Remodeling of mouse kidney phospholipid classes and subclasses by diet

Robert S. Chapkin and Kimberly J. Coble

Department of Animal Science, Molecular and Cell Biology Section, and the Graduate Faculty of Nutrition, Texas A&M University and Texas Agricultural Experiment Station, College Station, TX, USA

Diets containing gammalinolenic acid (18:3n-6) and n-3 polyunsaturated fatty acids (PUFA) can improve renal function and favorably modulate glomerular injury as a result of various inflammatory reactions. In order to elucidate the mechanisms by which dietary n-6 and n-3 PUFA influence renal metabolism, the composition of kidney phospholipid classes and subclasses from mice fed either corn oil (CO) containing 18:2n-6, borage oil (BO) containing 18:2n-6 and 18:3n-6, black currant seed oil (BL) containing 18:2n-6, 18:3n-6 and 18:3n-3, fish-corn oil mix (F/C) containing 18:2n-6, 20:5n-3 and 22:6n-3, and a fish-borage oil mix (F/B) containing 18:2n-6, 18:3n-6, 20:5n-3 and 22:6n-3 were determined. Three weeks of feeding the different lipids produced no significant change in the relative percentage of any phospholipid classes or subclasses of the choline (ChoGpl) and ethanolamine (EtnGpl) glycerophospholipids. In general, FO and F/B diets increased 20:5n-3 and 22:6n-3 in all phospholipids except sphingomyelin (CerPCho). BL feeding produced a smaller relative increase in 20:5n-3 and 22:6n-3. Dietary supplementation with 18:3n-6 (BO, BL and F/B diets) resulted in the accumulation of 20:3n-6 in diacyl-glycerophosphocholine (PtdCho), diacyl-glycerophosphoethanolamine (PtdEtn), phosphatidylinositol (PtdIns), and phosphatidylserine (PtdSer). The combination of 18:3n-6 and n-3 PUFA (F/B diet) elevated the diacyl phospholipid 20:3n-6/20:4n-6 ratio relative to CO, BO, BL, and F/C diets. These findings demonstrate that marked differences exist in the ability of kidney phospholipid classes and subclasses to incorporate dietary PUFA.

Keywords: kidney; phospholipids; dietary oils; polyunsaturated fatty acids

Introduction

A plethora of experimental evidence supports the view that dietary enrichment with specific polyunsaturated fatty acids (PUFA) has beneficial effects on renal function, morphology, and hypertension.[1-5] Diets containing fish oil, rich in eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), reduce cyclosporine induced renal dysfunction in the rat,[1] reduce blood pressure in essential hypertension in humans,[3] and suppress autoimmune lupus in mice.[6] In addition, diets containing the n-6 PUFA, gamma-linolenic acid (18:3n-6) and dihomogamma-linolenic acid (20:3n-6) may favorably alter hypertension,[4] renal function, and plasma lipids.[5]

Dietary PUFA are incorporated into tissue phospholipids and are potential antecedents of eicosanoids. It is now well established that alterations in the dietary content of PUFA are an important method for modulating kidney eicosanoid production[2,5,7] because, in general, eicosanoid biosynthesis is dependent upon the size and composition of the phospholipid precursor pool(s).[8] The alteration of cellular eicosanoid metabolism by specifically remodeling the phospholipid precursor levels of 20:3n-6, arachidonic acid (20:4n-6), 20:5n-3, and 22:6n-3 may in part be responsible for the attenuation of renal diseases, hypertension, and acute organ rejection in renal transplant recipients.[1-3,9,10] However, no studies to date have determined the ability of dietary gamma-linolenic acid and n-3 PUFA to modify kidney eicosanoid precursor reservoirs (i.e., phospholipid subclasses which have distinct metabolic and physical properties.)[11] The sub-

Address reprint requests to Dr. Robert S. Chapkin at the Department of Animal Science, Molecular and Cell Biology Section, Texas A&M University and Texas Agricultural Experiment Station, College Station, TX 77843-2471, USA.
Received June 11, 1990; accepted August 30, 1990.

classes of phospholipids consist of diacyl, alkylacyl, and alkenylacyl fatty acid moieties, each differing in the sn-1 linkage to the glycerol backbone.

The purpose of the present work was to determine PUFA composition of kidney individual phospholipid classes and subclasses from mice fed either corn oil (CO) containing primarily linoleic acid (18:2n-6), borage oil (BO) containing 18:2n-6 and 18:3n-6, blackcurrant seed oil (BL) containing 18:2n-6, 18:3n-6, and alpha-linolenic acid (18:3n-3), fish oil-corn oil mixture (F/C) containing 18:2n-6, 20:5n-3, and 22:6n-3, and fish oil-borage oil mixture (F/B) containing 18:2n-6, 18:3n-6, 20:5n-3, and 22:6n-3. We have demonstrated previously that combined borage and fish oil feeding produces the highest 20:3n-6/20:4n-6 ratios.[12] Our results demonstrate that although kidney phospholipids possess a similar prototypic structure, they are in reality a family of related molecules with distinct fatty acid compositions and metabolic properties.

Materials and methods

Materials

Monopentadecanoin and fatty acid standards were from Nu Check Prep (Elysian, MN). Silica gel plates were from E. Merck (Darmstadt, FRG). Phospholipase C (*Bacillus cereus*) was from Sigma (St. Louis, MO). Benzoic anhydride and 4-dimethylaminopyridine were purchased from Aldrich (Milwaukee, WI). Corn, borage, and blackcurrant seed oils were provided by Traco Labs (Champaign, IL). Menhaden fish oil was obtained from Zapata-Haynie (Reedville, VA). All chemicals were of optima grade (Fisher Scientific, Fair Lawn, NJ).

Dietary treatments

Twenty female (C57BL/6) mice (Harlan, Indianapolis, IN), weighing 15-18 g (4-6 weeks) were fed, ad libitum, one of five purified diets which were adequate in all nutrients.[13] The diets varied only in the oil composition (i.e., either corn, borage, blackcurrant, fish/borage, or fish/corn mixtures (1:1, w/w) at 10% of the diet by weight) (*Table 1*). The fatty acid composition of the diets is shown in *Table 2*. After a three-week feeding period, the animals were killed by CO_2 asphyxiation and the kidneys were immediately removed. Kidneys were decapsulated, rinsed with phosphate buffered saline, snap frozen in dry ice-acetone, and stored at −80° C.

Phospholipid analysis

Kidneys were thawed and homogenized in 0.1 M potassium chloride and extracted by the method of Folch et al.[14] The individual phospholipid classes were separated by thin-layer chromatography (TLC) on silica gel 60 plates using chloroform/methanol/acetic acid/water (50:37.5:3.5:2, v/v)[13] and detected under ultraviolet light after spraying with 0.1% anilinonaphthalensulfonate (ANS). A known amount of monopentadecanoin was added subsequently to the isolated phospholipid bands which were scraped into tubes containing 6% methanolic HCl and incubated at 75° C for 16 h.[15] The resultant fatty acid methyl esters (FAME) were further purified on silica gel plates using toluene prior to gas chromatographic analysis on a 30 m capillary DB-225 column (J&W Scientific, Folsom, CA).[15] The isolated choline and ethanolamine glycerophospholipid fractions were converted to benzoate derivatives following phospholipase C hydrolysis as previously described.[16] Briefly, phospholipid was suspended in 2 ml peroxide free diethyl ether and 2 ml

Table 1 Composition of diets

Ingredient[a]	Amount (g/100 g diet)
Oil(s)[b]	10.00
Casein (vitamin free)	20.00
DL-Methionine	0.30
Sucrose	44.00
Corn starch	14.98
Cellulose	6.00
Mineral mix[c]	3.50
Vitamin mix[d]	1.00
Choline chloride	0.20
t-Butylhydroquinone[e]	0.02

[a] All dietary components were purchased from U.S. Biochemicals (Cleveland, OH), except where noted.
[b] All diets provide approximately 22% energy from lipid.
[c] Provided at the following amount in grams/kilogram of salt mix as per AIN 76 mixture: $CaHPO_4$, 500.0; NaCl, 74.0; K-citrate, 220.0; K_2SO_4, 52.0; MgO, 24.0; manganous CO_3, 3.5; ferric citrate, 6.0; $ZnCO_3$, 1.6; $CuCO_3$, 0.3; KIO_3, 0.01; Na_2SeO_3, 0.01; $CrK(SO_4)_2$, 0.55.
[d] Provided at the following amount in grams/kilogram of vitamin mix (except as noted) as per AIN 76 mixture: thiamin HCl, 0.6; riboflavin, 0.6; pyridoxine HCl, 0.7; nicotinic acid, 3.0; Ca pantothenate, 1.6; folic acid, 0.2; biotin, 0.02; cyanocobalamin, 1 mg/kg; retinyl palmitate, 1.6 (250,000 I.U./g); dl-alpha-tocopheryl acetate, 20 (250 I.U./g); cholecalciferol, 2.5 mg (400,000 I.U./g); menaquinone, 5.0 mg/kg.
[e] Eastman Kodak Chemicals (Rochester, NY).

Table 2 Fatty acid composition of diets

Fatty acid[a]	CO	BO	BL	F/C	F/B
14:0	tr	0.3	tr	4.5	4.5
16:0	12.1	10.3	5.6	14.1	13.4
16:1n-7	0.2	0.1	tr	6.1	5.9
18:0	1.7	3.2	1.4	2.6	3.5
18:1n-9	25.7	14.7	8.8	16.4	10.8
18:2n-6	59.0	39.2	47.4	30.6	20.4
18:3n-6	tr	25.6	17.4	0.3	13.0
18:3n-3	tr	tr	13.1	tr	tr
18:4n-3	tr	tr	3.1	0.9	0.4
20:4n-6	tr	tr	tr	0.4	0.4
20:5n-3	tr	tr	tr	7.6	6.8
22:5n-3	tr	tr	tr	1.0	1.0
22:6n-3	tr	tr	tr	3.9	4.1

[a] Values are expressed as mg/100 mg total fatty acids present. Only the major fatty acids are presented (tr = trace amounts, less than 0.1%).
CO, corn oil; BO, borage oil; BL, black currant seed oil; F/C, fish-corn oil mixture; F/B, fish-borage oil mixture.

Table 3 Percent distribution of kidney phospholipids

	CO	BO	BL	F/B	F/C
ChoGpl					
Diacyl	29.85 ± 0.31	29.39 ± 1.23	31.36 ± 0.71	29.67 ± 0.38	28.69 ± 0.51
Alkylacyl	10.34 ± 2.21	13.48 ± 1.62	11.14 ± 1.62	14.12 ± 2.67	14.43 ± 0.98
Alkenylacyl	1.67 ± 0.15	1.71 ± 0.15	2.69 ± 0.81	1.96 ± 0.17	2.28 ± 0.36
EtnGpl					
Diacyl	19.94 ± 1.33	19.99 ± 0.37	20.18 ± 1.53	19.11 ± 0.66	19.16 ± 1.07
Alkylacyl	3.88 ± 1.56	1.79 ± 0.80	1.84 ± 0.83	2.27 ± 0.90	3.75 ± 0.53
Alkenylacyl	8.32 ± 0.57	9.16 ± 0.80	8.51 ± 0.21	7.32 ± 0.72	8.44 ± 0.36
PtdIns	7.01 ± 0.22	7.22 ± 0.75	6.18 ± 0.77	6.82 ± 0.33	6.68 ± 0.54
PtdSer	8.72 ± 0.67	7.92 ± 0.61	7.80 ± 1.19	7.77 ± 0.43	7.52 ± 0.38
CerPCho	11.55 ± 1.65	9.32 ± 0.56	10.29 ± 0.61	10.14 ± 0.67	9.04 ± 0.44

Values represent means ± SEM (n = 4), 2 kidneys pooled per analysis.
Results are expressed as mol %.
Row values with no superscripts are not significantly different ($P > 0.05$).
CO, corn oil; BO, borage oil; BL, black currant seed oil; F/B, fish-borage oil mixture; F/C, fish-corn oil mixture.

Tris-HCl buffer, pH 7.4, containing 5 mM calcium chloride and 10 U phospholipase C (*Bacillus cereus*). The mixture was shaken for 3 h at 20° C. Ether extracts were dried under nitrogen and redissolved in benzene containing 10 mg benzoic anhydride and 4 mg 4-dimethylaminopyridine and incubated for 1 h at 20° C. The reactions were terminated by adding concentrated ammonium hydroxide and extracted using hexane. The resultant diradylglycerobenzoates were separated by TLC using benzene/hexane/diethyl ether (50:45:4, v/v) as solvents.[16,17] The alkenylacyl (plasmalogen), alkylacyl and diacylglycerobenzoates were scraped from the TLC plates and transmethylated in the presence of known amounts of monopentadecanoin for mass determinations.[12,16] The fatty acid composition of phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), sphingomyelin (CerPCho) and subclasses of choline (ChoGpl) and ethanolamine (EtnGpl) glycerophospholipids were determined by gas chromatography.[12,15] The molar amounts of total FAME from the kidney diacyl-containing phospholipids were divided by two for determination of the relative phospholipid composition.[16]

Statistical analysis

The data were analyzed by one-way analysis of variance using multiple comparisons with the upper level significance chosen at $P < 0.05$.

Results

The effect of dietary treatments on the mol % distribution of kidney phospholipids is shown in *Table 3*. The choline and ethanolamine glycerolipids were the major phospholipid classes. Kidney ChoGpl was characterized by a high content of diacyl (PtdCho) and alkylacyl (PakCho) subclasses and EtnGpl contained primarily diacyl (PtdEtn) and alkenylacyl (PlsEtn) subclasses (*Table 3*). The mol % distribution of phospholipid classes (*Table 3*), ChoGpl and EtnGpl subclasses, was not significantly altered ($P > 0.05$) by dietary treatment. The fatty acid composition of kidney ChoGpl is shown in *Table 4*. The major PUFA in CO and BO fed animals was 20:4n-6 in PtdCho, 22:6n-3 in PakCho, and 22:6n-3 in the minor PlsCho fraction. The introduction of dietary n-3 PUFA (BL, F/B and F/C groups) significantly increased ($P < 0.05$) docosapentaenoic acid (22:5n-3) and 22:6n-3 primarily in PtdCho. F/B and F/C significantly reduced ($P < 0.05$) 20:4n-6 levels in all ChoGpl subclasses relative to CO, BO, and BL fed animals. The largest increase in 22:6n-3 was observed in F/B and F/C animals, which contained 20:5n-3 and 22:6n-3. Dietary 18:3n-6 (BO, BL and F/B groups) significantly increased 20:3n-6 levels in PtdCho. In addition, BO feeding increased PakCho 22:5n-6 levels approximately 20-fold relative to CO, BL, F/B and F/C diets.

The fatty acid composition of EtnGpl is presented in *Table 5*. The major PUFA in PtdEtn and PakEtn was 20:4n-6. Interestingly, the PlsEtn fraction contained high levels (25.3-49.8%) of 22:6n-3. Animals fed 20:5n-3 and 22:6n-3 (F/B and F/C diets) exhibited significant reductions in 20:4n-6 in all EtnGpl subclasses. This was offset by a corresponding increase in PtdEtn and PlsEtn 20:5n-3 and 22:6n-3. In contrast, alpha-linolenic acid (18:3n-3) feeding (BL diet) did not lower EtnGpl 20:4n-6 levels relative to CO and BO diets. BL feeding did, however, significantly increase ($P < 0.05$) PtdEtn and PlsEtn 22:6n-3 levels. Similar to PakCho, the ether-containing PlsEtn had significantly elevated 22:5n-6 levels following BO feeding. The increase was abolished when 18:3n-6 and n-3 PUFA were fed simultaneously. Dietary supplementation of 18:3n-6 (BO, BL, and F/B diets) also increased 20:3n-6 in PtdCho. Comparable changes were observed in the compositions of PtdIns (*Table 6*) and PtdSer (*Table 7*) following dietary manipulation. Interestingly, PtdIns and PtdSer contained very similar fatty acid profiles, each containing primarily stearic acid (18:0) and 20:4n-6. A notable difference between these two phospholipids was the magnitude of increase of 20:3n-6 in the PtdIns

Table 4 Fatty acid composition of kidney choline glycerophospholipids

Fatty acid‡	CO	BO	BL	F/B	F/C
			Diacyl		
16:0	42.90 ± 2.03	41.15 ± 1.14	41.06 ± 1.36	43.21 ± 1.36	45.06 ± 0.79
16:1n-7	0.98 ± 0.35	0.61 ± 0.22	0.52 ± 0.20	1.14 ± 0.12	0.97 ± 0.05
18:0	11.80 ± 0.17^{b}	13.51 ± 0.46^{a}	13.26 ± 0.61^{a}	13.05 ± 0.56^{a}	10.52 ± 0.33^{b}
18:1n-9	8.12 ± 0.04^{a}	6.82 ± 0.26^{b}	5.70 ± 0.11^{c}	6.50 ± 0.15^{b}	6.88 ± 0.22^{b}
18:1n-7	2.32 ± 0.10^{a}	1.56 ± 0.16^{a}	1.10 ± 0.39^{b}	1.64 ± 0.03^{a}	1.28 ± 0.43^{b}
18:2n-6	12.86 ± 0.73^{a}	7.80 ± 0.57^{b}	9.44 ± 0.67^{b}	5.59 ± 0.15^{c}	8.29 ± 0.36^{b}
18:3n-6	0.06 ± 0.04^{c}	1.27 ± 0.26^{a}	0.72 ± 0.04^{b}	0.49 ± 0.17^{b}	tr^{c}
18:3n-3	0.09 ± 0.05^{b}	tr^{b}	0.32 ± 0.11^{a}	tr^{b}	tr^{b}
20:1n-9	0.13 ± 0.08^{bc}	0.45 ± 0.02^{a}	0.18 ± 0.06^{b}	tr^{c}	tr^{c}
20:3n-6	1.07 ± 0.10^{b}	2.76 ± 0.10^{a}	2.63 ± 0.13^{a}	2.46 ± 0.09^{a}	0.59 ± 0.04^{c}
20:4n-6	10.65 ± 0.34^{b}	14.90 ± 0.69^{a}	11.17 ± 0.28^{b}	6.48 ± 0.14^{c}	4.23 ± 0.19^{d}
20:5n-3	tr^{c}	tr^{c}	tr^{c}	2.40 ± 0.25^{b}	3.48 ± 0.13^{a}
22:4n-6	0.33 ± 0.22^{a}	0.54 ± 0.19^{a}	tr^{b}	tr^{b}	tr^{b}
22:5n-6	tr^{b}	3.25 ± 0.20^{a}	tr^{b}	tr^{b}	tr^{b}
22:5n-3	tr^{c}	tr^{c}	0.56 ± 0.09^{b}	0.28 ± 0.18^{c}	1.01 ± 0.34^{a}
22:6n-3	6.80 ± 0.73^{c}	4.74 ± 0.44^{c}	11.59 ± 0.47^{b}	15.72 ± 0.76^{a}	17.14 ± 0.91^{a}
			Alkylacyl		
16:0	21.26 ± 1.20^{a}	21.92 ± 1.88^{a}	14.09 ± 1.45^{ab}	18.97 ± 2.62^{a}	11.81 ± 1.34^{b}
16:1n-7	1.78 ± 1.36	0.78 ± 0.45	0.20 ± 0.20	2.29 ± 0.70	1.04 ± 1.04
18:0	5.78 ± 1.44	4.34 ± 0.41	4.56 ± 0.64	7.03 ± 1.53	3.91 ± 0.79
18:1n-9	4.08 ± 0.86	5.06 ± 1.84	4.27 ± 1.35	8.58 ± 1.94	6.26 ± 1.90
18:1n-7	tr	tr	tr	1.11 ± 0.79	tr
18:2n-6	3.88 ± 0.32^{ab}	3.62 ± 0.55^{ab}	2.97 ± 0.52^{b}	3.10 ± 0.63^{ab}	4.20 ± 0.30^{a}
20:4n-6	6.45 ± 0.92^{ab}	8.05 ± 0.83^{a}	4.99 ± 1.08^{b}	0.98 ± 0.57^{c}	0.74 ± 0.43^{c}
22:4n-6	tr	0.95 ± 0.55	tr	tr	tr
22:5n-6	2.21 ± 2.21^{b}	19.85 ± 1.99^{a}	tr^{b}	tr^{b}	tr^{b}
22:5n-3	tr	tr	1.25 ± 0.83	tr	0.81 ± 0.74
22:6n-3	51.09 ± 2.26^{b}	32.65 ± 2.66^{c}	65.11 ± 3.24^{a}	52.23 ± 4.83^{b}	68.21 ± 4.74^{a}
			Alkenylacyl		
16:0	33.89 ± 1.90	28.41 ± 1.18	34.09 ± 9.13	36.99 ± 1.87	30.69 ± 2.36
16:1n-7	tr^{b}	3.14 ± 3.14^{b}	tr^{b}	5.99 ± 5.99^{ab}	13.35 ± 4.60^{a}
18:0	20.09 ± 2.34	17.85 ± 1.85	15.82 ± 3.63	19.90 ± 1.95	19.97 ± 4.47
18:1n-9	8.61 ± 2.49	8.68 ± 0.43	9.13 ± 1.47	6.64 ± 1.19	11.39 ± 1.63
18:2n-6	tr	2.52 ± 2.52	3.95 ± 3.95	2.11 ± 2.11	4.56 ± 3.51
20:4n-6	15.25 ± 1.25^{a}	18.02 ± 1.33^{a}	14.72 ± 2.24^{a}	8.36 ± 0.51^{b}	5.39 ± 1.43^{b}
22:6n-3	22.17 ± 2.83	21.09 ± 1.37	22.71 ± 3.94	20.00 ± 1.97	14.66 ± 3.72

Values represent means ± SEM (n = 4) (2 kidneys pooled/analysis).
Results are expressed as mol %.
Row values with the same or no superscripts are not significantly different ($P > 0.05$).
tr, trace amounts, less than 0.1 mol %.
‡ Only selected fatty acids are presented.

fraction relative to PtdSer following 18:3n-6 feeding (BO, BL and F/B diets). The fatty acid composition of CerPCho is shown in *Table 8*. Dietary lipid treatments produced only minor alterations in the PUFA composition of CerPCho.

Discussion

Previous studies have demonstrated that diets rich in 18:3n-6 and n-3 PUFA may improve renal function and prevent the progression of renal disease, reverse hypertension, and favorably modulate tissue injury.[1-3,6] In view of the paucity of data detailing the incorporation and distribution of dietary PUFA into kidney membrane phospholipids, we have determined the fatty acid composition of mouse kidney phospholipid classes and subclasses following dietary n-6 and n-3 PUFA manipulation. The results from the present study indicate that similar to the human kidney,[18] mouse renal ChoGpl and EtnGpl contain primarily diacyl, alkylacyl and diacyl, alkenylacyl subclasses, respectively. In addition, the relative mol % composition of mouse kidney PtdSer, PtdIns, and CerPCho (*Table 3*) are also very similar to the human kidney.[18] Examination of the effects of dietary lipid modulation revealed that n-6 and n-3 PUFA did not alter the mol % distribution of ChoGpl and EtnGpl subclasses (*Table 3*). However, consumption of borage enriched in 18:3n-6, blackcurrant seed enriched in 18:3n-6 and 18:3n-3, and fish oils enriched in 20:5n-3 and 22:6n-3, significantly altered fatty acyl moieties of kidney phospholipids compared to animals fed corn oil (containing 18:2n-6). In general, fish oil supplementation (FO and F/B diets) increased 20:5n-3 and 22:6n-3 in all phospholipids except PlsEtn and CerPCho. These phospholipid class data are consistent with earlier fish oil feeding studies (5,7,20). In addition, on a relative basis, BL feeding produced a smaller increase in

Table 5 Fatty acid composition of kidney ethanolamine glycerophospholipids

Fatty acid	CO	BO	BL	F/B	F/C
			Diacyl		
16:0	13.05 ± 0.51^{ab}	11.28 ± 0.46^{bc}	12.39 ± 0.99^{bc}	13.68 ± 0.25^{ab}	14.63 ± 0.64^{a}
16:1n-7	0.36 ± 0.22	0.35 ± 0.20	0.51 ± 0.18	1.17 ± 0.53	1.58 ± 0.62
18:0	29.79 ± 0.38^{b}	33.39 ± 1.07^{a}	32.26 ± 0.95^{ab}	31.88 ± 1.15^{ab}	31.01 ± 0.45^{ab}
18:1n-9	11.37 ± 0.43^{a}	9.53 ± 0.42^{b}	7.97 ± 0.48^{c}	7.34 ± 0.39^{c}	8.10 ± 0.51^{c}
18:1n-7	0.62 ± 0.36	0.26 ± 0.26	0.99 ± 0.38	0.55 ± 0.32	0.54 ± 0.22
18:2n-6	5.81 ± 0.10^{a}	3.26 ± 0.24^{bc}	4.10 ± 0.33^{b}	2.92 ± 0.40^{bc}	2.52 ± 0.85^{c}
18:3n-6	tr^{bc}	0.38 ± 0.15^{a}	0.26 ± 0.09^{a}	tr^{c}	tr^{c}
20:1n-9	0.08 ± 0.08	0.40 ± 0.13	0.14 ± 0.09	0.24 ± 0.24	0.05 ± 0.05
20:3n-6	0.30 ± 0.17^{bc}	1.08 ± 0.04^{a}	1.15 ± 0.09^{a}	1.23 ± 0.10^{a}	0.07 ± 0.07^{c}
20:4n-6	30.64 ± 0.38^{a}	31.04 ± 1.11^{a}	30.57 ± 0.36^{a}	23.15 ± 0.50^{b}	19.01 ± 0.33^{c}
20:5n-3	tr^{c}	tr^{c}	0.26 ± 0.16^{c}	5.10 ± 0.58^{b}	8.98 ± 0.17^{a}
22:6n-3	5.69 ± 0.50^{c}	3.30 ± 0.29^{d}	8.11 ± 0.13^{b}	10.82 ± 0.71^{a}	12.46 ± 0.87^{a}
			Alkylacyl		
16:0	32.66 ± 2.60	32.41 ± 3.42	31.00 ± 2.51	29.46 ± 0.58	31.37 ± 1.89
16:1n-7	5.62 ± 2.43	4.42 ± 2.55	3.54 ± 1.18	10.56 ± 5.01	4.26 ± 1.61
18:0	23.98 ± 1.67^{ab}	26.09 ± 1.53^{ab}	31.02 ± 3.09^{a}	20.97 ± 1.50^{c}	23.28 ± 3.16^{bc}
18:1n-9	13.79 ± 1.50^{b}	14.35 ± 1.28^{b}	14.15 ± 2.02^{b}	15.87 ± 4.00^{ab}	21.68 ± 3.98^{a}
18:2n-6	2.49 ± 1.93^{a}	tr^{b}	tr^{b}	2.89 ± 2.09^{ab}	5.64 ± 3.26^{a}
20:4n-6	14.50 ± 2.08^{a}	15.91 ± 0.29^{a}	15.57 ± 2.88^{a}	8.96 ± 0.92^{b}	8.71 ± 1.82^{b}
			Alkenylacyl		
16:0	8.25 ± 0.93^{ac}	9.19 ± 0.61^{ac}	8.46 ± 1.46^{ac}	11.00 ± 1.38^{a}	6.85 ± 0.17^{bc}
18:0	8.00 ± 0.89^{ac}	7.49 ± 1.26^{ac}	6.09 ± 0.74^{ac}	8.94 ± 1.01^{a}	5.89 ± 0.45^{bc}
18:1n-9	4.19 ± 0.89^{a}	3.75 ± 0.22^{a}	3.83 ± 0.84^{a}	1.46 ± 0.84^{b}	3.36 ± 0.22^{ab}
18:1n-7	1.63 ± 0.56^{a}	tr^{b}	1.26 ± 0.57^{a}	tr^{b}	0.37 ± 0.37^{b}
18:2n-6	5.76 ± 1.35^{a}	2.46 ± 0.21^{b}	2.81 ± 0.49^{b}	4.07 ± 2.35^{a}	1.97 ± 0.17^{b}
20:4n-6	32.56 ± 3.25^{a}	28.50 ± 0.61^{a}	29.24 ± 2.01^{a}	18.82 ± 0.86^{b}	13.40 ± 0.70^{c}
20:5n-3	tr^{c}	tr^{c}	tr^{c}	6.35 ± 0.36^{b}	9.81 ± 0.80^{a}
22:4n-6	1.58 ± 0.92^{b}	5.28 ± 0.31^{a}	tr^{c}	tr^{c}	tr^{c}
22:5n-6	3.00 ± 1.78^{b}	16.11 ± 1.39^{a}	tr^{c}	tr^{c}	tr^{c}
22:6n-3	35.16 ± 0.69^{c}	25.27 ± 0.60^{d}	42.34 ± 2.59^{b}	43.84 ± 1.86^{b}	49.81 ± 1.13^{a}

Refer to *Table 4* for legend details.

Table 6 Fatty acid composition of kidney phosphatidylinositol

Fatty acid	CO	BO	BL	F/B	F/C
16:0	12.51 ± 0.30	13.98 ± 1.58	12.00 ± 0.79	13.05 ± 0.37	13.10 ± 0.50
16:1n-7	0.52 ± 0.25	1.19 ± 0.92	0.11 ± 0.11	0.67 ± 0.25	0.53 ± 0.20
18:0	39.95 ± 0.33	38.81 ± 0.86	40.94 ± 0.98	40.55 ± 0.83	38.48 ± 0.59
18:1n-9	2.70 ± 0.51	2.13 ± 0.33	1.58 ± 0.09	2.01 ± 0.62	1.87 ± 0.32
18:1n-7	0.59 ± 0.07^{ab}	0.88 ± 0.42^{a}	0.18 ± 0.10^{b}	0.21 ± 0.12^{ab}	0.38 ± 0.14^{ab}
18:2n-6	3.97 ± 0.18^{a}	2.15 ± 0.12^{b}	2.63 ± 0.40^{b}	1.66 ± 0.23^{b}	2.86 ± 0.30^{b}
18:3n-6	tr^{b}	0.31 ± 0.02^{a}	tr^{b}	0.14 ± 0.14^{b}	tr^{b}
20:3n-6	3.96 ± 0.42^{c}	7.03 ± 0.27^{b}	8.54 ± 0.82^{a}	8.68 ± 0.29^{a}	2.85 ± 0.19^{c}
20:4n-6	28.98 ± 0.56^{a}	26.73 ± 1.78^{ab}	24.62 ± 1.33^{b}	16.87 ± 0.71^{c}	17.40 ± 0.86^{c}
20:5n-3	tr^{c}	tr^{c}	tr^{c}	2.07 ± 0.16^{b}	4.04 ± 0.09^{a}
22:1n-9	0.69 ± 0.43	tr	tr	0.91 ± 0.63	0.48 ± 0.29
22:4n-6	0.28 ± 0.16^{b}	1.08 ± 0.24^{a}	tr^{b}	tr^{b}	tr^{b}
22:5n-6	0.62 ± 0.36^{b}	1.70 ± 0.59^{a}	tr^{c}	tr^{c}	tr^{c}
22:5n-3	tr^{b}	tr^{b}	0.24 ± 0.24^{b}	0.42 ± 0.25^{b}	1.28 ± 0.11^{a}
22:6n-3	5.25 ± 0.45^{d}	2.60 ± 0.21^{e}	7.76 ± 0.23^{c}	11.62 ± 0.10^{b}	14.76 ± 0.83^{a}

Refer to *Table 4* for legend details.

Table 7 Fatty acid composition of kidney phosphatidylserine

Fatty acid	CO	BO	BL	F/B	F/C
16:0	12.22 ± 0.27	11.38 ± 0.72	11.66 ± 1.07	12.25 ± 0.45	12.87 ± 0.73
16:1n-7	0.47 ± 0.34	0.34 ± 0.21	0.23 ± 0.15	0.96 ± 0.61	0.61 ± 0.10
18:0	40.54 ± 0.39^{ab}	41.37 ± 0.66^{a}	40.62 ± 0.63^{a}	41.65 ± 1.29^{a}	38.85 ± 0.23^{b}
18:1n-9	6.29 ± 0.28	5.13 ± 0.17	4.79 ± 0.18	6.64 ± 0.88	5.18 ± 0.12
18:1n-7	tr	tr	tr	tr	tr
18:2n-6	3.83 ± 0.11^{a}	1.66 ± 0.05^{b}	3.04 ± 0.37^{a}	1.42 ± 0.48^{b}	3.85 ± 0.13^{a}
18:3n-6	tr^{b}	0.31 ± 0.10^{a}	0.37 ± 0.04^{a}	0.30 ± 0.10^{a}	tr^{b}
18:3n-3	tr	tr	tr	tr	tr
20:0	0.08 ± 0.08	0.22 ± 0.08	0.26 ± 0.09	0.24 ± 0.08	0.23 ± 0.08
20:3n-6	1.14 ± 0.10^{c}	2.18 ± 0.15^{b}	2.58 ± 0.07^{a}	2.58 ± 0.09^{a}	0.72 ± 0.04^{d}
20:3n-3	tr	tr	tr	tr	tr
20:4n-6	27.39 ± 0.79^{a}	26.67 ± 1.22^{a}	27.21 ± 1.28^{a}	18.15 ± 1.22^{b}	14.84 ± 0.74^{c}
20:5n-3	tr^{c}	tr^{c}	0.24 ± 0.15^{c}	4.25 ± 0.32^{b}	7.16 ± 0.19^{a}
22:1n-9	tr	0.91 ± 0.08	0.14 ± 0.08	0.61 ± 0.47	0.45 ± 0.16
22:4n-6	1.12 ± 0.22^{a}	1.52 ± 0.10^{a}	0.42 ± 0.24^{b}	tr^{b}	tr^{b}
22:5n-6	1.00 ± 0.44^{b}	4.66 ± 0.24^{a}	tr^{c}	tr^{c}	tr^{c}
22:5n-3	0.35 ± 0.35^{c}	tr^{c}	0.22 ± 0.22^{c}	0.94 ± 0.31^{b}	1.73 ± 0.07^{a}
22:6n-3	4.10 ± 0.20^{d}	2.29 ± 0.18^{e}	6.88 ± 0.48^{c}	9.21 ± 0.25^{b}	12.31 ± 0.36^{a}

Refer to *Table 4* for legend details.

20:5n-3 and 22:6n-3. The ineffectiveness of BL relative to FO and F/B diets, with regard to the relative increase in phospholipid levels of 20:5n-3 and 22:6n-3, may be explained in part by the competition of 18:3n-3 and 18:3n-6 for the rate-limiting Δ 6 desaturase.[19] Consistent with earlier observations,[20,21] mouse kidney phospholipids were enriched in 22:6n-3 even when fed CO and BO diets which lack n-3 PUFA. These data suggest the presence of a selective mechanism for esterifying 22:6n-3. A relative preference for specific fatty acids by a deacylation-reacylation cycle has been reported previously in platelets.[22] The accumulation of 20:5n-3 and 22:6n-3 in kidney phospholipids is noteworthy because dietary fish treatment seems to have a beneficial role in the progression of renal disease.[1,3,6,23] Although the mechanisms by which lipids affect renal function remain nebulous, 20:4n-6-derived cyclooxygenase products have been implicated in the development and progression of renal disease.[5,7,24] It is now well established that diets rich in n-3 PUFA can reduce kidney dienoic eicosanoid production.[5,7] In addition, 20:5n-3 can be metabolized to prostaglandin E_3 (PGE_3)[25] and P-450 monooxygenase products,[26] although the function of these metabolites remains unknown. Interestingly, fish oil feeding can also suppress kidney platelet-activating factor (PAF) synthesis.[27] This is significant because elevated PAF synthesis has been associated with renal pathophysiology.[28]

Dietary consumption of 18:3n-6 (BO, BL, and F/B diets) results in the accumulation of 20:3n-6 in PtdCho, PtdEtn, PtdIns, and PtdSer. The elevation in kidney phospholipid levels of 20:3n-6 may be relevant in terms of enhancing the synthesis of the 1-series prostaglandins, most notably PGE_1. PGE_1 suppresses immune complex-induced nephritis,[29] reduces the incidence of acute rejection in renal transplant recipients,[10] inhibits effector T-cell induction and tissue damage in experimental interstitial nephritis,[9] and may ameliorate hypertension.[4] The present results also demonstrate that the dietary combination of 18:3n-6 and twenty carbon n-3 PUFA (F/B diet) produced the highest 20:3n-6/20:4n-6 ratio in diacyl phos-

Table 8 Fatty acid composition of kidney sphingomyelin

Fatty acid	CO	BO	BL	F/B	F/C
16:0	49.92 ± 2.92	46.05 ± 1.39	44.51 ± 1.66	46.73 ± 2.31	46.19 ± 2.76
16:1n-7	0.49 ± 0.29	tr	0.44 ± 0.36	0.28 ± 0.28	0.10 ± 0.10
18:0	4.38 ± 1.15	3.01 ± 0.34	3.30 ± 0.39	5.25 ± 1.62	2.59 ± 0.18
18:1n-9	1.14 ± 0.56	0.43 ± 0.31	0.74 ± 0.56	1.11 ± 0.99	0.61 ± 0.11
18:2n-6	0.59 ± 0.34	0.75 ± 0.43	0.32 ± 0.20	0.14 ± 0.14	0.31 ± 0.20
20:0	3.01 ± 0.32^{a}	2.92 ± 0.08^{a}	2.74 ± 0.07^{a}	2.26 ± 0.27^{b}	2.56 ± 0.12^{a}
22:0	12.05 ± 0.94^{ab}	9.54 ± 0.11^{b}	12.54 ± 0.28^{a}	9.34 ± 1.50^{b}	12.41 ± 0.25^{a}
20:1n-9	0.21 ± 0.21	0.85 ± 0.49	0.27 ± 0.16	0.47 ± 0.29	0.38 ± 0.38
22:4n-6	2.92 ± 0.21^{a}	1.65 ± 0.55^{c}	3.36 ± 0.07^{ab}	2.34 ± 0.27^{ab}	3.01 ± 0.13^{a}
24:0	15.10 ± 1.52^{ab}	10.69 ± 0.16^{c}	19.57 ± 1.31^{a}	14.23 ± 2.29^{b}	19.91 ± 1.56^{a}
24:1n-9	9.96 ± 1.22^{c}	22.36 ± 0.65^{a}	10.31 ± 0.30^{c}	14.03 ± 1.86^{b}	10.62 ± 0.41^{c}

Refer to *Table 4* for legend details.

pholipids. Such a combination may favorably alter renal eicosanoid synthesis in advanced renal disease.[5]

In conclusion, results from the present study indicate that kidney phospholipids respond differently to specific dietary PUFA regimens. Therefore, although kidney phospholipids collectively share a common prototypic structure, they are in fact distinct molecules with unique metabolic properties.

Acknowledgments

This research was supported by NIH grant DK 41693 and the USDA Cooperative State Research Service, Project No. H-6983. We wish to thank Mr. Sid Tracy of Traco Labs and Mr. Tony Bimbo of Zapata-Haynie for generously providing the dietary oils. We would like to acknowledge the excellent technical and secretarial assistance of Mr. Richard Ishizaki and Ms. Judy Davidson, respectively.

References

1 Homan van der Heide, J.J., Bilo, H.J.G., Tegzess, A.M., and Donker, A.J.M. (1990). The effect of dietary supplementation with fish oil on renal function in cyclosporine-treated renal transplant recipients. *Transplant.* **49,** 523–527

2 Ito, Y., Barcelli, U., Yamashita, W., Weiss, M., Glas-Greenwalt, P., and Pollak, V.E. (1988). Fish oil has beneficial effects on lipids and renal disease of nephrotic rats. *Metabolism* **37,** 352–357

3 Knapp, H.R. and Fitzgerald, G.A. (1989). The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. *N. Engl. J. Med.* **320,** 1037–1043

4 Hassall, C.H. and Kirtland, S.J. (1984). Dihomogammalinolenic acid reverses hypertension induced in rats by diets rich in saturated fat. *Lipids* **19,** 699–703

5 Barcelli, U.O., Beach, D.C., Thompson, B., Weiss, M., and Pollak, V.E. (1988). A diet containing n-3 and n-6 fatty acids favorably alters the renal phospholipid, eicosanoid synthesis and plasma lipids in nephrotic rats. *Lipids* **23,** 1059–1063

6 Kelley, V.E., Ferretti, A., Izui, S., and Strom, T.B. (1985). A fish oil diet rich in eicosapentaenoic acid reduces cyclooxygenase metabolites and suppresses lupus in MRL-1pr mice. *J. Immunol.* **134,** 1914–1919

7 Barcelli, U.O., Beach, D.C., and Pollak, V.E. (1988). The influence of n-6 and n-3 fatty acids on kidney phospholipid composition and on eicosanoid production in aging rats. *Lipids* **23,** 309–312

8 Willis, A.L. (1981). Nutritional and pharmacological factors in eicosanoid biology. *Nutr. Res.* **39,** 289–301

9 Kelly, C.J., Zurier, R.B., Krakauer, K.A., Blanchard, N., and Nelson, E.G. (1987) Prostaglandin E_1 inhibits effector T-cell induction and tissue damage in experimental murine interstitial nephritis. *J. Clin. Invest.* **79,** 782–789

10 Moran, M., Mozes, M.F., Maddux, M., Veremis, S., Bartkus, C., Ketel, B., Pollak, R., Wallemark, C., and Jonasson, O. (1990). Prevention of acute graft rejection by the prostaglandin E_1 analog misoprostol in renal transplant recipients treated with cyclosporine and prednisone. *N. Engl. J. Med.* **322,** 1183–1188

11 Brown, M.L., Jakubowski, J.A., Leventis, L.L., and Deykin, D. (1987). Ionophore induced metabolism of phospholipids and eicosanoid metabolism in porcine aortic endothelial cells: Selective release of arachidonic acid from diacyl and ether phospholipids. *Biochim. Biophys. Acta* **921,** 159–166

12 Chapkin, R.S., Somers, S.D., and Erickson, K.L. (1988). Dietary manipulation of macrophage phospholipid classes: Selective increase of dihomogammalinolenic acid. *Lipids* **23,** 766–770

13 National Research Council (1978). Nutrient requirements of laboratory animals. National Academy of Sciences, Washington, D.C., 38–53

14 Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **22,** 497–509

15 Chapkin, R.S. and Ziboh, V.A. (1986). Metabolism of essential fatty acids by human epidermal enzyme preparations: Evidence of chain elongation. *J. Lipid Res.* **27,** 945–954

16 Chapkin, R.S. and Carmichael, S.L. (1990). Effect of dietary blackcurrant seed oil on macrophage subclasses of choline and ethanolamine glycerophospholipids. *J. Nutr.* **120,** 825–830

17 Blank, M.L., Robinson, M., Fitzgerald, V., and Synder, F. (1984). Novel quantitative method for determination of molecular species of phospholipids and diglycerides. *J. Chrom.* **298,** 473–482

18 Diagne, A., Fauvel, J., Record, M., Chap, H., and Douste-Blazy, L.D. (1984). Studies on ether phospholipids. I. Comparative composition of various tissues from human, rat and guinea pig. *Biochim. Biophys. Acta* **793,** 221–231

19 Brenner, R.R. (1984). Effect of unsaturated acids on membrane structure and enzyme kinetics. *Prog. Lipid Res.* **23,** 69–96

20 Swanson, J.E., Black, S.M., and Kinsella, J.E. (1987). Dietary n-3 polyunsaturated fatty acids: Rate and extent of modification of fatty acyl composition of lipid classes of mouse lung and kidney. *J. Nutr.* **117,** 824–832

21 Lefkowith, J.B., Flippo, V., Sprecher, H., and Needleman, P. (1985). Paradoxical conservation of cardiac and renal arachidonate content in essential fatty acid deficiency. *J. Biol. Chem.* **260,** 15736–15744

22 Inoue, M., Murase, S., and Okuyama, H. (1984). Acyl coenzyme A: phospholipid acyltransferase in porcine platelets discriminate between w-3 and w-6 unsaturated fatty acids. *Arch. Biochem. Biophys.* **231,** 29–37

23 Prickett, J.D., Robinson, D.R., and Steinberg, A.D. (1983). Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB x NZW/F1 mice. *Arthrit. Rheum.* **26,** 133–139

24 Klahr, S. and Harris, K. (1989). Role of dietary lipids and renal eicosanoids on the progression of renal disease. *Kid. Int.* **36,** S27–S31

25 Ferretti, A., Schoene, N.W., and Flanagan, V.P. (1981). Identification and quantitation of prostaglandin E_3 in renal medullary tissue of three strains of rats fed fish oil. *Lipids* **16,** 800–804

26 VanRollins, M., Frade, P.D., and Carretero, O.A. (1988). Oxidation of 5,8,11,14,17-eicosapentaenoic acid by hepatic and renal microsomes. *Biochim. Biophys. Acta* **966,** 133–149

27 Yeo, Y.K., Philbrick, D.J., and Holub, B.J. (1989). The effect of long-term consumption of fish oil on platelet-activating factor synthesis in rat renal microsomes. *Biochem. Biophys. Res. Comm.* **160,** 1238–1242

28 Bertani, T., Livio, M., Macconi, D., Morigi, M., Bisogno, G., Patrono, C., and Remuzzi, G. (1987). Platelet activating factor (PAF) as a mediator of injury in nephrotoxic nephritis. *Kid. Int.* **31,** 1248–1256

29 Fantone, J.C., Kunkel, S.L., Ward, P.A., and Zurier, R.B. (1980). Suppression by prostaglandin E_1 of vascular permeability induced by vasoactive inflammatory mediators. *J. Immunol.* **125,** 2591–2596