Incorporation of dietary 5,11,14-icosatrienoate into various mouse phospholipid classes and tissues

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To investigate the basis of acyl specificity in the phosphatidylinositol (PI) lipid class, we fed mice fatty acids lacking the usual methylene interrupted double bonds. Mice were fed 10 wt% diets containing either 2.9 or 16% 5,11,14-icosatrienoate (5,11,14-20:3) as a component of seed oil mixtures, or control oil mixtures in which either 18:1n-9 or 18:3n-3 replaced the 5,11,14-20:3 content of the seed oils, for a 2-week period. 5,11,14-20:3 was found to be maximally incorporated into cardiac and hepatic PI (15–17 area%), and hepatic phosphatidylcholine (13%), but minimally incorporated into neutral lipids and those phospholipids that contain small amounts of 20:4n-6, such as hepatic sphingomyelin and cardiolipin. Within the PI class, there were important differences in the tissue distribution of 5,11,14-20:3: liver>heart>kidney = spleen>thymus = visceral fat. There was a clear selectivity for the incorporation of this fatty acid into PI as compared with other phospholipids. 5,11,14-20:3 was also extensively incorporated into hepatic phosphatidylinositol bisphosphate (PIP_2), a precursor of second messengers. In hepatic PI, 5,11,14-20:3 replaced 20:4n-6, resulting in a 50% reduction in the level of 20:4n-6. By contrast, in phosphatidylcholine and phosphatidylethanolamine lipid classes, 5,11,14-20:3 replaced several polyenes, including 18:2n-6, 20:4n-6, and 20:5n-3. In comparison with dietary 18:3n-3, 5,11,14-20:3 was found to be more effective at decreasing hepatic PI 20:4n-6 levels. Because leukotrienes and prostaglandins cannot be formed from 5,11,14-20:3 due to the lack of an internal Δ8 double bond, and because 20:4n-6 was dramatically reduced in some PI pools, we expect that 5,11,14-20:3 may alter eicosanoid signaling.

Keywords: 5,11,14-icosatrienoate; fatty acid; *Juniperus chinensis;* phosphatidylinositol; phospholipid; *Platycladus orientalis*

Introduction

The predominant molecular species of phosphatidylinositol (PI) is the 1-stearoyl-2-arachidonoyl-*sn*-PI molecular species.[1,2] The conservancy of this particular molecular species may be linked to the important second messenger roles of phospholipase derived products such as 1-stearoyl-2-arachidonyl-*sn*-glycerol, 1-stearoyl-2-arachidonyl-*sn*-glycerol 3-P, and inositol triphosphate. PI and its derived products are also important sources of phospholipase A_2- and diacylglycerol lipase-derived 20:4n-6 for the synthesis of eicosanoids. Additionally, the acyl composition of glycosylated PI may be a determinant in the anchoring to proteins[3] (See the accompanying "Pathways of Nutritional Biochemistry" figure entitled, "Acyl specificity of phosphatidylinositol"). In comparison with other phospholipids, the acyl composition of PI is resistant to most dietary fatty acid modifications,[4–6] with exception of essential fatty acid deficiency.[7] The 1-stearoyl-2-arachidonoyl-*sn*-PI species is maintained even in cold-water fish, which consume a diet rich in 20:5n-3 and 22:6n-3.[8] In contrast, in vitro 20:5n-3 is readily incorporated into PI in platelets,[9] C3H 10T 1/2 cells,[10] and myocytes.[11]

To investigate the basis of this in vivo selectivity for

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20:4n-6, we fed mice fatty acids with a differing chain length and number and arrangement of double bonds.[4,12,13] Any dissimilarity to 20:4n-6 was found to preclude significant incorporation into PI.[4] In contrast, we found that the non-methylene interrupted fatty acid (NMIFA) 5,11,14-20:3, which lacks the Δ8 double bond essential for prostaglandin and leukotriene synthesis, replaced a significant quantity of 20:4n-6 in PI, but not phosphatidylcholine (PC) and phosphatidylethanolamine (PE).[12] Because 5,11,14-20:3 represented only 3% of the total fatty acids in saponified *Platycladus orientalis* (PO) seed oil, we concluded that this fatty acid is selectively incorporated into PI pools, and that the feeding of 5,11,14-20:3 represents a unique nutritional tool for investigating the basis of PI acyl specificity and for determining the metabolic consequences of acyl alteration in vivo.

The purposes of the present investigation were: (1) to determine whether dietary 5,11,14-20:3 esterified to glycerol in intact PO seed oil would be incorporated into phospholipid classes similarly to the free acid; (2) to establish a dose-response relationship for the incorporation of 5,11,14-20:3 into phospholipid classes; (3) to evaluate the incorporation of 5,11,14-20:3 into a wider range of tissues and neutral lipid and phospholipid classes, including phosphatidylinositol bisphosphate (PIP_2); (4) to determine whether the previously observed decline in 20:4n-6 following PO oil feeding was due predominately to replacement by 5,11,14-20:3 or to replacement by n-3 fatty acids that were also present in the PO oil; and (5) to compare the efficacy of dietary 18:3n-3 to 5,11,14-20:3 with respect to suppressing 20:4n-6 levels in PI.

Methods and materials

Animals and diets

The experimental animals consisted of 4–5-wk-old pathogen-free, female, C57BL/6 mice (Simonsen Laboratories, Gilroy, CA USA) weighing 14–15 g initially. There were five mice per treatment, housed in groups of five in clear plastic cages (27 × 17 × 13 cm) with bedding. The mice received ad libitum amounts (4 g/mouse–week 1; 5 g/mouse–week 2) of the experimental diets in ceramic feed cups each evening in a humidity-controlled room with a dark cycle from 1800–0700 hr, for a 2-week period. Uneaten food was discarded each evening. Deionized water was provided through a pressure-sensitive nozzle. Feed cups, cages, and bedding were cleaned every 2–4 days.

The diets consisted of 10 wt% of *Platycladus orientalis*[14,15] or *Juniperus chinensis* (JC) seed oils.[16] (F.W. Schumacher Co., Sandwich, MA USA); or control mixtures of olive oil (G. Sensat, Extra Virgin #5, Specialty Food and Beverage Sales, West Milford, NJ USA), safflower oil (Dyets Inc., Bethlehem, PA USA), and linseed oil (Spectrum Marketing, Petaluma, CA USA), added to fat-free AIN 76A meal (Dyets) (*Table 1*). The PO and JC oils contained 2.9 and 16 area% 5,11,14-20:3, respectively. The proportion of 18:1n-9 was similar in these two diets, although PO contained more 18:3n-3 and less 18:2n-6 than JC. "PO 18:1" contained the same level of 18:2n-6 and 18:3n-3 as PO, and 18:1n-9 replaced the sum of the NMIFA in PO. "JC 18:1" was analogously paired to JC. Because dietary 18:1n-9 and its desaturation/elongation products are minimally incorporated into mouse phospholipid classes (excluding cardiolipin),[13] the extent to which 5,11,14-20:3 directly replaced 20:4n-6 could be ascertained by comparing PO and JC with their respective controls. "JC 18:3" contained the same level of 18:2n-6 and 18:1n-9 as JC, and 18:3n-3 replaced the sum of the NMIFA in JC. The relative abilities of n-3 fatty acids and NMIFA to replace 20:4n-6 in phospholipid classes could thus be determined by comparing JC to JC 18:3. The JC 18:3 and PO 18:1 control diets contained similar amounts of 18:3n-3; the 18:2n-6 content in JC 18:3 was replaced by 18:1n-9 in PO 18:1.

Extraction of seed oils

Seeds were sieved, freeze-dried, suspended in liquid nitrogen, ground in a hammer mill, extracted with hot isopropanol:$CHCl_3$, evaporated in a rotary evaporator, redissolved in hexane, then purified by 1% NaCl washes and centrifugation.[12] *Tert*-butylhydroquinone (0.02 wt%) was added to all lipid extracts to minimize autoxidation.[17] Seed oil aliquots were methylated and analyzed for fatty acid composition by gas chromatography.[12]

Extraction of tissue lipids

Phospholipids were extracted, separated into classes by high-performance thin-layer chromatography (HPTLC), visualized, scraped from the plates, methylated, and fatty acid methyl esters resolved by gas chromatography as previously described,[12] with the modifications described below.

Fatty acid methyl esters were injected with a Hewlett Packard (Palo Alto, CA USA) model 7673 autosampler and resolved with Hewlett Packard gas chromatograph Model 5890A equipped with a DB-23 capillary column (25 m × 0.25 μm i.d., 50% cyanopropyl phase, 0.25 μm film thickness; J&W Scientific, Folsom, CA USA). Integrator data were electronically transferred to a Macintosh computer (Apple Corp., Cuperton, CA USA) where the data were automatically sorted based on the retention time of fatty acid methyl ester standards (NuChek Prep; Elysian, MN USA; Matreya Marine Mix, Pleasant Gap, PA USA; Biomol Research Laboratories, Plymouth Meeting, PA USA), and then converted to area percentages in Microsoft Excel (Redmond, WA USA). NMIFA were identified by retention time and by GC-MS.[15]

To analyze thymus and white visceral fat phospholipids by HPTLC, neutral lipids were first removed. Lipid extracts from 300 mg of tissue were dissolved in 2 mL hexane and applied to 500 mg silica columns equipped with stainless-steel frits and 10 mL reservoirs (Analytichem International, Harbor City, CA USA). Columns were mounted to a Supelco Visiprep Solid Phase Extraction Vacuum Manifold (Bellefonte, PA USA). Neutral lipids were eluted with 12 mL $CHCl_3$; phospholipids were eluted with 4 mL of $CHCl_3$/MeOH (2:1 vol/vol) followed by 4 mL MeOH.[18]

The neutral lipid extracts from visceral fat and from hepatic lipid extracts (300 mg) were then redissolved in hexane/methyltertiarybutylether (200:3 vol/vol; Burdick and Jackson, Muskegon, MI USA), and applied to 500 mg silica columns (Analytichem) using the solvent system of Hamilton and Comai[19] to obtain cholesterol ester, free fatty acid, and triacylglycerol lipid classes. The purity of the cholesterol ester and triacylglycerol fractions was confirmed by HPTLC using 10 × 10 cm Silica Gel 60 plates (E. Merck, Darmstadt, Germany) and hexane/methyltertiarybutylether (200:3 vol/vol). A solvent system of benzene/ether/ethyl acetate/acetic acid (80:10:10:0.2, by vol) was utilized to confirm the purity of the

Table 1 Fatty acid composition of the experimental diets

Fatty acid Olive/Saf/Lin. (wt%)	PO	PO 18:1 15:15:70	JC	JC 18:1 35:35:30	JC 18:3 0:27:73
18:1n-9	11.3	24.9	13.0	36.5	15.1
18:2n-6	24.0	23.9	32.9	34.0	33.1
20:2n-6	0.0	0.0	2.0	0.0	0.0
5,11-20:2	0.6	0.0	0.8	0.0	0.0
5,11,14-20:3	2.9	0.0	16.0	0.0	0.0
5,11,14,17-20:4	9.1	0.0	6.8	0.0	0.0
18:3n-3	40.6	40.8	17.9	17.7	42.3
20:3n-3	0.5	0.0	0.5	0.0	0.0
Others*	10.9	10.4	10.1	11.8	9.5
SUM NMIFA	12.6	0.0	23.6	0.0	0.0
NMIFA + 18:3n-3	53.2	40.8	41.5	17.7	42.3
NMIFA + 18:1n-9	23.9	24.9	36.6	36.5	15.1

Results are expressed as the average area % of two determinations. Abbreviations: NMIFA, non methylene interrupted fatty acids; PO, *Platycladus orientalis;* JC, *Juniperus chinensis;* PO 18:1, JC 18:1 and JC 18:3, see Methods and materials section; Saf, safflower; Lin, linseed oil.
*Includes: 14:0, 16:0, 18:0, 20:0, 20:1n-9, 22:0, and 24:0.

free fatty acid fraction and to obtain hepatic 1,2-diacylglycerol nad 1,3-diacylglycerol fractions (*Table 2*).[20]

For the analysis of the combined isomers of PIP_2, livers from five mice (2 g) were pooled; extracted with $CHCl_3$, MeOH, and HCl mixtures; redissolved in $CHCl_3$/MeOH/1 M HCl/0.2 M ammonium formate in MeOH (1.0:0.5:0.5:2.0, by vol), and applied to neomycin columns.[21-23] The columns consisted of pasteur pipettes containing neomycin sulfate (Sigma Chemical Co., St. Louis, MO USA) reductively coupled to 2420 nm diameter oxidized glass beads (CPG Inc., Fairfield, NJ USA). Twelve column volumes of 0.2 M NH_4-formate in $CHCl_3$/MeOH/$H_2$0 (5:10:2 vol/vol/vol) were added to elute all phospholipids except phosphatidylinositol monophosphate (PIP) and PIP_2 (fraction 1), followed by 12 column volumes of 1 M NH_4-formate in $CHCl_3$/MeOH/$H_2$0 (5:10:2 vol/vol/vol) to elute PIP and PIP_2 (fraction 2).[22] The PIP and PIP_2 fractions were resolved by HPTLC with a $CHCl_3$/MeOH/ammonia/H_2O (45:35:8:4, by vol) solvent system[24] and 10 × 10 cm Silica Gel 60 plates (E. Merck), and identified by comparison with phospholipid standards (Sigma). The R_f values for PIP_2, PIP, and other phospholipids were, respectively: 0.03, 0.14, and 0.44–0.75. PIP and PIP_2 were the only phospholipids that were

Table 2 Fatty acid composition of neutral lipid classes from liver and visceral fat

	Liver										Visceral fat					
	Platycladus orientalis					*Juniperus chinensis*					*Platycladus orientalis*			*Juniperus chinensis*		
Fatty acids	1,2-DAG	1,3-DAG	CE	FFA	TAG	1,2-DAG	1,3-DAG	CE	FFA	TAG	CE	FFA	TAG	CE	FFA	TAG
16:0	27.5	28.1	14.6	28.0	23.0	27.5	17.7	20.6	26.9	18.3	22.9	32.2	28.5	24.0	30.4	23.3
18:0	8.3	25.3	3.3	10.6	2.8	5.4	10.3	4.7	14.7	3.9	24.6	54.9	4.8	15.6	49.7	5.3
18:1n-9 + 7*	41.6	32.3	55.2	34.6	46.2	41.8	44.1	47.2	30.2	41.9	15.8	4.1	32.1	30.5	7.1	32.9
18:2n-6	12.0	11.1	8.8	10.9	14.0	12.2	16.9	10.5	7.7	13.9	11.6	1.2	10.7	13.9	2.6	15.6
20:2n-6	0.0	0.0	0.1	0.4	0.3	0.4	0.5	0.0	0.3	0.1	0.00	0.1	0.2	0.0	0.2	0.3
20:3n-6	0.0	0.0	0.1	0.3	0.2	0.2	0.5	0.1	0.3	0.1	0.00	0.0	0.1	0.0	0.0	0.1
20:4n-6	0.0	0.0	0.7	0.8	0.2	0.3	2.2	0.3	0.5	0.4	0.00	0.0	0.1	0.0	0.1	0.1
22:4n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:5n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5,11-20:2	0.0	0.0	0.0	0.1	0.2	0.3	0.0	0.0	0.1	0.1	0.3	0.0	0.2	0.0	0.1	0.1
5,11,14-20:3	1.7	0.0	1.8	1.2	1.0	1.8	0.0	0.6	0.4	0.7	1.3	0.1	0.6	0.0	0.5	2.3
5,11,14,17-20:4	0.7	0.0	1.3	0.5	0.6	0.7	0.7	0.5	0.6	1.1	0.0	0.0	1.1	0.0	0.1	0.9
18:3n-3	3.5	3.2	4.4	5.4	5.1	3.4	3.0	7.3	9.1	11.4	1.2	0.5	8.4	4.1	0.8	5.5
20:3n-3	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.1	0.5	0.3	0.0	0.0	0.1	0.0	0.0	0.1
20:5n-3	0.0	0.0	0.2	0.4	0.3	0.2	0.6	0.3	0.9	0.9	3.0	0.1	0.1	0.0	0.1	0.1
22:5n-3	0.0	0.0	0.0	0.2	0.2	0.2	0.0	0.1	0.5	0.4	0.0	0.1	0.1	0.0	0.1	0.0
22:6n-3	0.0	0.0	1.6	1.1	0.6	0.5	0.7	0.7	1.3	1.2	0.0	0.0	0.1	0.0	0.1	0.1
Others†	4.6	0.0	7.8	5.6	5.0	5.2	2.8	7.2	5.9	5.2	19.2§	6.7	12.8	11.9†	7.9	13.4

Results are expressed as the area % of each fatty acid in the designated lipid class and represent the average of 2–4 determinations. A value of 0.0 indicates that the area % was less than 0.05% or not detectable. Abbreviations: DAG, diacylglycerol; CE, cholesteryl ester; FFA, free fatty acid; and TAG, triacylglycerol.
*See *Table 3*.
†Includes: 14:0, 14:1n-5, 15:0, 16:1n-9 + 7, 18:3n-6, 20:3n-9, 18:4n-3, 20:0, 22:0, 24:0, 20:1n-9, 22:1n-9, and 24:1n-9.
§Approximately half of the fatty acids in the Others category were 22:1n-9.

detectable in fraction 2 after charring with a $CuSO_4$ solution; all other phospholipid classes were detectable in fraction 1.

Statistics

Values represent the average area% of 3–7 determinations for each lipid class. Data were evaluated by analysis of variance combined with Fisher's protected least significant difference multiple comparison test ($P < 0.05$), using the Macintosh computer program SuperANOVA (Abacus Concepts, Berkeley, CA USA). Data for sphingomyelin (SPH), phosphatidylserine (PS), cardiolipin (CL), and PI of kidney, thymus, and visceral fat are not included in tables for brevity.

Results and discussion

Weight gain

There were no differences in feed consumption between the groups, with exception of experimental days 1–2. After 2 weeks of feeding the experimental diets, the final weights of PO- and JC-fed mice were significantly less ($P < 0.05$) than their respective PO 18:1 and JC 18:1 controls, although quantitatively these differences were small (e.g., ≤ 2.0 g). In a related long-term study concerned with the metabolism of PO oil, no mortalities have been observed in mice fed PO oil for a 5-month period (unpublished results).

Esterification of 5,11,14-20:3: a factor affecting incorporation into phospholipid classes?

Previously we found that when 5,11,14-20:3 was fed as a free fatty acid component of saponified PO oil, it was incorporated into hepatic PI at a level of 9.8 area%.[12] In the present investigation, 5,11,14-20:3 fed as a component of intact PO oil was similarly incorporated to a level of 9.2%. The free acid of 5,11,14-20:3 may therefore be absorbed and metabolized similarly to the natural esterified form of 5,11,14-20:3.

Tissue differences in the incorporation of dietary fatty acids into phospholipid classes

There were significant differences in the tissue distribution of 5,11,14-20:3 in phospholipid classes. There was more 5,11,14-20:3 incorporated into PI of the liver and heart than the spleen and kidney at the higher dosage level. At the lower dosage level there was clearly more 5,11,14-20:3 incorporated into hepatic PI (9.2%) than cardiac, renal, and splenic PI (3.1–4.6%) (*Table 3; Figure 1*).[12] Levels of 5,11,14-20:3 in visceral fat and thymic PI were uniquely low (2.0%). Conceivably, levels of 5,11,14-20:3 in these non-hepatic tissues may have become further enriched had a longer feeding duration been employed.

There was more 5,11,14-20:3 incorporated into PC of the liver (13.0%) than other tissues (6.4–8.3%) at the higher dosage level (*Table 4*). Interestingly, in PE, the incorporation of 5,11,14-20:3 was greatest in the spleen at both dosage levels (*Table 5*).

Regarding the incorporation of n-3 fatty acids, the greatest organ difference was the more extensive incorporation of 22:6n-3 into PC and PE from the heart relative to the liver. In PE 22:5n-3 levels were threefold higher in the spleen than in other tissues and 20:5n-3 levels were highest in the liver.[4]

Differences in the incorporation of dietary fatty acids into hepatic phospholipid classes

Among hepatic phospholipid classes, the incorporation of 5,11,14-20:3 was greatest in PI compared with SPH, CL, PS, PE, and PC (*Figure 2*). A similar level of incorporation of 5,11,14-20:3 into PI and PIP_2 was evident at both dosage levels (PO data shown, *Figure 3*). 5,11,14-20:3 appears to be excluded from SPH and CL, which are phospholipids having only small amounts of 20:4n-6. At the higher dosage level, the differences between PC and PI were less pronounced. The level of 5,11,14-20:3 in hepatic PI increased from 9.2 to 16.7% as the dietary concentration of 5,11,14-20:3 was increased from 2.9% (PO oil) to 16% (JC oil). A similar proportional increase of 5,11,14-20:3 (slope = 0.6) was found in PC. Overall, the main 5,11,14-20:3 containing phospholipids were: hepatic and cardiac PI, 15–17%; hepatic PC, 13%, splenic PE, 10%; and hepatic PS, 7%. In human endothelial cells incubated with 5,11,14[^{14}C]-20:3, the area% distribution of 5,11,14-20:3 was similarly found to be: PI/PS≈PC>PE.[25]

In *Figure 3*, only those fatty acids with two or more double bonds were included in calculating the area percentage of n-6, n-3, and non-methylene interrupted fatty acids in different hepatic phospholipid classes following PO oil feeding. Previous work suggests that polyenes are largely incorporated into the *sn*-2 position of phospholipids[26,27] (excluding CL),[6] where they may be released as eicosanoid precursors. PC contained about the same proportions of n-6 fatty acids (largely 18:2n-6 and 20:4n-6) as n-3 fatty acids (20:5n-3, 22:6n-3). CL, PI and PIP_2 contained the greatest area% of n-6 fatty acids as 18:2n-6 (CL) or 20:4n-6 (PI/PIP_2). However, the n-6 NMIFA 5,11,14-20:3 accumulated most extensively in PI and PIP_2. n-3 fatty acids accumulated most extensively in PS and PE, but were excluded from PI. Although n-3 fatty acids did not accumulate extensively in hepatic CL, 22:6n-3 can accumulate in cardiac CL to a level of 48% when fish oil is the n-3 dietary source.[13]

Incorporation of other NMIFA and minor fatty acids into phospholipid classes

The NMIFA 5,11-20:2 (up to 0.8% in the PO/JC diets), and particularly 5,11,14,17-20:4 (7–9% in the diets), were essentially excluded from all phospholipid classes examined (*Tables 3–5*).[12] It is noteworthy that in the cardiac PE lipid class, the n-3 polyene 22:6n-3 accumulated to a level of 43%;[4,28] whereas the n-3 NMIFA 5,11,14,17-20:4 was excluded, and the n-6 NMIFA 5,11,14-20:3 accumulated to a level of only 2.6%. In all tissues and phospholipids examined, 18:3n-3 did not accumulate extensively.[4] 22:4n-6 and

Table 3 Fatty acid composition of phosphatidylinositol from heart, liver, and spleen

	Heart					Liver					Spleen				
Fatty acids	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC
16:0	2.6^{ab}	3.4^{b}	1.8^{a}	1.8^{a}	2.0^{a}	3.5	2.7	2.8	3.4	3.7	4.9	4.4	4.2	3.3	4.7
18:0	60.9^{b}	58.5^{b}	57.4^{b}	58.0^{b}	45.4^{a}	49.9^{a}	51.0^{a}	56.1^{b}	52.6^{ab}	52.1^{ab}	61.4	59.2	59.0	56.9	64.4
18:1n-9+7*	3.4^{c}	2.1^{a}	2.5^{ab}	2.9^{b}	2.7^{b}	2.2	2.4	1.7	2.2	2.6	3.1^{c}	2.6^{b}	2.4^{ab}	2.9^{c}	2.1^{a}
18:2n-6	4.1^{b}	2.7^{a}	3.6^{ab}	3.7^{b}	4.0^{b}	1.1	1.1	1.2	1.2	1.3	1.5	1.3	1.4	1.0	1.1
20:2n-6	0.0^{a}	0.3^{b}	0.3^{bc}	0.6^{d}	0.5^{cd}	1.0^{b}	0.6^{ab}	0.0^{a}	0.5^{ab}	0.4^{a}	0.0	0.1	0.0	0.0	0.0
20:3n-6	2.6^{b}	1.9^{a}	2.8^{b}	3.0^{b}	2.1^{a}	3.2^{c}	2.5^{b}	3.2^{c}	2.7^{b}	1.6^{a}	1.7^{c}	1.3^{ab}	1.7^{c}	1.6^{bc}	1.1^{a}
20:4n-6	21.6^{a}	19.9^{a}	25.2^{b}	25.8^{b}	20.6^{a}	29.2^{c}	21.9^{b}	28.4^{c}	31.9^{c}	16.5^{a}	22.3^{a}	23.0^{ab}	25.9^{b}	29.9^{c}	20.5^{a}
22:4n-6	0.1^{a}	0.2^{a}	0.1^{a}	0.2^{a}	0.5^{b}	0.1	0.0	0.1	0.1	0.0	0.5^{a}	0.6^{a}	0.6^{a}	1.0^{b}	0.6^{a}
22:5n-6	0.0	0.3	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5,11-20:2	0.0^{a}	0.2^{b}	0.0^{a}	0.0^{a}	0.3^{c}	0.0	0.1	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0
5,11,14-20:3	0.0^{a}	4.6^{b}	0.0^{a}	0.0^{a}	15.0^{c}	0.2^{a}	9.2^{b}	0.1^{a}	0.1^{a}	16.7^{c}	0.0^{a}	3.2^{b}	0.0^{a}	0.0^{a}	3.6^{b}
5,11,14,17-20:4	0.0^{a}	0.3^{c}	0.0^{a}	0.0^{a}	0.2^{b}	0.1^{a}	1.5^{b}	0.0^{a}	0.1^{a}	0.6^{a}	0.0^{a}	0.4^{b}	0.0^{a}	0.0^{a}	0.0^{a}
18:3n-3	0.3^{c}	0.1^{b}	0.0^{a}	0.0^{a}	0.0^{a}	0.1	0.3	0.1	0.4	0.3	0.2	0.2	0.1	0.0	0.0
20:3n-3	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.2^{b}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:5n-3	0.5^{ab}	0.8^{b}	0.5^{ab}	0.1^{a}	0.1^{a}	6.6^{c}	3.5^{b}	3.4^{b}	1.1^{a}	0.8^{a}	1.6^{b}	1.3^{b}	1.4^{b}	0.5^{a}	0.0^{a}
22:5n-3	1.1^{ab}	1.2^{ab}	1.7^{c}	1.1^{a}	1.5^{bc}	1.7^{c}	1.0^{b}	0.6^{ab}	0.4^{a}	0.6^{ab}	1.4	1.3	1.5	1.1	0.6
22:6n-3	1.4	2.8	3.4	2.6	3.3	1.7^{b}	1.6^{ab}	1.3^{ab}	1.0^{a}	1.3^{ab}	0.7^{a}	0.7^{a}	0.9^{ab}	1.0^{b}	1.1^{b}
Others†	1.3	0.6	0.4	0.4	1.7	0.2	0.5	0.8	2.3	1.2	0.7	0.4	0.7	0.8	0.2

See legend for *Table 1* for abbreviations. Results are expressed as area % of each fatty acid in the phospholipid, and represent the average of 3–7 determinations. A value of 0.0 indicates that the area % was less than 0.05%, or not detectable. Statistics are presented for the main effect, Diet. For a given organ, values sharing a common superscript, or lacking any superscript, are not significantly different at $P > 0.05$ (Fisher's protected least significant difference multiple comparison test).
*Combined isomers of 18:1n-9 and 18:1n-7.
†Includes: 14:0, 14:1n-5, 15:0, 16:1n-9+7, 18:3n-6, 18:4n-3, 20:0, 22:0, 24:0, 20:1n-9, 22:1n-9, and 24:1n-9.

22:5n-6 were minor components in all phospholipid classes.

Incorporation of dietary fatty acids into neutral lipid classes

In both visceral fat and liver there was minimal accumulation of 5,11,14-20:3 and 5,11,14,17-20:4 into cholesterol ester, free fatty acid, diacylglycerol, and triacylglycerol pools, with levels reaching 1.8% (*Table 2*). In all the neutral lipid classes examined, the major saturated fatty acids were 16:0 and 18:0, the major monoene was 18:1n-9, the major n-6 polyene was 18:2n-6 and the major n-3 polyene was 18:3n-3.[29] In contrast to phospholipids, the polyenes 20:4n-6, 20:5n-3, and 22:6n-3 were only very minor constituents in neutral lipid classes. There was no evidence that 20:4n-6 was shifted from phospholipid to cholesterol ester pools[30] based on the low levels of 20:4n-6 in cholesterol esters from mice fed PO and JC oils. The major fatty acid in hepatic cholesterol esters was 18:1n-9.[30] In the visceral fat cholesterol ester pool, 16:0, 18:0, 18:1n-9, 18:2n-6, and 22:1n-9 were abundant fatty acids.[29]

Evidence for the direct replacement of 20:4n-6 with 5,11,14-20:3 in phospholipid pools

There are several lines of evidence to suggest that 5,11,14-20:3 directly replaced 20:4n-6 in hepatic PI.

First, although JC oil contained more 18:2n-6 (a precursor of 20:4n-6) and less 18:3n-3 (a precursor of 20:5n-3, which could compete with 20:4n-6 for *sn*-2 acylation), the area% of 20:4n-6 decreased from 31.9% to 16.5% (a 48% reduction), as the concentration of dietary 5,11,14-20:3 was increased.

Second, by replacing dietary 18:1n-9 with 5,11,14-20:3 (PO 18:1 versus PO; JC 18:1 versus JC), there was a significant decrease in the area% of 20:4n-6 ($P < 0.05$). There was also some replacement of 20:5n-3 with 5,11,14-20:3 following PO oil feeding.

Third, by replacing dietary 18:3n-3 with 5,11,14-20:3 (JC 18:3 versus JC), there was a significant decrease in 20:4n-6 ($P < 0.05$). This suggests that dietary 5,11,14-20:3 was more effective than dietary 18:3n-3 at decreasing hepatic PI 20:4n-6 levels. It would be interesting to compare JC oil with a paired control in which 20:5n-3 and/or 22:6n-3 (the fatty acids found in fish oils) replaced 5,11,14-20:3. Fish-oil feeding is reported to reduce 20:4n-6 levels in renal,[31] splenic,[32] and cardiac[33] PI pools in mice. Lokesh et al.[32] and Swanson et al.[33] found that PI was, however, combined with PS, which contains a large proportion of n-3 fatty acids.

In addition to the direct replacement of 20:4n-6 with 5,11,14-20:3, dietary 5,11,14-20:3 (or any other component in the PO and JC oils) could alternatively inhibit the $\Delta 6$ or $\Delta 5$ desaturases that are responsible for the conversion of dietary 18:2n-6 to 20:4n-6. Although we did not directly assess these enzyme activities, precursor to product ratios can be utilized to assess changes in apparent activity. Values for the ratio of 20:3n-6 to 20:4n-6 in hepatic PI were 0.084, 0.097, and 0.113 for JC 18:1, JC, and JC 18:3, respectively. This represents a quantitatively small (13.4%), but significant ($P < 0.05$) decline in apparent $\Delta 5$ desaturase activity with JC feed-

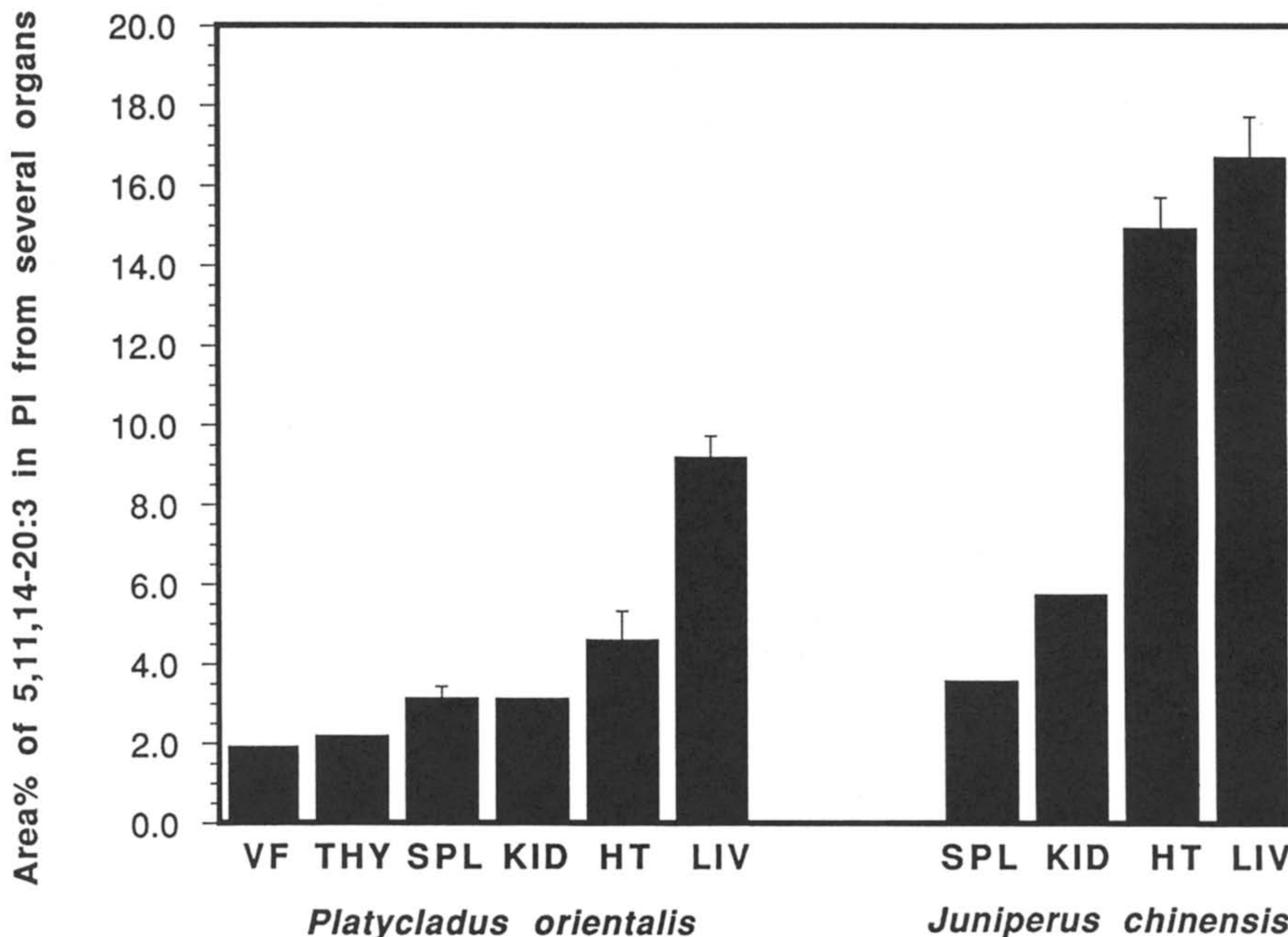

Figure 1 Incorporation of 5,11,14-icosatrienoate into phosphatidylinositol from different tissues. Values represent the average area % of 4–7 determinations, except in cases where tissue samples were pooled, and $n = 1$. Error bars denote 1 SE. Abbreviations: HT, heart; KID, kidney; LIV, liver; SPL, spleen; THY, thymus; VF, visceral fat.

ing relative to JC 18:1 feeding. The lack of increase in 18:2n-6 levels in hepatic PI following JC feeding and the lack of significant increase ($P > 0.05$) in 22:5n-3 to 22:6n-3 ratios, is an indication that Δ6 desaturase activity was probably not inhibited. These results suggest that the decline in 20:4n-6 levels in hepatic PI is due largely to replacement with 5,11,14-20:3 rather than to a decreased substrate supply of 20:4n-6.

In contrast to hepatic PI, in splenic, renal, and cardiac PI 20:4n-6 levels were significantly lowered only when dietary 5,11,14-20:3 levels were 16% (JC oil).

In PS, PC, and PE, 5,11,14-20:3 also replaced 20:4n-6. However, in contrast to PI, in hepatic and splenic PC and PE, the reductions in 20:4n-6 with 5,11,14-20:3 feeding were less dramatic because 5,11,14-20:3 replaced several polyenes, including 18:1n-9, 18:2n-6, 20:4n-6, and 20:5n-3 ($P < 0.05$). JC 18:3 feeding resulted in replacement of 20:4n-6 with 20:5n-3, and was as effective as JC feeding in reducing 20:4n-6 levels in PC and PE.

Biochemical mechanisms for the extensive incorporation of 5,11,14-20:3 into phospholipids

There are several biochemical mechanisms that might account for the extensive incorporation of 5,11,14-20:3 into arachidonate-containing phospholipids, most notably PI and PC. Such mechanisms would also need to account for the exclusion of other eicosatrienes, such as 11,14,17 20:3.[4]

First, there may be an abundance of 5,11,14-20:3 substrate available for phospholipid acylation because 5,11,14-20:3 was not converted to other fatty acid metabolites based on gas chromatographic data. On the basis of the position of the double bonds, 5,11,14-20:3 is not expected to be a substrate for the Δ9, Δ6, Δ5, or Δ4 desaturases, if the latter actually exists.[34] Furthermore, 5,11,14-20:3 cannot be converted to 20:4n-6 because mammalian cells do not appear to have a Δ8 desaturase.[35–37] There was also no evidence from the present study and others[12,25,35,38] that 5,11,14-20:3 was exten-

Table 4 Fatty acid composition of phosphatidylcholine from heart, liver, and spleen

	Heart					Liver					Spleen				
Fatty acid	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC
16:0	18.2[ab]	21.0[c]	19.5[bc]	18.4[ab]	17.0[a]	26.5[b]	23.1[a]	23.8[a]	23.2[a]	22.6[a]	38.6[ab]	37.8[a]	43.1[b]	39.8[ab]	42.7[b]
18:0	22.8[ab]	23.5[b]	23.2[b]	21.3[a]	21.9[ab]	15.7[a]	18.2[bc]	18.5[c]	17.0[ab]	18.9[c]	12.4	12.1	12.1	10.9	11.1
18:1n-9+7	10.5[c]	8.9[bc]	7.9[b]	9.6[c]	5.9[a]	12.5	11.1	10.3	11.4	11.0	14.3[c]	10.9[a]	12.1[b]	13.4[c]	9.8[a]
18:2n-6	8.4[b]	8.0[b]	7.6[b]	7.4[b]	5.7[a]	13.1[b]	13.8[b]	19.0[d]	17.3[c]	10.9[a]	11.0[bc]	11.2[bc]	11.9[c]	9.8[b]	8.2[a]
20:2n-6	0.2[a]	0.7[b]	0.3[a]	0.3[a]	0.8[c]	0.2	0.3	0.2	0.3	0.3	0.9[a]	1.2[b]	0.9[a]	1.0[ab]	1.4[c]
20:3n-6	0.6[b]	0.5[a]	0.7[b]	0.8[c]	0.4[a]	1.9[ab]	1.7[a]	2.0[b]	2.9[c]	2.0[b]	1.4[bc]	1.2[b]	1.5[c]	1.6[c]	1.0[a]
20:4n-6	5.5[a]	5.6[a]	6.2[a]	7.7[b]	5.8[a]	4.4[a]	4.7[a]	5.7[b]	9.8[d]	6.5[c]	8.6[a]	9.0[a]	8.7[a]	12.5[b]	8.2[a]
22:4n-6	0.2[a]	0.2[a]	0.2[a]	0.3[b]	0.3[b]	0.0	0.1	0.2	0.1	0.0	0.4[a]	0.4[a]	0.4[a]	0.8[c]	0.6[b]
22:5n-6	0.4[a]	0.5[a]	0.4[a]	0.6[b]	0.7[b]	0.0	0.0	0.0	0.1	0.0	0.1	0.2	0.1	0.1	0.1
5,11-20:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5,11,14-20:3	0.0[a]	3.0[b]	0.0[a]	0.0[a]	6.4[c]	0.1[a]	5.3[b]	0.1[a]	0.1[a]	13.0[c]	0.1[a]	3.9[b]	0.1[a]	0.1[a]	7.5[c]
5,11,14,17-20:4	0.0[a]	0.2[b]	0.0[a]	0.0[a]	0.2[b]	0.0	0.5	0.0	0.0	0.4	0.0[a]	0.6[c]	0.0[a]	0.0[a]	0.3[b]
18:3n-3	0.8[c]	0.2[a]	0.6[b]	0.3[a]	0.3[a]	1.5[b]	1.5[b]	1.3[b]	0.5[a]	0.4[a]	1.3[b]	1.2[b]	0.6[a]	0.4[a]	0.5[a]
20:3n-3	0.0[ab]	0.0[ab]	0.1[c]	0.1[b]	0.2[c]	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.3
20:5n-3	0.5[d]	0.4[c]	0.4[c]	0.2[b]	0.1[a]	9.1[d]	5.6[c]	5.6[c]	2.8[b]	1.6[a]	1.9[c]	1.7[c]	1.3[b]	0.7[a]	0.4[a]
22:5n-3	3.7[bc]	3.2[ab]	4.2[c]	2.9[a]	2.6[a]	1.0[d]	1.0[cd]	0.8[bc]	0.6[ab]	0.5[a]	2.1[d]	1.8[cd]	1.6[bc]	1.4[ab]	1.1[a]
22:6n-3	27.1[b]	23.1[a]	28.0[bc]	29.2[bc]	30.4[c]	12.1[b]	11.4[ab]	10.3[a]	12.4[b]	10.7[a]	2.8	2.8	2.0	3.1	3.3
Others	1.1	1.1	0.8	1.0	1.3	1.6	1.4	1.8	1.6	0.9	4.0	3.7	3.4	4.4	3.5

See *Table 3* for abbreviations and statistical methodology.

Table 5 Fatty acid composition of phosphatidylethanolamine from heart, liver, and spleen

	Heart					Liver					Spleen				
Fatty acid	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC	PO 18:1	PO	JC 18:3	JC 18:1	JC
16:0	5.0	5.6	5.1	4.6	5.1	14.7	15.3	14.8	13.9	14.9	7.2	7.3	7.1	6.7	7.9
18:0	28.7[c]	28.9[c]	26.1[ab]	25.1[a]	26.6[b]	26.5	24.9	28.0	26.3	25.7	27.8	25.3	25.4	24.6	25.1
18:1n-9+7	7.1[b]	5.8[a]	6.6[b]	7.7[c]	5.8[a]	7.7[ab]	8.9[c]	7.3[a]	8.3[abc]	8.6[bc]	7.9[bc]	6.1[bc]	6.4[ab]	7.1[b]	5.7[a]
18:2n-6	2.5[a]	2.6[a]	4.9[bc]	6.0[c]	3.6[ab]	4.8[b]	3.9[a]	5.1[b]	5.1[b]	3.4[a]	4.7[bc]	4.8[bc]	5.1[c]	3.4[a]	3.8[ab]
20:2n-6	0.0[a]	0.2[b]	0.2[b]	0.3[b]	0.4[c]	0.0[a]	0.1[ab]	0.0[a]	0.0[a]	0.1[b]	0.6	0.8	0.6	0.7	0.9
20:3n-6	0.3[a]	0.2[a]	0.4[bc]	0.4[c]	0.3[ab]	0.6[b]	0.3[a]	0.7[bc]	0.9[c]	0.6[ab]	1.4[c]	1.2[b]	1.5[d]	1.4[c]	0.9[a]
20:4n-6	6.9	7.7	7.0	7.3	6.4	12.7[ab]	11.9[a]	14.0[bc]	19.7[d]	14.8[c]	20.5[a]	20.3[a]	23.2[b]	26.9[c]	19.4[a]
22:4n-6	0.4[a]	0.4[b]	0.4[ab]	0.5[c]	0.6[d]	0.2[a]	0.1[a]	0.2[a]	0.5[b]	0.2[a]	2.1[a]	2.3[ab]	2.6[b]	4.2[d]	3.4[c]
22:5n-6	1.2[a]	1.5[b]	1.2[a]	1.5[b]	1.6[b]	0.0[ab]	0.0[a]	0.0[ab]	0.1[c]	0.1[bc]	0.1[a]	0.3[b]	0.2[b]	0.3[b]	0.4[b]
5,11-20:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5,11,14-20:3	0.0[a]	1.3[b]	0.0[a]	0.0[a]	2.6[c]	0.1[a]	2.9[b]	0.0[a]	0.1[a]	7.1[c]	0.0[a]	5.3[b]	0.0[a]	0.0[a]	9.8[c]
5,11,14,17-20:4	0.0[a]	0.2[c]	0.0[a]	0.0[a]	0.1[b]	0.0[a]	0.8[c]	0.0[a]	0.0[a]	0.2[b]	0.0[a]	0.7[c]	0.0[a]	0.0[a]	0.4[b]
18:3n-3	0.3[b]	0.3[bc]	0.4[c]	0.2[a]	0.2[a]	0.9[b]	0.7[b]	0.7[b]	0.3[a]	0.3[a]	0.6[b]	0.7[b]	0.1[a]	0.1[a]	0.3[a]
20:3n-3	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.1
20:5n-3	0.8[c]	0.7[b]	0.6[b]	0.3[a]	0.2[a]	10.3[e]	7.1[c]	7.7[d]	3.5[b]	1.9[a]	3.9[c]	3.0[b]	3.1[b]	1.3[a]	0.8[a]
22:5n-3	3.3[c]	3.1[b]	3.2[bc]	2.3[a]	2.2[a]	1.6[c]	1.3[b]	1.4[bc]	1.0[a]	0.9[a]	10.3[d]	8.9[c]	10.2[d]	6.9[b]	5.7[a]
22:6n-3	43.0	40.8	43.4	43.4	43.1	18.9	21.3	19.3	19.1	20.3	12.3[a]	12.0[a]	13.4[ab]	15.3[c]	14.4[bc]
Others	0.5	0.5	0.5	0.6	1.1	0.9	0.7	0.8	1.1	0.8	0.5	1.0	1.0	1.1	1.1

See *Table 3* for abbreviations and statistical methodology.

sively retroconverted to 3,9,12-18:3, a theoretical precursor of 20:4n-6. 5,11,14-20:3 has also been found to be poorly elongated compared with other fatty acid substrates in vitro.[39] If 5,11,14-20:3 is metabolized similarly to 20:4n-6, we also expect 5,11,14-20:3 to be a poor substrate for β-oxidation.[40]

Supporting these claims for the minimal metabolism of 5,11,14-20:3, Garcia et al.[39] found that approximately 73% of the 5,11,14[^{14}C]-20:3 administered to human endothelial cells was incorporated intact into total phospholipids. Schlenk et al.[35] found that in rats previously fed a fat-free diet for 37 weeks and then administered 5,11,14[^{14}C]-20:3 via a stomach tube, 97% of 5,11,14[^{14}C]-20:3 was incorporated into PC intact (PI was not examined).

In addition to being minimally metabolized, we found that 5,11,14-20:3 was preferentially incorporated into phospholipids rather than neutral lipids (*Table 2*). Similarly, Schlenk et al.[35] found there to be 100-fold more 5,11,14[^{14}C]-20:3 incorporated into hepatic PC than into triacylglycerol and cholesterol ester pools of mice.

Second, on the basis of our data it is plausible that in some cell types (e.g., hepatocytes) 5,11,14-20:3 may effectively compete with 20:4n-6 for acyl CoA synthe-

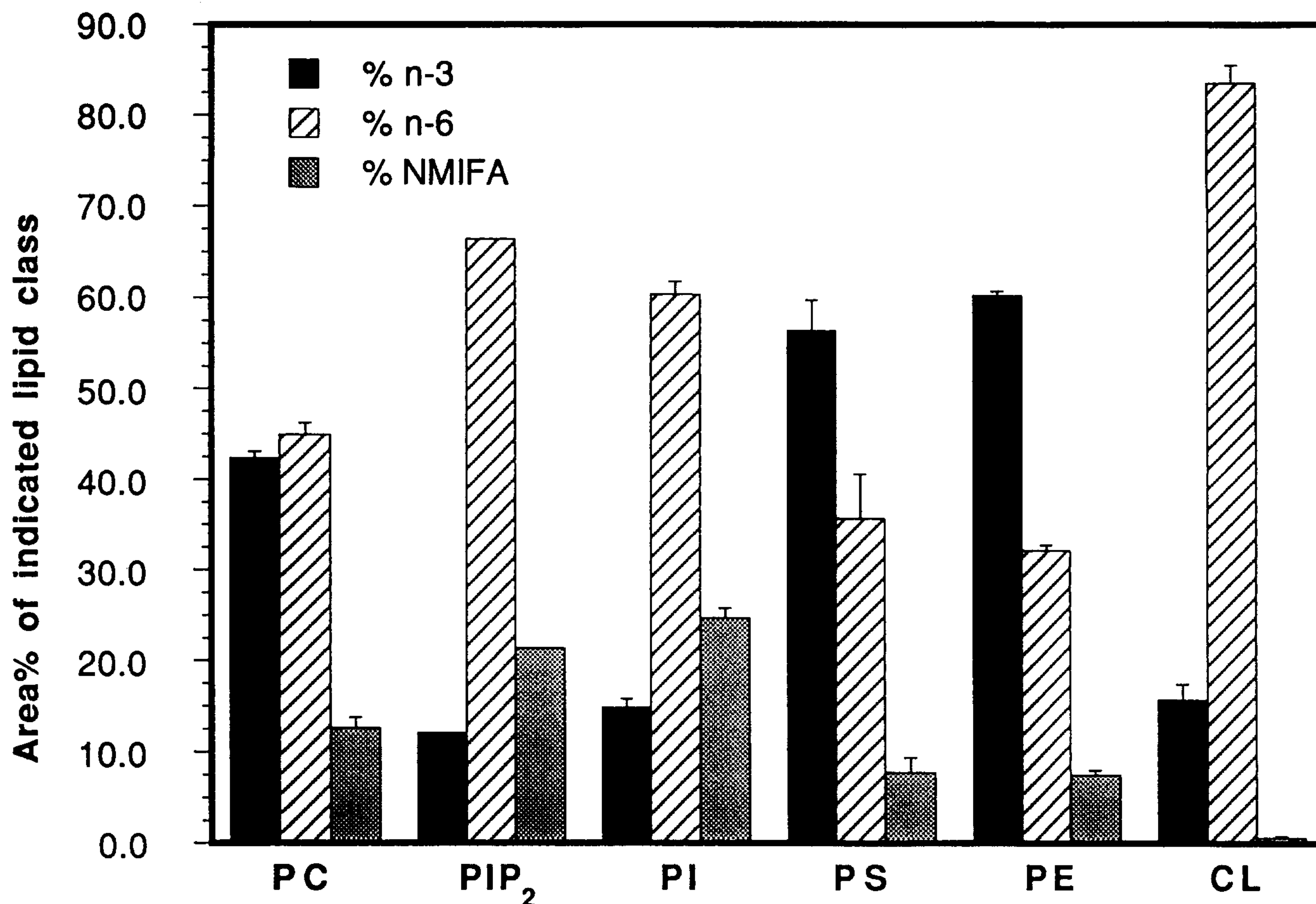

Figure 2 Incorporation of 5,11,14-icosatrienoate into hepatic phospholipid classes. Values represent the average area % of 3–7 determinations. Error bars denote 1 SE. Within each dietary group *(Platycladus orientalis* or *Juniperus chinensis)* bars sharing a letter in common are not significantly different at $P > 0.05$ (Fisher's protected least significant difference multiple comparison test). Abbreviations: SPH, sphingomyelin; CL, cardiolipin; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol.

tase or arachidonyl-CoA synthetase, the enzymes that catalyze the first step in the incorporation of fatty acids into phospholipids.[41] Interestingly, Neufeld et al.[42] found that the apparent K_i of 5,11,14-20:3 was however 5.5 times higher than 20:4n-6 for human platelet membrane arachidonoyl-CoA synthetase, and 3.2 times higher in calf brain extracts. In comparison with the K_i values of various other fatty acid derivatives and the acyl CoA synthesis rate of labeled fatty acids, arachidonoyl-CoA synthetase was concluded to have a specificity for 18-20 carbon fatty acids with Δ11 unsaturation, which includes 5,11,14-20:3.

Third, it is also plausible that 5,11,14-20:3 may compete with 20:4n-6 for binding to lysoPI acyl transferase during the de novo synthesis and retailoring of PI. In studies with bovine heart muscle microsomes[43] and human platelets,[44] this enzyme showed a definite preference for arachidonyl-CoA. Alternatively, this competition may occur during the synthesis of acyl-specific phosphatidic acid,[45] and during base exchange, CoA-independent and CoA-dependent transacylase reactions.[27,46–48] Retailoring at both the *sn*-1 and *sn*-2 positions is an important means for producing 1-stearoyl-2-arachidonoyl-*sn*-PI from its monounsaturated cytidine diphosphodiacylglycerol precursor (itself derived from phosphatidic acid).[2,27,46] There was no evidence that the feeding of diets rich in 5,11,14-20:3 perturbed retailoring at the *sn*-1 position in hepatocytes because 18:0 was the predominant saturated fatty acid, and its levels were not changed.[2,49]

Fourth, during retailoring operations, 5,11,14-20:3 might be a poor substrate for phospholipase A_2. In studies with unstimulated human endothelial cells there were relatively small differences in the basal release of 5,11,14-20:3, 20:4n-6, and other polyenes from phospholipids.[39] However, when these cells were stimulated with the phospholipase A_2 agonists, histamine and thrombin, the release of [^{14}C]-labeled fatty acids from total phospholipids was as follows: 5,8,11,14-20:4≈ 5,8,11-20:3>5,8,14-20:3»5,11,14-20:3.[25] Thus, the lack of a *cis* Δ8 double bond in 5,11,14-20:3 resulted in diminished agonist stimulated phospholipase A_2 release of this fatty acid from cellular phospholipids. In vivo, it is possible that 5,11,14-20:3 might be a poor substrate for

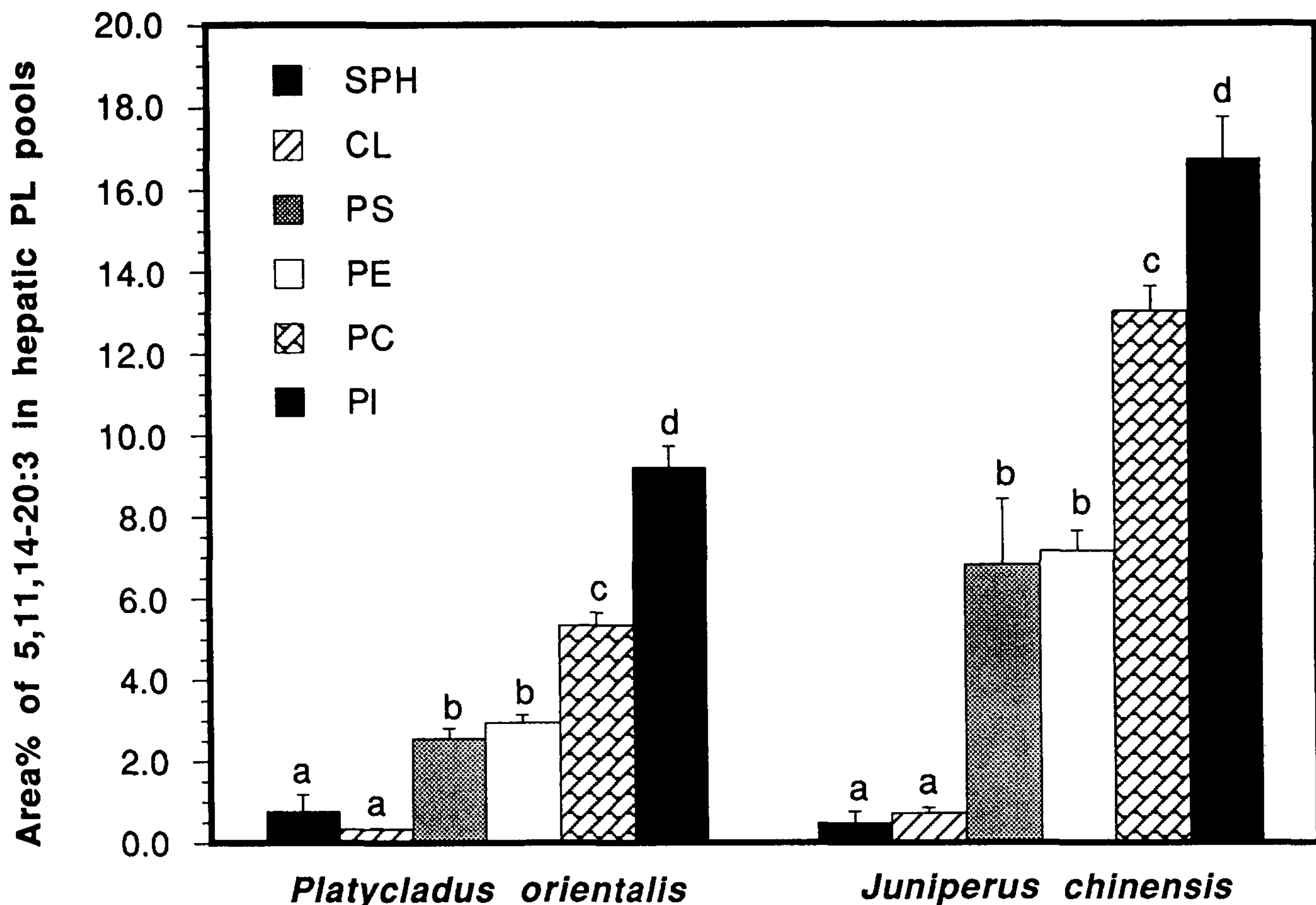

Figure 3 Relative amounts (area %) of polyunsaturated fatty acids (n-3, n-6, and non-methylene interrupted fatty acids–NMIFA) incorporated into hepatic phospholipid classes after feeding PO oil. Only those fatty acids with two or more double bonds were included in calculating the area percentage of n-6, n-3, and NMIFA. The percentage of n-3 includes: 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3; the percentage of n-6 includes: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, and 22:5n-6; and the percentage of NMIFA includes: 5,11,14-20:3, 5,11-20:2 and 5,11,14,17-20:4 (the latter two accumulated insignificantly). Values represent the average area % of 4–7 determinations, except for PIP_2, where five tissue samples were pooled. Error bars denote 1 SE. Abbreviations: refer to *Figure 1;* PIP_2, phosphatidylinositol bisphosphate.

phospholipase A_2 in some phospholipid pools under some conditions.

Exclusion of 5,11,14,17-20:4 from phospholipid and neutral lipid classes

The absence of 5,11,14,17-20:4 from all phospholipid and neutral lipid pools examined is intriguing. Clearly, not all eicosatetraenes (e.g., 5,8,11,14 20:4 and 5,11, 14,17 20:4) are incorporated equivalently into phospholipid pools. There was not evidence that 5,11,14,17-20:4 was extensively converted to 20:5n-3 on the basis that 20:5n-3 levels were not influenced by the feeding of 5,11,14,17-20:4. As stated, mammalian cells appear to lack the Δ8 desaturase. Other proposed pathways for the conversion of 5,11,14,17-20:4 to 20:5n-3 are as follows: (a) 5,11,14,17-20:4→3,9,12,15-18:4→3,6,9,12,15-18:5→5,8,11,14,17-20:5[37]; and (b) 5,11,14,17-20:4→11,14,17-20:3→9,12,15-18:3→6,9,12,15-18:4→8,11,14,17-20:4→5,8,11,14,17-20:5.[37,38] There was also no gas chromatographic evidence, such as the presence of major unknown peaks with appropriate retention times, to indicate that a docosapentaene metabolite of 5,11,14, 17-20:4 was extensively incorporated into lipid classes. Proposed pathways for the conversion of 5,11,14,17-20:4 to docosapentaene derivatives are as follows: (a) 5,11,14,17-20:4→7,13,16,19-22:4→9,15,18,21-24:4→6, 9,15,18,21-24:5→ 4,7,13,16,19-22:5[34]; and (b) 5,11,14, 17-20:4→5,9,11,14,17-20:5→7,11,13,16,19-22:5.[50]

There are several possible explanations to account for the absence of 5,11,14,17-20:4 in lipid classes. This fatty acid could be poorly absorbed, rapidly oxidized, and/or actively removed from phospholipids via phospholipase A_2. Alternatively, 5,11,14,17-20:4 might be a poor substrate for CoA synthetases or acyltransferases.

Physiological consequences of altering the acyl composition of PI and PIP_2

We have demonstrated that the feeding of JC oil containing 16 area% 5,11,14-20:3, resulted in a 48% reduction in the area% of 20:4n-6 in hepatic PI. In contrast to the methylene interrupted fatty acid 20:5n-3, which is a major component of fish oil,[51] the NMIFA 5,11,14-

20:3 is not a substrate for prostaglandin synthesis,[52,53] leukotriene synthesis,[54] or for the 12 lipoxygenase.[52] Aborted 11- and 15-hydroxyicosatrienoate eicosanoid side products may however be formed from 5,11,14-20:3.[52,53] Additionally, studies with human endothelial cells showed that the agonist-stimulated release of 5,11,14[^{14}C]-20:3 from phospholipids was comparatively less than for other polyenes evaluated.[25] For these reasons we expect that the consumption of 5,11,14-20:3 could have a major impact on eicosanoid production. Previously we demonstrated that the feeding of PO oil to mice for 2 weeks resulted in a significant reduction in the 12-hyroxyeicosatetraenoate production of homogenized lung tissue.[12]

Acyl alterations in PIP_2 may be important because PIP_2 is a precursor of the second messengers, diacylglycerol and inositol triphosphate. The extent to which dietary fat may modify the acyl composition of PIP_2 is not well characterized. Murine liver, brain,[1,26] and erythrocytes[55] have been found to have a similar acyl composition as PI. Palmer[56] found that lysophosphatidylinositol 4-phosphate and lysophosphatidylinositol 4,5-bisphosphate could not be enriched with ^{3}H arachidonate in rat brain and liver microsomes, which suggests that PIP_2 is not retailored following its synthesis. In contrast, Augert et al.[57] found that PIP_2 isolated from rat hepatocytes had higher levels of 16:0 ($P < 0.05$) and 18:0 (although not statistically significant), and significantly lower levels of 20:4n-6 (25% versus 37%, respectively), when compared with PI.

The physiological consequences of altering the acyl composition of PIP_2 and producing novel diacylglycerol and phosphatidic acid second messengers (e.g., 1-stearoyl-2-(5,11,14)-icosatrienoyl-*sn*-glycerol) are unknown in vivo.[58,59] It is also not known whether the observed acyl changes in PIP_2 will affect the enzyme kinetics for phospholipase C, leading to an altered rate of formation of inositol triphosphate and diacylglycerol.[60,61] There is in vitro evidence to suggest that different molecular species of diacylglycerol might activate protein kinase C differently.[61–64] In addition, PS,[62] PE,[65] and free fatty acids[66] may be structurally associated with protein kinase C, and these components are also responsive to dietary fat manipulation.[62] PC was also sensitive to dietary fat modification and there is evidence that PC may be structurally associated with protein kinase C[67] and an important source of diacylglycerol for the activation of protein kinase C.[27,57,68,69]

In summary, we have demonstrated that 5,11,14-20:3 was extensively incorporated into cardiac and hepatic PI (15–17%), and hepatic PC (13%) following the feeding of JC oil, but was minimally incorporated into neutral lipids and those phospholipids that contain only small amounts of 20:4n-6 (e.g., hepatic SPH and CL). Within the PI class, there were important differences in the tissue distribution of 5,11,14-20:3 over the 14-day feeding period: liver>heart>kidney = spleen>thymus = visceral fat. At the lower dose of 5,11,14-20:3, this fatty acid was preferentially incorporated into PI as compared with other phospholipids. In hepatic PI, 5,11,14-20:3 replaced 20:4n-6, resulting in a 50% reduction in the area% of 20:4n-6. By contrast, in PC and PE 5,11,14-20:3 replaced several polyenes including 18:2n-6, 20:4n-6, and 20:5n-3. In comparison with dietary 18:3n-3, 5,11,14-20:3 feeding resulted in lower levels of 20:4n-6 in hepatic PI. Because leukotrienes and prostaglandins cannot be formed from 5,11,14-20:3 due to the lack of an internal $\Delta 8$ double bond, and because 20:4n-6 was dramatically reduced in some PI pools, we expect that dietary intake of 5,11,14-20:3 may alter eicosanoid metabolism. 5,11,14-20:3 was also extensively incorporated into hepatic PIP_2. It will be important to assess whether the acyl change in PIP_2 will affect the kinetics of phospholipase C in vivo, and whether the novel molecular species of diacylglycerol generated may affect cellular signaling differently than 1-stearoyl-2-arachidonoyl-*sn*-diacylglycerol.

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