT: Foods which increase tissue arachidonic acid levels have been proposed to increase thrombosis tendency, presumably through increased platelet aggregation. This study examined the effect of doubling the dietary arachidonic acid (20:4n-6) using meat- or fish-based diets on the systemic production of prostacyclin (PGI_2) and thromboxane (TXA_2) in 29 healthy, nonsmoking adults. There were three, 3-wk low-fat dietary periods (<15% energy as fat) in which subjects consumed a vegetarian diet for 1 wk followed by 2 wk on diets containing meat or fish as sources of 20:4n-6. Between each diet period, there was a 3-wk washout period, during which subjects returned to their normal diets. The level of 20:4n-6 consumed during the last 2 wk of each study was approximately double the usual intake (mean 140 mg/d), while the mean eicosapentaenoic acid (20:5n-3) content of the diets varied from 1 mg/d on the white meat diet to 70 mg/d on the red meat diet and to 847 mg/d on the fish diet. The serum phospholipid (PL) 20:4n-6/20:5n-3 ratios were 11:1 on the vegetarian diet, 15:1 on the white meat diet, 8:1 on the red meat diet, and 2:1 on the fish diet ($P < 0.001$). Neither white nor red meat diets affected platelet 20:4n-6 levels, platelet aggregation, *ex vivo* platelet TXB_2 production, or the systemic PGI_2 or TXA_2 production as measured by gas chromatography–mass spectrometry analysis of the excretion levels of the principal urinary metabolites 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGI_2-M) and 11-dehydro-TXB_2 (TXA_2-M), respectively. The fish diet decreased the 20:4n-6/20:5n-3 ratio in platelet PL from the baseline level of 45:1 to 13:1 ($P < 0.001$), had no effects on platelet aggregation, but significantly decreased platelet TXB_2 production (collagen-stimulated) and TXA_2-M production, while PGI_2-M levels were unaltered. These results indicate that short-term diets which double the usual 20:4n-6 intake using white meat (175–330 g/d) or red meat (275–530 g/d) are not associated with an increased TXA_2 production, but this does not rule out the adverse effects of 20:4n-6 at higher levels in the diet, or for more prolonged periods. Short-term diets containing fish (100–200 g/d with 90–210 mg/d 20:4n-6 and approximately 650–1000 mg/d 20:5n-3) led to significant increases in platelet 20:5n-3 levels and a decrease in the *ex vivo* and systemic TXA_2 production.

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Eicosanoids derived from arachidonic acid (20:4n-6) are thought to play a role in the pathogenesis of coronary artery disease. The two most powerful vasoactive eicosanoids issuing from the cyclooxygenase pathway are the platelet-derived thromboxane (TXA_2) (1) and prostacyclin (PGI_2), derived from arterial endothelial cells (2). Thromboxane is both a potent pulmonary vasoconstrictor and a procoagulant, whereas PGI_2 is a major endogenous vasodilator and an inhibitor of platelet aggregation (3,4). It is believed that an imbalance between the release of these two mediators could be involved in the pathogenesis of thrombogenesis and atherosclerosis (5).

It has been proposed that the low incidence of myocardial infarction and ischemic heart disease in Greenland Eskimos is due to the high content of long-chain (LC) n-3 polyunsaturated fatty acids (PUFA) in their diet (6,7). The beneficial effects may be due to a decrease in TXA_2 derived from 20:4n-6 and an increase in the 3-series prostanoids which have an attenuated spectrum of biological activity (8). The thromboxane/prostacyclin ratio has subsequently been shown to be reduced in Greenland Eskimos compared with Danes (9).

Since dietary LC n-3 PUFA modify eicosanoid formation from the more dominant precursor fatty acid, 20:4n-6, it has been argued that the ingestion of foods which raise tissue levels of 20:4n-6 will lead to an increased thrombotic potential (10). This view has been based to a large extent on the detrimental findings of two studies, the first of which involved rabbits being injected intravenously with 20:4n-6, resulting in death within 3 min (11). The second was a human study in which four subjects were fed 6 g/d of ethyl arachidonate for a period of 2–3 wk. The threshold concentration of ADP re-

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Abbreviations: 20:4n-6, Arachidonic acid; DHA, docosahexaenoic acid (22:6n-3); LA, linoleic acid; LC, long-chain; PAP, platelet adjusted plasma; PEG, polyethylene glycol; PGI_2, prostacyclin; PGI_2-M, 2,3-dinor-6-keto-$PGF_{1\alpha}$; PL, phospholipid; PPP, platelet-poor plasma; PRP, platelet-rich plasma; P/S, polyunsaturated/saturated; PUFA, polyunsaturated fatty acid; TXA_2-M, 11-dehydro-TXB_2; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

quired to induce secondary irreversible aggregation of platelet-rich plasma (PRP) dropped significantly in two of the four subjects, indicating increased platelet reactivity (12).

The Western diet is rich in 18:2n-6, containing 10–30 g/d, compared with 1–3 g/d 18:3n-3 (13) and about 50–70 mg/d 20:5n-3 and 100 mg/d docosahexaenoic acid (DHA) (14). The 20:4n-6 intake in the Western diet has been postulated to be between 100–1000 mg/d (15,16). We have reported the 20:4n-6 intake in the Australian diet to be approximately 130 mg/day for adult males and 96 mg/day for adult females (14), based on average national food intake figures. Thus, the relatively high level of n-6 PUFA in the diet, coupled with the body's natural tendency to conserve tissue arachidonic acid, leads to 20:4n-6 being the most abundant 20-carbon PUFA available in tissue membrane phospholipids (PL) for eicosanoid synthesis.

The relative importance of dietary 20:4n-6, compared with dietary linoleic acid, in contributing to tissue 20:4n-6 levels is not fully understood; however, we have shown that short-term diets (1 to 3 wk) rich in foods containing up to 500 mg/day of 20:4n-6 (red meat, tropical seafood, eggs, liver) are associated with significant increases in the plasma levels of 20:4n-6 (17–20). Furthermore, we have recently reported data from a pilot study indicating that such diets are associated with alterations in thromboxane and prostacyclin production (21).

The objectives of this study were to investigate the effect of three diets containing 20:4n-6 (from foods) on serum and platelet PUFA levels, *in vitro* platelet thromboxane production, and *in vivo* whole body production of PGI_2 and TXA_2. The three diets with elevated but constant levels of 20:4n-6 contained low, medium, and high levels of LC n-3 PUFA. Rather than using isolated oils in pharmacological doses, a more realistic dietary approach was used: The three diets all contained the same level of 20:4n-6 (mean of 140 mg/day) which was approximately double the usual consumption level of 20:4n-6 for these particular subjects, whose normal diets were relatively low in meat and eggs, the main source of dietary 20:4n-6. The first diet was rich in white meat which provided little LC n-3 PUFA, the second diet was rich in red meat which provided a similar level of n-3 PUFA to the usual diet, and the third diet was rich in fish which provided approximately 3 g/day of n-3 PUFA.

MATERIALS AND METHODS

Subjects. A total of 29 free-living subjects took part in the three phases of the intervention study (14 males, 15 females), with an age range of 22–52 yr. Subjects were screened for any existing medical condition and requested not to consume garlic, which is known to affect lipid and cholesterol levels and platelet activity (22), or use any form of medication during the study, particularly corticosteroids and nonsteroidal anti-inflammatory drugs such as aspirin, which could interfere with cyclooxygenase activity (23), thus altering platelet function. Subjects were also requested to abstain from extreme exercise and the consumption of vitamin and mineral supplements (particularly vitamin E) during the study and 2 wk prior to the start.

The study consisted of three 3-wk low-fat (<15% energy as fat) dietary intervention phases with a 3-wk washout period between each, at which time subjects returned to their own "baseline or normal" diet. Individual phases consisted of 1 wk of precooked vegetarian food, followed by 2 wk of meat consumption (either white meat, red meat, or fish). Vegetarian meals were precooked for subjects due to their lack of familiarity with vegetarian cooking. Meat was provided raw and cooked by subjects to their own tastes (no added fats) and consumed with vegetables if desired. The white meat consisted of fat-trimmed turkey fillets (<7.2% fat) and turkey roll (1% fat); the red meat consisted of fat-trimmed beef (<2.8% fat) and lamb (<4.2% fat), and the fish was Atlantic salmon (<10% fat) (14). The average intakes were 231 ± 52 g/day of white meat, 351 ± 104 g/day red meat, and 133 ± 32 g/day of fish (Fig. 1). The short duration of each study (2 wk) was largely due to the difficulty that subjects found in maintaining the consumption levels of meat continuously over an extended period of time.

The fish diet was the last carried out, as tissue DHA levels are known to take up to 3 wk to return to pre-dietary intervention levels, whereas other LC PUFA return to normal levels more quickly (24). No other fat sources were allowed and energy percentage as fat was made up to 15% for each individual with olive oil (77% oleic acid, Moro Spanish Olive oil; Conga Foods, Melbourne, Australia) or Brio™ margarine (Brio Foods, Pireas, Greece) containing 61% oleic acid and 13% *trans*-18:1.

The daily energy intake of each subject was determined from pre-intervention study weighed food records, and the dietary 20:4n-6 intake was designated to be proportional to the total energy intake of each subject. The white meat, red meat, and fish stages of the study were thus controlled for 20:4n-6 intake, but with varying levels of LC n-3 PUFA. No LC PUFA were consumed during the vegetarian periods, so that all subjects entered each dietary period from the same background PUFA intake, giving a valuable reference point for the purpose of examining comparative changes on each diet. Also no LC PUFA were available from any dietary source other than the meat or fish during the study periods. Dietary composition was calculated using SODA (System for On-line Dietary Analysis, version 5.0; Computer Models, Cottesloe, WA., Australia) based on Australian food composition data.

Blood collection. Subjects reported to the clinical rooms of the University in the morning, following a 12-h overnight fast on three occasions during each phase of the study. These were at the beginning of each phase, at the end of week one (vegetarian diet), and again at the end of week three, after 2 wk of meat or fish consumption. Weight was recorded to the nearest 0.1 kg. At these times they received dietary counseling and their records of food consumed (by weight) were collected, checked and any relevant changes in diet for the following week explained to them. Subjects were requested to

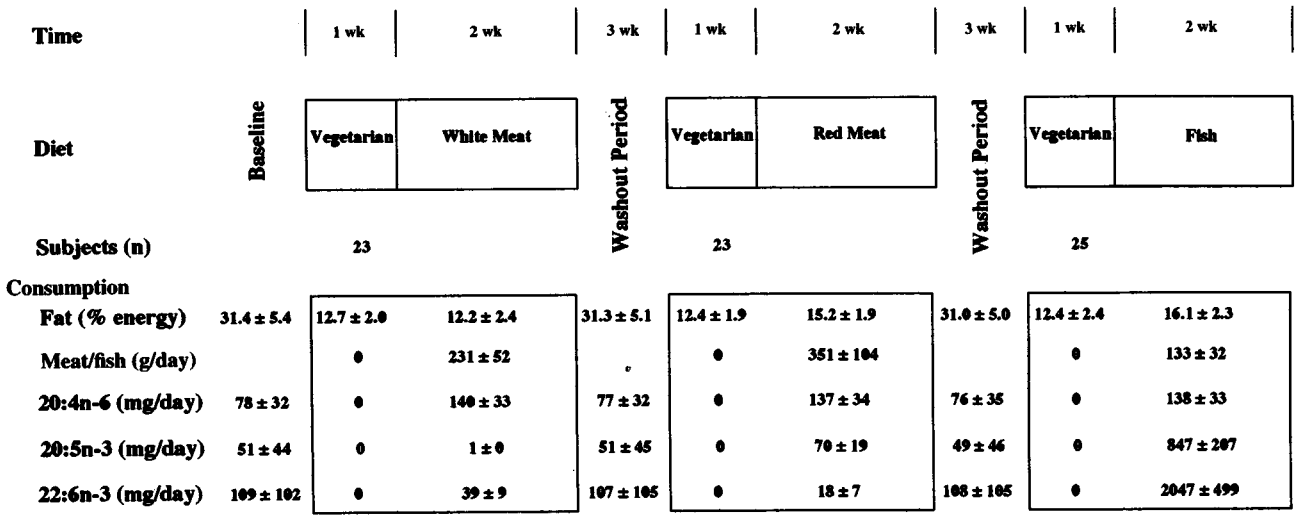

FIG. 1. Experimental design. Three dietary regimes were examined, each consisting of a 1-wk vegetarian diet followed by 2 wk of white meat, red meat, or fish consumption. Each phase was separated by a 3-wk washout period to allow serum phospholipid fatty acid levels to return to baseline. The percentage energy derived from fat in each stage of the study is indicated along with a summary of the amount of meat or fish consumed, with the relevant content of arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3). Values are mean ± SD.

report any deviations from the dietary protocol and any signs of illness.

Blood was collected following 15 min of quiet relaxation, with minimum stasis. Approximately 34 mL of blood was collected sequentially into a 10-mL plain vacuette (Griener Interpath, Melbourne, Australia), followed by two 5-mL CTAD vacuettes (citric acid, theophylline, adenosine, dipyridamole tubes; Becton Dickinson, Oxford, United Kingdom) pre-warmed to 37°C, and finally three 5-mL sodium citrate vacuettes (Griener Interpath). The serum was removed and frozen at −20°C for later determination of serum fatty acid composition. The blood collected in the three citrate tubes was used for full blood examination and platelet aggregation studies.

Serum PL fatty acid analysis. Lipids were extracted from the serum, and the total PL were separated by thin-layer chromatography and fatty acid methyl esters (FAME) were prepared by saponification in 0.68 M KOH in methanol, followed by methyl esterification with 20% BF_3 in methanol (25). The amounts were quantified using diheptadecanoyl phosphatidylcholine as an internal standard. The FAME were separated by capillary gas chromatography using a 50 m × 0.32 mm i.d. BPX70, WCOT fused-silica capillary column (SGE, Melbourne, Australia) as described previously (26).

Platelet PL fatty acid analysis. Platelets were separated from 10 mL of blood collected in CTAD vacutainers (27) as described by Naughton *et al.* (28), and extracted in 10 mL of 1:1 chloroform/methanol. All platelet separation steps were carried out in polypropylene or silanized glass tubes, and PL fatty acid analysis proceeded as for serum PL fatty acids.

Plasma preparation for platelet aggregation. Platelet-poor plasma (PPP) and PRP were prepared as described previously by Steel *et al.* (29). Platelet aggregation studies required plasma with equal quantities of platelets for each subject, hence requiring concentration adjustment. The platelet-adjusted plasma (PAP) used was obtained by individual dilution of each subject's PRP with their own PPP until a platelet concentration of 220×10^9 platelets/L was achieved. The PAP was then stored at 37°C until the aggregation test was performed, within 2 h of the blood collection.

Platelet aggregation and platelet thromboxane production. Platelet aggregation was determined in PAP using a dual channel, Payton Aggregation Module 300 BD-5 (Payton Scientific Pty. Ltd., Victor Harbour, South Australia). The method used was a turbidimetric method, and aggregation was measured using two different agonists, collagen (Hormon Chemie GMBH, Munich, Germany) and thrombin (Thrombostat, Parke Davies Pty. Ltd., Caringbah, NSW, Australia), both of which initiate a TXA_2 release.

Following platelet aggregation, the supernatant of the aggregated PAP was removed and used for thromboxane determination as outlined by Butcher *et al.* (30). TXB_2 was determined in duplicate in each sample using a radioimmunoassay technique (Scintillation Proximity Assay, SPA, Amersham, Melbourne, Australia) and measured on a liquid scintillation counter. All samples were analyzed as one batch and a pooled serum sample was analyzed five times to determine the intraassay coefficient of variation (mean ± SD, 720.5 ± 8.0 cpm, coefficient of variation 1.1%).

Urine collection. Twenty-four-hour urine samples were collected by subjects, to which was added Indomethacin (Sigma Chemical Co., St. Louis, MO) (50 mg/50 mL urine). Aliquots of 50 mL were stored at −80°C until required for extraction and derivatization.

Prostacyclin measurement. The mean daily excretion of PGI_2-M (2,3-dinor-6-keto-$PGF_{1\alpha}$) derived from 20:4n-6 and

PGI_3-M (Δ^{17}-2,3-dinor-6-keto-$PGF_{1\alpha}$) derived from 20:5n-3 was determined as the methyloxime, pentafluorobenzyl ester, trimethylsilyl ether derivative in 5-mL aliquots of urine spiked with 10 ng of tetradeuterated [19,19,20,20-$^2H_4$] 2,3-dinor-6-keto-$PGF_{1\alpha}$ as an internal standard as described previously (31). The samples were separated using a 12-m, 0.22-mm i.d. BP-1 fused-silica capillary column (SGE, Melbourne, Australia), which interfaced directly with a Hewlett-Packard 5988A single quadrupole mass spectrometer (Palo Alto, CA) as outlined previously (32). The interassay coefficient of variation was 4.2%.

Thromboxane measurement. Quantitative analysis of 11-dehydro-TXB_2 in urine was used for the assessment of *in vivo* thromboxane production (33). A 2-mL aliquot of urine was spiked with 1 ng of tetradeuterated [19,19,20,20-$^2H_4$]-11-dehydro-TXB_2 as an internal standard. Gas chromatography was performed on a Varian 3400 (Palo Alto, CA) operated in splitless mode with a fused-silica capillary column, DB5-MS, 30 m, 0.25 mm i.d., 0.25 mm coating (J&W Scientific, Folsom, CA). The gas chromatograph was interfaced to a Finnigan MAT TSQ 70 triple-stage mass spectrometer (San Jose, CA) operated in NICI mode with methane used as ionization gas. Selected ion monitoring was used to monitor the molecular ion of the pentafluorobenzyl ester trimethylsilyl ether forms of the endogenous metabolite 11-dehydro-TXB_2 and the tetradeuterated [19,19,20,20-$^2H_4$]-11-dehydro-TXB_2 internal standard at *m/z* 511 and *m/z* 515, respectively.

Statistical analysis. We analyzed the results using analysis of variance on data blocks between baseline, vegetarian, and diet period during each study. As sample sizes were approximately equal, the Student's Newman Keul multiple comparison test was applied. All statistical analyses were carried out on a Sun Sparc Station, running Unix, using SAS (Statistical Analysis System, Cary, NC).

RESULTS

One male and one female subject failed to complete the white meat phase of the study, and their results were not recorded; another male only completed the white meat study. One extra female was recruited for the red meat phase, and all subjects completed this phase of the study. One male and two females did not continue with the third phase of the study. Two extra males and three females were recruited to take part in the fish phase of the study. Overall, nine females and 10 males completed all three phases of the study. All subjects remained within a 2-kg weight range during their participation. No statistically significant change occurred in group weight or body mass index in any phase of the study (Table 1). There were no significant differences in baseline levels of PL fatty acids or *in vivo* production of thromboxane or PGI_2 at the start of each study, indicating a return to normal levels during the washout periods.

Fat consumption as a percentage of energy intake on baseline diets was 31% (all figures quoted are for combined male and female results, unless otherwise stated) (Table 2). After 1 wk of vegetarian diet during each of the three diet phases, the fat levels were 12–13% of energy. Following 2 wk on each of the diets, the dietary fat levels were 12, 15, and 16% of energy intake, respectively. The marked fall in the proportion of energy derived from fat on all diets was compensated for by increased carbohydrate consumption.

There was a substantial decrease, relative to baseline, in saturated fat intake during each phase of the study (Table 2). The decrease in monounsaturated fatty acid, relative to baseline during each phase, was more modest owing to the use of olive oil in making up each individual diet to 15% energy as fat. The baseline polyunsaturated/saturated (P/S) ratio in each phase was 0.4, which increased to 0.9 during each of the three vegetarian periods. During the white meat phase, the P/S ratio remained at 0.9, but fell to 0.5 during the red meat phase. The fish was relatively rich in PUFA, and during this phase of the study the dietary P/S ratio increased to 1.3. Cholesterol intake was extremely low on the vegetarian diets relative to baseline ($P < 0.001$). During the white meat and fish phases of the study, cholesterol intakes rose relative to the vegetarian period ($P < 0.001$), but were still well below baseline intakes ($P < 0.001$). During the red meat phase of the study, cholesterol intake returned to baseline levels.

The quantity of meat or fish consumed by each subject and

TABLE 1
Subject Numbers, Age, Weight, and Body Mass Index (BMI) for the White Meat, Red Meat, and Fish Dietary Intervention Studies[a]

	Phase of Study											
	White meat				Red meat				Fish			
	n	Age (yr)	Weight (kg)	BMI	n	Age (yr)	Weight (kg)	BMI	n	Age (yr)	Weight (kg)	BMI
Males	12	34.9 ± 10.0	79.5 ± 5.2[c]	24.5 ± 1.3[b]	11	35.8 ± 9.9	80.2 ± 4.3[c]	24.7 ± 0.7[b]	12	34.8 ± 9.5	77.9 ± 4.4[c]	24.5 ± 0.8[b]
Females	11	34.7 ± 8.5	63.3 ± 7.6	22.8 ± 2.4	12	35.0 ± 8.1	63.4 ± 7.3	23.0 ± 2.3	13	35.8 ± 7.4	61.8 ± 8.5	22.5 ± 2.4
All subjects	23	34.8 ± 9.1	71.7 ± 10.4	23.7 ± 2.1	23	36.8 ± 13.2	71.4 ± 10.4	23.8 ± 1.9	25	35.4 ± 8.3	69.5 ± 10.6	23.5 ± 2.1

[a]Values are mean ± SD.

[b,c]Significant difference between males and females ([b]$P < 0.01$, [c]$P < 0.001$).

TABLE 2
Changes in Dietary Composition During the White Meat, Red Meat and Fish Dietary Intervention Studies

	Stage	Energy intake (kj/day)	Carbohy.	Protein	Alcohol	Fat	Sat.	Mono.	Poly.	Cholesterol (mg/day)	Fiber (g/day)
			(percentage of daily total energy intake)								
White meat (n = 23)	Baseline	9,637 ± 2,439	49.4 ± 5.2	16.7 ± 2.1	2.6 ± 3.8	31.4 ± 5.4	14.5 ± 3.0	11.8 ± 3.2	5.2 ± 1.7	235 ± 78	27.5 ± 9.8
	Week 1	9,918 ± 2,137	72.8 ± 5.6^{c}	11.8 ± 2.4^{c}	2.6 ± 3.9	12.7 ± 2.0^{c}	2.9 ± 0.5^{c}	7.5 ± 1.3^{c}	2.5 ± 0.5^{c}	15 ± 11^{c}	43.6 ± 11.1^{c}
	Week 3	9,673 ± 1,862	65.0 ± 6.4^{c,f}	20.7 ± 3.1^{c,f}	2.2 ± 2.7	12.2 ± 2.4^{c}	2.8 ± 0.5^{c}	6.9 ± 1.5^{c}	2.4 ± 0.5^{c}	144 ± 33^{c,f}	31.4 ± 8.4^{c,f}
Red meat (n = 23)	Baseline	9,451 ± 2,483	49.0 ± 6.0	16.8 ± 2.2	3.0 ± 4.0	31.3 ± 5.1	14.4 ± 3.2	11.8 ± 2.8	5.1 ± 1.7	227 ± 68	26.5 ± 10.4
	Week 1	9,611 ± 2,372	72.9 ± 4.2^{c}	12.0 ± 1.7^{c}	2.8 ± 3.9	12.4 ± 1.9^{c}	2.6 ± 0.4^{c}	7.6 ± 1.4^{c}	2.2 ± 0.4^{c}	14 ± 10^{c}	37.7 ± 11.4^{c}
	Week 3	9,404 ± 2,182	59.4 ± 4.3^{c,f}	22.7 ± 2.2^{c,f}	2.7 ± 3.0	15.2 ± 1.9^{c,f}	4.6 ± 0.5^{c,f}	8.5 ± 1.3^{c}	2.1 ± 0.3^{c}	224 ± 57^{f}	25.9 ± 8.2^{f}
Fish (n = 25)	Baseline	9,499 ± 2,421	49.7 ± 6.1	16.6 ± 2.0	2.8 ± 3.9	31.0 ± 5.0	14.9 ± 3.2	11.2 ± 2.2	4.9 ± 1.5	224 ± 70	25.7 ± 10.7
	Week 1	9,539 ± 2,024	73.0 ± 3.6^{c}	12.2 ± 2.0^{c}	2.4 ± 3.1	12.4 ± 2.4^{c}	2.6 ± 0.4^{c}	7.6 ± 1.8^{c}	2.2 ± 0.3^{c}	14 ± 12^{c}	38.1 ± 12.9^{c}
	Week 3	8,786 ± 1,872	66.7 ± 4.0^{c,f}	15.1 ± 2.1^{c,f}	2.1 ± 2.4	16.1 ± 2.3^{c,f}	3.6 ± 0.5^{c,f}	7.5 ± 1.4^{c}	5.0 ± 0.6^{c,f}	80 ± 22^{c,f}	25.6 ± 7.8^{f}

Values are mean ± SD. Abbreviations: Carbohy. = carbohydrate; Sat. = saturated fatty acids; Mono. = monounsaturated fatty acids; Poly. = polyunsaturated fatty acids. ^{a,b,c}Significantly different to baseline ($^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$). ^{d,e,f}Significantly different to Week 1-vegetarian ($^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$)

the intake of LC PUFA during the white meat, red meat, and fish dietary periods are shown in Table 3. The amounts consumed daily were 231 ± 52 g of white meat (range 150–270 g white meat for females and 205–330 g white meat for males), 351 ± 104 g of red meat (range 255–400 g red meat for females and 330–500 g red meat for males), 133 ± 32 g of fish (range 90–155 g fish for females and 115–200 g fish for males). The mean 20:4n-6 intake was approximately 77 mg/day during the baseline period, and this almost doubled during the meat and fish diet phases of the study (Table 3). The 20:5n-3 levels of about 50 mg/day on the baseline diet declined significantly on the white meat phase and increased by a factor of 16 in the fish period. The DHA values of about 100 mg/day fell significantly on the meat diets to less than 40 mg/day and increased to about 2050 mg/day on the fish diet.

Significant changes in serum PL fatty acid concentration occurred for only six fatty acids during the study (Table 4). Oleic acid (18:1) levels increased during the vegetarian week of the white and red meat phases, which coincided with the highest consumption period of 18:1 by subjects. After 2 wk on each diet, the 18:1 levels decreased, particularly during the fish phase. Linoleic acid (LA, 18:2n-6) decreased during each vegetarian stage and remained low after two weeks of white meat, red meat or fish consumption. Arachidonic acid increased significantly during the white meat and red meat phases of the study, but decreased during the fish phase.

Eicosapentaenoic acid levels decreased during the vegetarian week of each phase of the study. During the 2 wk of white meat consumption, the level of 20:5n-3 did not change; however, a small but significant increase occurred during the 2 wk of red meat consumption and a large increase occurred during the fish phase of the study. Docosapentaenoic acid (22:5n-3) remained unchanged during the vegetarian week of each phase, but increased during the 2 wk of red meat and fish consumption. DHA (22:6n-3) was only significantly elevated during the fish phase of the study. As anticipated from dietary composition, the serum ratio of 20:4n-6/20:5n-3 changed substantially during the course of the study. During the vegetarian stages, the average ratio was 11:1; this increased to 15:1 on the white meat diet (poor in 20:5n-3) then decreased to 8:1 on red meat and 2:1 on the fish diet.

The changes in platelet fatty acids were relatively minor compared with the serum PL, apart from substantial increases in 20:5n-3 and 22:6n-3 after the fish phase (Table 5). The level of stearic acid (18:0) in the platelet PL decreased significantly during the fish phase of the study ($P < 0.001$), while LA decreased in the final 2 wk of each study. During the white and red meat phases of the study, 20:4n-6 levels remained unchanged, but fell significantly during the fish phase.

TABLE 3
Estimated Daily Intake of Long-Chain Fatty Acids (mg/day) During the Three, Two-Week Periods of Meat and Fish Consumption, Compared with the Mean Baseline Intake Levels Prior to the Study[a]

		Long-chain polyunsaturated fatty acid intake							
		AA[b]		EPA[c]		DPA[d]		DHA[e]	
	Meat (g)	Baseline	Diet	Baseline	Diet	Baseline	Diet	Baseline	Diet
White meat	231 ± 52	78 ± 32	140 ± 33[f]	51 ± 44	1 ± 0	33 ± 18	19 ± 7	109 ± 102	39 ± 9
Red meat	351 ± 104	77 ± 32	137 ± 34	51 ± 45	70 ± 19	33 ± 18	73 ± 19	107 ± 105	18 ± 7
Fish	133 ± 32	76 ± 35	138 ± 33	49 ± 46	847 ± 207	32 ± 21	487 ± 119	108 ± 105	2047 ± 499

[a]Values are means ± SD.
[b]Arachidonic acid, 20:4n-6, [c]Eicosapentaenoic acid, 20:5n-3.
[d]Docosapentaenoic acid, 22:5n-3, [e]Docosahexaenoic acid, 22:6n-3.
[f]Arachidonic acid intake kept constant on each phase of the study.

TABLE 4
Serum Phospholipid Fatty Acid Concentrations (mg/100 mL Serum) During Each Study

		Serum phospholipid fatty acid concentration					
Study	Stage	18:1	18:2n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3
White	Baseline	15.9 ± 2.5	30.3 ± 6.6	13.0 ± 3.5	1.5 ± 0.6	1.3 ± 0.3	4.7 ± 1.2
meat	Week 1	17.8 ± 3.4[c]	23.9 ± 4.5[c]	13.5 ± 3.9	1.2 ± 0.5[c]	1.7 ± 1.7	5.0 ± 1.6
(*n* = 23)	Week 3	17.4 ± 4.1[c]	23.8 ± 4.2[c]	16.0 ± 4.1[c,f]	1.1 ± 0.7[c]	1.3 ± 0.3	5.3 ± 1.2[a]
Red	Baseline	15.4 ± 3.3	29.1 ± 7.9	13.3 ± 4.1	1.5 ± 0.6	1.2 ± 0.3	4.8 ± 1.7
meat	Week 1	16.4 ± 3.8[b]	22.5 ± 5.0[c]	13.7 ± 3.9	1.2 ± 0.4[b]	1.2 ± 0.3	5.3 ± 1.8[b]
(*n* = 23)	Week 3	15.4 ± 2.9[e]	22.4 ± 5.6[c]	16.5 ± 5.0[c,f]	2.0 ± 0.8[f]	1.5 ± 0.5[b,e]	5.1 ± 1.8[b]
Fish	Baseline	15.0 ± 2.7	30.7 ± 7.5	14.2 ± 4.0	1.9 ± 1.0	1.4 ± 0.4	5.1 ± 1.4
(*n* = 25)	Week 1	15.9 ± 3.1	24.3 ± 5.8[c]	13.7 ± 3.8	1.3 ± 0.5[b]	1.4 ± 0.4	5.3 ± 1.4
	Week 3	12.8 ± 3.1[c,f]	18.5 ± 4.9[c,f]	12.0 ± 2.9[c]	6.5 ± 2.2[c,f]	1.7 ± 0.6[b,e]	13.3 ± 3.7[c,f]

Values are mean ± SD. No change was observed in the concentration of 14:0, 16:0, 16:1, 18:0, or 18:3n-3 fatty acids.
[a,b,c]Significantly different to baseline ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$).
[d,e,f]Significantly different to week 1—vegetarian ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$).

TABLE 5
Platelet Phospholipid Fatty Acid Composition (percentage of total fatty acids) During Each Study

		Platelet phospholipid fatty acid composition						
Study	Stage	18:0	18:1	18:2n-6	20:4n-6	20:5n-3	22:5n-3	22:6n-3
White	Baseline	17.3 ± 2.0	14.3 ± 1.4	6.2 ± 0.8	27.5 ± 3.1	0.7 ± 0.2	1.9 ± 0.4	2.1 ± 0.3
meat	Week 1	16.8 ± 2.3	15.1 ± 2.1	5.5 ± 0.7[c]	26.8 ± 4.4	0.5 ± 0.2[c]	1.7 ± 0.4	2.2 ± 0.3
(*n* = 23)	Week 3	16.1 ± 1.6	16.1 ± 2.1[b]	5.3 ± 0.6[c,f]	27.1 ± 2.7	0.4 ± 0.1[c,f]	1.5 ± 0.3	2.2 ± 0.3
Red	Baseline	14.7 ± 2.0	14.6 ± 1.1	6.3 ± 0.9	29.6 ± 2.8	0.7 ± 0.2	1.9 ± 0.4	2.3 ± 0.4
meat	Week 1	14.3 ± 1.7	15.4 ± 1.3[b]	5.7 ± 0.7[c]	29.8 ± 3.1	0.5 ± 0.2[c]	1.8 ± 0.3	2.3 ± 0.4
(*n* = 23)	Week 3	14.3 ± 1.9	15.7 ± 1.2[b]	5.5 ± 0.5[c]	29.3 ± 2.4	0.7 ± 0.2[f]	1.9 ± 0.3	2.1 ± 0.3[b,e]
Fish	Baseline	16.8 ± 1.4	15.4 ± 0.9	6.3 ± 0.8	27.0 ± 2.4	0.6 ± 0.1	1.9 ± 0.3	2.0 ± 0.3
(*n* = 25)	Week 1	16.1 ± 1.0[c]	16.4 ± 1.2[c]	5.6 ± 0.6[c]	27.1 ± 2.1	0.4 ± 0.1[a]	1.7 ± 0.3	2.0 ± 0.3
	Week 3	15.5 ± 1.1[c,f]	16.9 ± 1.1[c]	5.1 ± 0.5[c,f]	24.1 ± 2.1[c,f]	1.9 ± 0.5[c,f]	1.8 ± 0.2	3.9 ± 0.5[c,f]

Values are mean ± SD. No change was observed in the concentration of 14:0, 16:0, 16:1, or 18:3n-3 fatty acids.
[a,b,c]Significantly different to baseline ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$).
[d,e,f]Significantly different to week 1—vegetarian ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$).

During each vegetarian period, 20:5n-3 levels decreased significantly; however, the 20:5n-3 level increased significantly during the red meat and fish phases. Platelet levels of 22:6n-3 decreased during the red meat phase and doubled during the fish phase of the study. No significant change was detected in the platelet PL ratio of 20:4n-6/20:5n-3 during the white or red meat diets. However, the fish diet resulted in a decrease from 45:1 (baseline diet) to 13:1.

There were no significant changes in platelet aggregation (data not shown) on any of the studies. However, when all baseline data for the three studies were grouped and compared with the grouped vegetarian data, a significant but unexplained increase in aggregation due to collagen was observed after 1 wk of low-fat vegetarian diet relative to baseline (baseline 64.0% ± 2.4%, vegetarian 71.0% ± 2.3%, $P < 0.01$).

Using collagen as an agonist (Table 6), the *ex vivo* platelet TXB_2 production increased during the vegetarian period of the white meat study ($P < 0.05$) and returned to baseline following 2 wk of white meat consumption ($P < 0.05$). During the vegetarian weeks of the white meat and fish studies, the

TABLE 6
Thromboxane B_2 Produced by Platelets During Aggregation, Using the Agonists Collagen (C), Thrombin (T)

Study	Stage	Platelet TXB_2 (C) (ng/mL)	Platelet TXB_2 (T) (ng/mL)
White	Baseline	10.6 ± 2.2	4.6 ± 3.3
meat	Week 1	12.1 ± 1.5[a]	7.0 ± 2.5[b]
(*n* = 23)	Week 3	10.6 ± 1.1[d]	6.7 ± 3.6[a]
Red	Baseline	7.9 ± 2.2	5.2 ± 3.4
meat	Week 1	8.5 ± 2.8	6.2 ± 3.4
(*n* = 23)	Week 3	8.8 ± 1.5	7.2 ± 3.7
Fish	Baseline	9.1 ± 2.2	5.5 ± 3.2
(*n* = 25)	Week 1	9.6 ± 2.4	6.6 ± 3.2[a]
	Week 3	9.3 ± 2.4	5.1 ± 3.5[d]

[a,b,c]Significantly different to baseline ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$).
[d,e,f]Significantly different to week 1—vegetarian ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$). Values are mean ± SD.

TABLE 7
Urinary Prostacyclin and Thromboxane Metabolite Levels Measured as a Total Daily Excretion

	White meat (n = 23)	Red meat (n = 23)	Fish (n = 25)
Daily PGI_2-M excretion (ng/day)			
Baseline	445 ± 198	442 ± 188	397 ± 130
Week 1	345 ± 157[c]	373 ± 213	333 ± 140[b]
Week 3	339 ± 140[c]	410 ± 187	326 ± 108[b]
Daily TXA_2-M excretion (ng/day)			
Baseline	898 ± 422	804 ± 305	807 ± 358
Week 1	834 ± 276	873 ± 396	834 ± 297
Week 3	851 ± 340	937 ± 367	715 ± 263[d]

Week 1 = end of vegetarian diet; week 3 = end of meat or fish diet.
[a,b,c]Significantly different to baseline ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$).
[d,e,f]Significantly different to Week 1—vegetarian ([d]$P < 0.05$, [e]$P < 0.01$, [f]$P < 0.001$).
Values are mean ± SD.

thrombin-stimulated platelets showed an increased TXB_2 production ($P < 0.01$ and $P < 0.05$, respectively). This decreased during the 2 wk of fish consumption ($P < 0.05$), but remained elevated on the white meat diet.

The daily urinary excretion of PGI_2-M decreased during the 1-wk vegetarian period of each study, reaching significance in the white meat ($P < 0.001$) and fish studies ($P < 0.01$) and remaining depressed, relative to baseline values, during the 2 wk of white meat ($P < 0.001$) and fish consumption ($P < 0.01$) (Table 7). No significant change in PGI_2 output was recorded during the 2 wk of meat or fish consumption relative to the first-week low-fat vegetarian diet. Prostacyclin (PGI_3), derived from the n-3 PUFA 20:5n-3, was detected in most subjects following 2 wk of fish consumption only, and reached a mean value 12 ± 9 ng/day.

The daily urinary excretion of TXA_2-M showed large interindividual variation, with no significant changes during the vegetarian week of each study. No effect of white or red meat consumption was evident, but a significant reduction occurred during the 2 wk of fish consumption ($P < 0.05$) relative to the vegetarian diet.

DISCUSSION

In the Western diet, the main dietary PUFA is linoleic acid, and this fatty acid is almost certainly the major source of the 20:4n-6 which is found in high proportions in most cell membranes of the body (34). High intakes of fatty fish or fish oil rich in LC n-3 PUFA are known to increase membrane PL levels of these LC n-3 PUFA, with such diets often leading to simultaneous reductions in the 20:4n-6 levels in the membranes. These changes have been associated with beneficial effects on thrombosis tendency, including reduced TXA_2 production, reduced platelet aggregation, and lowered plasma triglyceride levels (35).

We have been interested in the converse situation, that is, what are the consequences of ingesting diets containing 20:4n-6 (21,30). In the early 1960s Mohrhauer and Holman (36) showed that on a gram for gram basis, dietary 20:4n-6 led to substantially higher tissue 20:4n-6 levels than dietary LA. The purpose of the present study was to examine the effects of the ingestion of a constant dietary level of 20:4n-6 combined with varying levels of LC n-3 PUFA (supplied from meat or fish) on serum and platelet PL 20:4n-6 levels and subsequent conversion to vasoactive eicosanoids in studies of two weeks' duration. By using white meat, red meat, and fish, we were able to supply a constant level of dietary 20:4n-6 at double the usual intake for these subjects and a varying LC n-3 PUFA intake (from very low to very high).

The meat diets were associated with approximately 20% increases in the serum PL 20:4n-6 concentration and little or no change in n-3 PUFA levels; however, there were no changes in the platelet 20:4n-6 levels on these diets in the short-term period of this study. In contrast, the fish diet led to very large increases in 20:5n-3 and 22:6n-3 in both plasma and platelet PL and to significant decreases in 20:4n-6 levels in both tissues. An obvious explanation for the changes in the platelet lipids on the fish diet (but not on the meat diets) was the increase in the dietary intake of n-3 PUFA by about 3 g/day compared with the increase in 20:4n-6 of only 60–70 mg/day on the meat diets.

There were no effects from any of these diets on *ex vivo* platelet aggregation. This is not surprising since the platelet PL 20:4n-6 on the fish diet was still 12 times the 20:5n-3 level, compared with Eskimos, where the levels of 20:4n-6 and 20:5n-3 in platelet lipids are about the same (37).

However, the fish diet was associated with a significant reduction ($P < 0.05$) in *ex vivo* TXB_2 production from thrombin-stimulated platelets and in the principal urinary metabolite of thromboxane (11-dehydro-TXB_2, TXA_2-M). This decrease in TXA_2-M daily excretion, following the 2 wk of fish consumption, coincided with a decrease in platelet PL 20:4n-6, an increase in 20:5n-3 and 22:6n-3, and a change in the 20:4n-6/20:5n-3 ratio in platelet PL from 68:1 (vegetarian diet) to 13:1. These changes could account for the 20% decrease in TXA_2 production by a number of mechanisms, firstly, replacement of 20:4n-6 in PL by LC n-3 PUFA, effectively lowering the amount of substrate for TXA_2 production

(38); secondly, inactivation of cyclooxygenase by LC n-3 PUFA (36); finally, release of 20:5n-3 from platelet PL, which competes with 20:4n-6 for access to cyclooxygenase and produces an alternative form of thromboxane (TXA_3) (39). Decreases in TXA_2 following fish consumption have been observed in other studies. Knapp and FitzGerald (40), found a 29% reduction in TXA_2 production, measured as the urinary metabolite 2,3-dinor-TXB_2, following 50 mL/day of fish oil for 4 wk. A small amount of TXA_3 was detected (~14% of the total thromboxane level). Ferretti *et al.* (41) observed a 38% reduction in TXA_2 production, measured as the urinary metabolite, 11-dehydro-TXB_2, following 10 wk of fish oil consumption (15 g/day). A small quantity of the TXA_3 metabolite was also detected, but not quantified. In the 2 wk of fish consumption in the present study, no TXA_3 products were detected, possibly owing to the lower level of 20:5n-3 consumed (~847 mg/day), compared with ~9 g/day and 4.5 g/day in the above two studies, respectively. Like many other studies involving fish oil consumption, the intake of LC n-3 PUFA the above two studies is at a pharmacological level and has little resemblance to normal human nutrition. It is likely that TXA_3 is not normally produced in humans consuming a modern Western diet even when modest amounts of fish are consumed, as in the 2-wk fish diet of the present study. White and red meat consumption did not result in a substantial change in platelet 20:4n-6/20:5n-3 ratio or TXA_2 production, even though both these diets significantly increased the 20:4n-6 concentration of the plasma PL. Presumably the low intake of 20:4n-6 and the short time frame of the study contributed to the failure to alter platelet 20:4n-6 levels. However the study was designed to test the impact of high 20:4n-6 intake from foods, in this case specifically meats. The limiting factors proved to be the actual physical quantity of meat that subjects could comfortably consume on a daily basis and the period of time that they could maintain this level of consumption.

The decrease in PGI_2-M recorded during the low-fat vegetarian period of each study is consistent with observations by Nordoy *et al.* of decreased prostacyclin production on low-fat diets (42), the suggested reason being that fatty acids (saturates in particular) stimulate PGI_2 production by endothelial cells. An *in vitro* study by Zhang *et al.* (43) showed stearic acid to increase PGI_2 production by endothelial cells in culture medium, but this increase was associated with morphological signs of cellular damage and reduced viability in culture. No further changes in PGI_2-M production were observed after the 2 wk of meat or fish diets. Previous studies have shown conflicting effects of LC n-3 PUFA on *in vivo* PGI_2 production. Mann *et al.* (32) showed a decreased level of urinary PGI_2-M in rats following 2 wk of dietary supplementation with 20:5n-3, and Ferretti *et al.* (31,41), in a study involving the feeding of 15g/day of fish oil to human subjects, found a 20% decrease in urinary PGI_2-M. In other studies, no change in urinary PGI_2-M was observed when fish oil or fish was fed to human subjects (38,44,45). Prakash *et al.* (45) found a decrease in TXA_2-M in subjects fed a similar diet to this study, containing 450 g/day of salmon (rich in 20:4n-6 and LC n-3 PUFA). In two other human studies involving fish consumption (46,47), urinary PGI_2-M levels were increased. However, it should be noted that in these two studies the level of 20:4n-6 contained in the fish consumed was high (~250 mg/day and ~380 mg/day, respectively). In the present study, a small amount of PGI_3-M (~4% of total prostacyclin) derived from 20:5n-3 was measured in the urine following 2 wk of fish consumption. Knapp and FitzGerald (40), feeding 50 mL/day of fish oil for 4 wk, also found an increase in the PGI_3-M level (from zero to ~15% of the total prostacyclin production).

In conclusion, it can be stated that under the circumstances of this study, there was no increase in TXA_2 production on diets containing 20:4n-6 and relatively low levels of n-3 PUFA (white and red meat), but there was a decrease on a fish diet (100–200 g/day), containing 90–210 mg/day 20:4n-6 and approximately 3 g of LC n-3 PUFA. However, this does not rule out the adverse effects of 20:4n-6 at higher levels in the diet or for more prolonged periods. The LC n-3 PUFA from fish had a beneficial effect on platelet reactivity by lowering *in vivo* TXA_2 production without significantly affecting PGI_2 production; however, the maintenance of PGI_2 levels on fish diets may be dependent on the simultaneous consumption of 20:4n-6, which is relatively high in salmon (14) compared with other sources such as white or red meat, and certainly supplies more 20:4n-6 than is present in fish oil feeding studies. White and red meat consumption within the ranges used in this study does not appear to change prostacyclin production; however, the low-fat diets did decrease prostacyclin production, and future studies will have to take this phenomenon into account.

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