

# Meats and Fish Consumed in the American Diet Contain Substantial Amounts of Ether-Linked Phospholipids<sup>1</sup>

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**ABSTRACT** The primary goal of this study was to determine the amounts of ether-containing phospholipids, along with their concentration of certain polyunsaturated acyl groups, from selected, commonly consumed foods of animal origin (salmon, catfish, pork, beef, turkey and chicken). Levels of ether-linked glycerolipids in the samples were of particular interest, because ingestion of ether lipids could contribute to the production of platelet-activating factor (PAF; 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), one of the most potent biological mediators known. Alkylacyl-*sn*-glycero-3-phosphocholine was found in all of the meats, with pork loin having the highest levels (0.9  $\mu\text{mol/g}$  tissue) and chicken breasts the lowest (0.1  $\mu\text{mol/g}$  tissue). Although choline plasmalogens were not as evident as the ubiquitous ethanolamine plasmalogens, substantial amounts (1.0  $\mu\text{mol/g}$  tissue) of alk-1-enylacyl-*sn*-glycero-3-phosphocholine were found in tissues from beef and turkey. Triacylglycerols contained greater proportions of saturated fatty acids than phospholipids, and the ether-linked phospholipids were generally more unsaturated than diacyl species of the same phospholipid. Our data indicate that in addition to the phospholipid fraction of commonly eaten animal tissues supplying substantial amounts of polyunsaturated fatty acids, they are also a rich source of ether-linked lipids. Dietary ether-linked phospholipids could influence the lipid composition of host tissues to the extent that biological responses produced by ether lipid mediators would be affected. *J. Nutr.* 122: 1656-1661, 1992.

#### INDEXING KEY WORDS:

- plasmalogens • dietary glycerylethers
- polyunsaturated fatty acids
- ether-containing phospholipids
- edible animal tissues

Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; alkylacetyl-GPC)<sup>3</sup> exhibits many diverse biological activities, ranging from those involving inflammatory and allergic reactions

and antihypertensive responses to those of a physiological nature such as those related to reproduction, fetal development and parturition (Snyder 1990). Upon agonist stimulation, PAF can be produced by a number of different cell types via remodeling of the biologically inactive precursor, 1-*O*-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC) (Snyder 1990). The ability to produce PAF is attenuated in cells that are deficient in arachidonic acid, but PAF biosynthesis can be restored by repletion with either arachidonic acid (Ramesha and Pickett 1986, Suga et al. 1990) or certain other polyunsaturated fatty acids (Suga et al. 1990). Therefore, polyunsaturated fatty acyl groups are important not only as precursors of eicosanoids but also for the optimum production of PAF. The cells or tissues that are responsible for increasing the circulating levels of PAF, under specific conditions of stimulation, have not been unequivocally established. Because of the high biological potency of PAF [levels as low as 2 nmol/L can activate human platelets (Nunez et al. 1989)], even small increases in cellular levels of alkylacyl-GPC could potentiate the production of PAF. Platelet-activating factor can also be biosynthesized by a *de novo* pathway (Snyder 1990) beginning with alkylglycerols that could be produced from dietary lipids containing the basic 1-*O*-alkyl-*sn*-glycerol structure as a part of more complex lipid structures.

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<sup>3</sup>Abbreviations used: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GPI, *sn*-glycero-3-phosphoinositol; GPS, *sn*-glycero-3-phosphoserine; PAF, platelet-activating factor, alkylacetyl-GPC.

We have previously shown that alkylacyl-GPC levels in rat tissues can be increased with dietary supplements of alkylglycerol diacetates (Blank et al. 1991). However, the contribution of naturally occurring alkylglycerolipids in the American diet to tissue levels of alkylacyl-GPC are presently unknown. This report represents the first step in understanding this process, through the determination of ether-content and composition of some commonly consumed meats and fish in the American diet.

## MATERIALS AND METHODS

Muscle tissues from the middorsal area of pond-raised catfish and Norwegian salmon, beef ribeye steaks, pork loin chops, and breasts of chicken and turkey were used for lipid analyses. Approximately 20 g of each food sample (purchased fresh from a local supermarket) was excised, with care taken to exclude any gross areas of adipose tissue. Each sample was obtained from three different animals of each species and analyzed separately. After measuring the wet weight, the tissues were placed into 100 mL of chloroform and homogenized using a Polytron homogenizer (Brinkman Instruments, Westbury, NY); an equal volume (100 mL) of methanol was then added and the samples were again homogenized. The chloroform-methanol homogenates were then shaken for 20 min, centrifuged ( $560 \times g$  for 10 min at room temperature), and the supernatants containing the lipid fractions were removed. The protein pellets were extracted once more using 100 mL of chloroform-methanol (1:1, v/v) and, after centrifugation, this extract was pooled with the first supernatant. One-half volume of water was added to the chloroform-methanol solution of lipids and, after centrifugation, the lower chloroform layer containing the lipids was recovered. This extraction technique was based on the principle originally described by Bligh and Dyer (1959). The total lipids were weighed and then dissolved in 3 mL of chloroform and stored at  $-20^{\circ}\text{C}$  until analyzed (usually within 1 wk).

Approximately 250  $\mu\text{g}$  of each sample of total lipids was analyzed by TLC on 250- $\mu\text{m}$  layers of Silica Gel G using a solvent system of hexane-diethyl ether-acetic acid (80:20:1 by volume); the developed TLC plates were then sprayed with sulfuric acid and charred at  $180^{\circ}\text{C}$  for 1 h. Samples were visually compared with a known amount of palmitic acid, chromatographed in an adjacent lane, and only extracts that were estimated to contain  $<2\%$  free fatty acids by weight were used. Based on addition of known amounts of hexadecyldipalmitoylglycerol to 250  $\mu\text{g}$  of total lipids from the beef steak, we would also be able to detect levels of  $\geq 1\%$  alkylacylglycerols in the total lipids with this TLC technique. Phospholipids were separated by TLC on layers of Silica Gel H in a

TABLE 1

*Lipid concentration of muscle tissue from selected fish, meat and poultry products consumed by humans<sup>1</sup>*

Animal	Total lipids	Total phospholipids
	<i>mg/g wet wt</i>	
Salmon	32.8 $\pm$ 7.2	5.76 $\pm$ 0.35
Catfish	18.1 $\pm$ 3.2	4.37 $\pm$ 0.45
Pork	38.5 $\pm$ 17.2	3.68 $\pm$ 0.16
Beef	33.1 $\pm$ 4.6	4.86 $\pm$ 0.35
Chicken	27.0 $\pm$ 2.4	5.85 $\pm$ 0.17
Turkey	20.0 $\pm$ 4.5	5.10 $\pm$ 0.04

<sup>1</sup>Values are means  $\pm$  SEM from three animals of each species. The milligrams of total phospholipids were calculated by multiplying the milligrams of phospholipid phosphorus by 25. Total lipids were determined gravimetrically.

solvent system of chloroform-methanol-acetic acid-water (50:25:6:2 by volume). After chromatographic development, the plates were either sprayed with sulfuric acid and charred for determination of phosphorus (Rouser et al. 1966) or (with preparative TLC plates) the layers were exposed to ammonia vapor for  $\sim 3$  min before spraying with 2,7-dichlorofluorescein (0.1% in ethanol) to locate the separated phospholipid bands under UV light for their subsequent extraction by the method of Bligh and Dyer (1959). Subclasses of diradyl-GPC and diradyl-GPE were quantified as diradylglycerobenzoate derivatives by normal-phase HPLC as previously described (Blank et al. 1987). Diradylglycerobenzoate derivatives from the GPC- and GPE-containing phospholipids were also separated into subclasses by preparative TLC and then subjected to methanolysis for the analysis of acyl groups as their methyl ester derivatives by gas-liquid chromatography (Blank et al. 1984). Composition of the alkyl and alk-1-enyl ether chains was determined by reverse-phase HPLC of the dibenzoate derivatives of the alkyl- and alk-1-enyl-glycerols (Blank et al. 1983).

Statistically significant differences in the data were based on Student's *t* test for unpaired variables; *P* values  $> 0.05$  were not considered statistically significant.

## RESULTS

As indicated by the standard errors, there were large variations in the amount of total lipid per gram of wet tissue; the meat from catfish and turkey contained the lowest amounts of lipid (Table 1). No neutral ether lipids (e.g., alkylacylglycerols) were detected in any of the tissue lipids when they were examined by TLC using the nonpolar solvent system consisting of hexane-diethyl ether-acetic acid (80:20:1

TABLE 2

*Distribution of acyl groups in triacylglycerols from selected fish, meat and poultry products consumed by humans<sup>1</sup>*

Acyl group	Salmon	Catfish	Pork	Beef	Turkey	Chicken
	<i>g/100 g acyl groups</i>					
16:0	20.6 ± 0.4	18.6 ± 0.5	28.1 ± 1.9	27.8 ± 0.3	23.7 ± 0.8	24.0 ± 0.5
18:0	5.3 ± 0.4	5.3 ± 1.0	10.9 ± 1.1	13.6 ± 0.7	9.1 ± 0.1	4.3 ± 0.4
18:1(n-9)	29.4 ± 0.9	51.3 ± 1.0	48.7 ± 2.3	44.7 ± 1.6	34.3 ± 1.3	39.3 ± 0.5
18:2(n-6)	4.7 ± 1.4	13.0 ± 0.7	3.0 ± 0.1	1.6 ± 0.5	23.2 ± 1.1	18.1 ± 0.2
20:4(n-6)	0.7 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	—	0.9 ± 0.7	0.3 ± 0.2
20:5(n-3)	4.8 ± 1.1	0.4 ± 0.1	—	—	0.1 ± 0.1	—
22:5(n-3)	2.4 ± 0.8	0.2 ± 0.1	—	—	1.3 ± 1.1	—
22:6(n-3)	8.5 ± 2.4	0.8 ± 0.5	—	—	0.6 ± 0.4	0.1 ± 0.1

<sup>1</sup>Values represent means ± SEM from three animals of each species. Dashes indicate the absence of that particular acyl group.

by volume). Levels of alkyldiacylglycerols that were <1% of the total lipid weight would probably not be detected by this technique. Triacylglycerols were the major lipid class of all samples analyzed, and the distribution of selected acyl groups from this fraction is summarized in Table 2. Of the samples analyzed, triacylglycerols from salmon had the highest percentages of polyunsaturated (>2 double bonds) acyl groups. Not unexpectedly, triacylglycerols from muscle tissues of the other animals contained either small (e.g., catfish and turkey) or <0.5% of fatty acids with four or more double bonds. Significantly greater amounts of linoleic acid were present in triacylglycerols from catfish (13%), chicken (18%) and turkey (23%) compared with the other three food-stuffs ( $P < 0.01$ ). Triacylglycerols from pork and beef were the most saturated, containing only small amounts of linoleic acid and virtually no other polyunsaturated acyl groups.

There was less variation in the phospholipid content per gram of wet tissue within an animal

species (Table 1 and Table 3) than was found with total lipid amounts. This is probably because the triacylglycerols, which are the major component of the total lipids, act as cellular lipid storage sites that are more responsive to the general nutritional status of the animal than the phospholipids, which have both structural and functional roles. Diacyl-GPC was the major phospholipid subclass in every sample analyzed and was highest in salmon tissue (Table 3). Whether expressed as the percentage of the choline phosphatides or per gram of wet weight, meat from pork had the highest levels of alkylacyl-GPC. Nearly one-third of the diradyl-GPC fraction from beef tissue was in the choline plasmalogens (the alk-1-enylacyl-GPC subclass). High levels of alk-1-enylacyl-GPC are also found in bovine heart muscle (Horrocks 1972), and greater amounts of choline plasmalogens (Davenport 1964) seem to be a general characteristic of bovine muscle tissue. The concentration of alk-1-enylacyl-GPC was also high in the diradyl-GPC

TABLE 3

*Phospholipid composition of muscle tissue from selected fish, meat and poultry products consumed by humans<sup>1</sup>*

Phospholipid	Salmon	Catfish	Pork	Beef	Turkey	Chicken
	<i>μmol/g tissue</i>					
Sphingomyelin	0.16 ± 0.04	0.10 ± 0.04	0.35 ± 0.04	0.39 ± 0.02	0.39 ± 0.01	0.53 ± 0.03
Diradyl-GPI/GPS	0.80 ± 0.06	0.51 ± 0.04	0.47 ± 0.07	0.62 ± 0.05	0.64 ± 0.20	0.90 ± 0.05
Alk-1-enylacyl-GPC	0.03 ± 0.00	0.20 ± 0.04	0.48 ± 0.08	0.96 ± 0.16	1.03 ± 0.09	0.22 ± 0.01
Alkylacyl-GPC	0.40 ± 0.07	0.37 ± 0.06	0.89 ± 0.12	0.17 ± 0.03	0.25 ± 0.02	0.13 ± 0.02
Diacyl-GPC	4.51 ± 0.32	3.12 ± 0.34	1.35 ± 0.23	2.48 ± 0.16	2.66 ± 0.08	3.55 ± 0.07
Alk-1-enylacyl-GPE	0.23 ± 0.02	0.64 ± 0.05	0.57 ± 0.02	0.98 ± 0.12	0.79 ± 0.04	1.00 ± 0.04
Alkylacyl-GPE	0.01 ± 0.00	0.10 ± 0.06	0.07 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
Diacyl-GPE	1.26 ± 0.06	0.56 ± 0.22	0.23 ± 0.01	0.56 ± 0.04	0.71 ± 0.05	1.15 ± 0.05

<sup>1</sup>Values are means ± SEM from three animals of each species. Phospholipids were separated by TLC and phosphorus was determined as described in Materials and Methods. Abbreviations used: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GPI, *sn*-glycero-3-phosphoinositol; GPS, *sn*-glycero-3-phosphoserine.

TABLE 4

*Distribution of alkyl and alk-1-enyl groups in ether-linked subclasses of ethanolamine and choline glycerophospholipids from selected fish, meat and poultry products consumed by humans<sup>1</sup>*

Animal	Ether group	Ethanolamine		Choline	
		Alk-1-enyl	Alkyl	Alk-1-enyl	Alkyl
<i>g/100 g ether groups</i>					
Salmon	16:1	1.6 ± 0.3	NA	NA	1.1 ± 0.1
	16:0	63.6 ± 2.3	NA	NA	81.4 ± 2.0
	18:1	18.8 ± 0.8	NA	NA	6.6 ± 1.1
	18:0	9.5 ± 1.3	NA	NA	1.2 ± 0.1
Catfish	16:1	5.2 ± 0.2	12.6 ± 0.0	4.8 ± 0.1	7.5 ± 0.3
	16:0	21.0 ± 1.1	15.2 ± 0.1	35.2 ± 0.7	24.8 ± 0.6
	18:1	54.2 ± 0.7	64.7 ± 0.3	45.8 ± 0.6	51.2 ± 1.3
	18:0	14.8 ± 0.6	3.9 ± 0.0	7.4 ± 0.2	5.9 ± 0.1
Pork	16:1	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	1.3 ± 0.2
	16:0	33.6 ± 1.9	44.5 ± 0.1	67.4 ± 2.0	63.9 ± 1.5
	18:1	20.2 ± 0.4	27.2 ± 1.4	17.4 ± 1.4	21.7 ± 0.7
	18:0	38.8 ± 1.1	24.8 ± 4.0	9.0 ± 0.5	7.3 ± 0.8
Beef	16:1	trace	NA	0.1 ± 0.0	trace
	16:0	41.6 ± 1.2	NA	73.1 ± 2.6	69.4 ± 0.1
	18:1	7.3 ± 0.2	NA	5.2 ± 0.3	9.0 ± 1.2
	18:0	41.5 ± 1.8	NA	9.3 ± 1.8	8.2 ± 1.2
Turkey	16:1	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.0	1.2 ± 0.0
	16:0	53.2 ± 0.2	59.4 ± 3.3	77.8 ± 1.4	72.0 ± 1.1
	18:1	9.9 ± 0.5	13.4 ± 0.6	7.2 ± 0.4	14.4 ± 0.7
	18:0	29.2 ± 0.0	19.8 ± 0.7	8.2 ± 1.2	7.1 ± 0.6
Chicken	16:1	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	1.6 ± 0.3
	16:0	58.5 ± 0.2	58.1 ± 0.1	82.4 ± 3.0	73.0 ± 0.9
	18:1	12.5 ± 0.9	19.1 ± 0.2	7.8 ± 0.8	18.3 ± 2.3
	18:0	24.8 ± 0.3	19.0 ± 1.1	7.8 ± 2.0	4.9 ± 1.5

<sup>1</sup>Values are means ± variations from the means of samples from two different animals of each species; NA signifies that the sample was not analyzed because insufficient material was available. Other ether groups were also present (e.g., 14:0, 15:0 and 17:0), but none represented >5.0% of the total.

fraction from turkey breasts. Unlike the diradyl-GPC phospholipids, the diradyl-GPE class contained only low levels of the alkylacyl-GPE subclass (0.01 to 0.10 µmol/g) in all tissues. As with many other animal tissues (Horrocks 1972), ethanolamine plasmalogens represented a large, and often the major (catfish, pork, beef and turkey), subclass of the diradyl-GPE phospholipids.

Analysis of the ether chain composition showed that 16:0, 18:0 and 18:1 moieties composed the bulk of ether groups present in the ether-linked phospholipids from these foodstuffs (Table 4). This is not surprising because these are the main components of the ether groups found in most animal species previously analyzed (Horrocks 1972). There was a striking similarity in the composition of ether chains from the two avian species. Ether lipids from catfish contained the highest amounts of the 16:1 and 18:1 alk-1-enyl and alkyl groups.

The distribution of selected acyl groups, with at least two double bonds per molecule, in subclasses of diradyl-GPC and diradyl-GPE from the six different edible tissues are shown in Table 5. These phospho-

lipid subclasses from fish, when compared with tissues from most of the other animals, contained significantly higher levels of 20:5 and 22:6 acyl moieties, with 22:6 being the most prominent of the two acyl groups. Although 18:2 and 20:4 were virtually absent in the phospholipids from salmon, phosphatides from catfish possessed reasonably high levels of these two fatty acids. This difference in acyl composition between salmon and catfish likely reflects the different food chains present in the environments (saltwater vs. freshwater) of the two species. All tissues from the warm-blooded animals contained high levels of 18:2 and 20:4 and lesser amounts of 20:5 and 22:6 in the subclasses of diradyl-GPE and diradyl-GPC. Significantly greater quantities of 22:6 were present in ethanolamine and choline phospholipids from turkey and chicken breasts than in the meat from beef and pork ( $P < 0.001$ ). Phospholipid subclasses of all animal tissues analyzed had a much higher content of polyunsaturated fatty acids (Table 5) than triacylglycerols (Table 2) from the same cut of meat. Except in salmon, the ether-linked subclasses generally contained more 22:6 than

TABLE 5

Distribution of selected polyunsaturated acyl groups in subclasses of ethanolamine and choline phospholipids from selected fish, meat and poultry products consumed by humans<sup>1</sup>

Animal	Acyl group	Diradyl-GPE			Diradyl-GPC		
		Alk-1-enyl-acyl	Alkyl-acyl	Diacyl	Alk-1-enyl-acyl	Alkyl-acyl	Diacyl
		<i>g/100 g acyl groups</i>					
Salmon	18:2( <i>n</i> -6)	0.3 ± 0.1	NA	1.7 ± 0.4	NA	0.3 ± 0.0	0.6 ± 0.2
	20:4( <i>n</i> -6)	2.0 ± 0.2	NA	1.4 ± 0.3	NA	0.9 ± 0.3	1.1 ± 0.4
	20:5( <i>n</i> -3)	5.7 ± 0.5	NA	5.3 ± 1.0	NA	5.0 ± 1.0	7.5 ± 1.6
	22:6( <i>n</i> -3)	49.9 ± 5.6	NA	46.0 ± 2.8	NA	71.3 ± 0.7	39.6 ± 3.5
Catfish	18:2( <i>n</i> -6)	2.4 ± 0.4	2.9 ± 0.5	10.5 ± 0.6	4.7 ± 0.6	5.0 ± 0.2	14.7 ± 1.2
	20:4( <i>n</i> -6)	8.0 ± 0.2	4.8 ± 0.6	8.6 ± 0.5	10.8 ± 0.5	11.3 ± 1.0	3.4 ± 1.0
	20:5( <i>n</i> -3)	2.8 ± 0.3	2.9 ± 0.6	3.5 ± 1.4	5.2 ± 0.1	8.4 ± 1.4	2.0 ± 0.7
	22:6( <i>n</i> -3)	42.6 ± 2.4	42.9 ± 3.2	14.5 ± 0.7	25.6 ± 2.4	32.6 ± 1.9	5.2 ± 0.9
Pork	18:2( <i>n</i> -6)	23.1 ± 0.7	12.0 ± 1.5	28.6 ± 1.4	41.4 ± 2.7	64.2 ± 1.4	25.5 ± 3.2
	20:4( <i>n</i> -6)	25.0 ± 3.3	19.5 ± 5.0	8.0 ± 1.2	8.2 ± 0.9	8.6 ± 2.2	1.5 ± 0.6
	20:5( <i>n</i> -3)	0.6 ± 0.0 <sup>ab</sup>	0.5 ± 0.2 <sup>b</sup>	tr <sup>a</sup>	— <sup>b</sup>	— <sup>ab</sup>	— <sup>ab</sup>
	22:6( <i>n</i> -3)	— <sup>ab</sup>	— <sup>b</sup>	— <sup>ab</sup>	— <sup>b</sup>	— <sup>ab</sup>	— <sup>ab</sup>
Beef	18:2( <i>n</i> -6)	23.4 ± 1.8	NA	19.4 ± 2.6	34.4 ± 9.5	32.8 ± 3.1	19.7 ± 6.0
	20:4( <i>n</i> -6)	18.3 ± 3.2	NA	14.1 ± 2.4	6.6 ± 3.3	5.7 ± 1.4	2.9 ± 1.2
	20:5( <i>n</i> -3)	2.0 ± 0.4 <sup>a</sup>	NA	0.9 ± 0.1 <sup>a</sup>	2.0 ± 1.5	0.8 ± 0.4 <sup>ab</sup>	0.4 ± 0.4 <sup>a</sup>
	22:6( <i>n</i> -3)	0.8 ± 0.8 <sup>ab</sup>	NA	tr <sup>ab</sup>	0.3 ± 0.3 <sup>b</sup>	— <sup>ab</sup>	0.2 ± 0.2 <sup>ab</sup>
Turkey	18:2( <i>n</i> -6)	3.1 ± 0.2	9.2 ± 0.6	16.6 ± 2.0	7.9 ± 0.7	9.0 ± 0.2	22.5 ± 1.0
	20:4( <i>n</i> -6)	22.2 ± 1.8	8.8 ± 0.7	14.3 ± 1.0	22.3 ± 1.0	21.8 ± 1.0	4.7 ± 0.7
	20:5( <i>n</i> -3)	1.4 ± 0.2 <sup>ab</sup>	0.6 ± 0.2 <sup>b</sup>	0.5 ± 0.0 <sup>a</sup>	1.5 ± 0.1 <sup>b</sup>	2.1 ± 0.2 <sup>b</sup>	tr <sup>ab</sup>
	22:6( <i>n</i> -3)	16.9 ± 0.7 <sup>ab</sup>	7.4 ± 0.2 <sup>b</sup>	2.7 ± 0.5 <sup>ab</sup>	7.5 ± 0.4 <sup>b</sup>	7.5 ± 0.1 <sup>ab</sup>	1.3 ± 0.2 <sup>ab</sup>
Chicken	18:2( <i>n</i> -6)	7.1 ± 0.7	11.1 ± 1.4	14.8 ± 1.0	10.1 ± 1.2	16.1 ± 1.2	23.3 ± 0.5
	20:4( <i>n</i> -6)	10.3 ± 1.4	7.7 ± 1.3	21.3 ± 2.2	10.1 ± 0.6	9.0 ± 0.3	3.1 ± 0.3
	20:5( <i>n</i> -3)	1.2 ± 0.1 <sup>ab</sup>	1.1 ± 0.3 <sup>b</sup>	1.3 ± 0.1 <sup>ab</sup>	0.7 ± 0.4 <sup>b</sup>	1.4 ± 0.3 <sup>ab</sup>	— <sup>ab</sup>
	22:6( <i>n</i> -3)	12.3 ± 1.3 <sup>ab</sup>	4.4 ± 1.0 <sup>b</sup>	1.9 ± 0.4 <sup>ab</sup>	3.2 ± 0.5 <sup>b</sup>	1.1 ± 0.6 <sup>ab</sup>	— <sup>ab</sup>

<sup>1</sup>Values are means ± SEM from three animals of each species. Dashes indicate the acyl group was not observed, and tr means trace amounts (e.g., <0.2%) were present; NA signifies that the sample was not analyzed because insufficient material precluded an acceptable accuracy. Acyl moieties from ether-linked subclasses represent only the *sn*-2 position; acyl groups from the diacyl subclasses are from both the *sn*-1 and *sn*-2 positions, meaning that if these unsaturated fatty acids are located exclusively at the *sn*-2 position of the diacyl subclasses, then the values for diacyls should be multiplied by two to be directly comparable with values of the ether subclasses. <sup>a</sup>Values are significantly lower than in the corresponding subclass from salmon ( $P < 0.05$ ). <sup>b</sup>Values are significantly lower than the corresponding subclass from catfish ( $P < 0.05$ ). Abbreviations used: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine.

was found in the corresponding diacyl subclasses. The alk-1-enylacyl-GPC subclass from pork ( $P < 0.05$ ), and the alk-1-enylacyl- and alkylacyl-GPC subclasses of turkey ( $P < 0.01$ ) and chicken ( $P < 0.02$ ), were significantly enriched in arachidonate when compared with their diacyl counterparts.

## DISCUSSION

The aim of this study was to quantify the amounts of ether-linked lipids, including the subclasses of diradyl-GPE and diradyl-GPC, in edible meats and fish, because such information is not available for this possibly important source of potent biologically active mediators. The amount of alkylacyl-GPC in the meats was of particular interest because (based on limited experimental results) the basic structure, alkylyso-GPC (lyso-PAF), would likely be produced

by digestive processes (Tso 1985). Platelet-activating factor could be produced directly by enzymatic acetylation of the lyso-PAF, or, alternatively, subsequent reacylation of lyso-PAF in tissues would produce alkylacyl-GPC, a precursor of PAF and a very rich source of eicosanoid precursors such as arachidonic acid. Ether-linked phospholipids were present in every animal tissue that we analyzed; however, no ether-linked neutral lipids were detected. Ether groups in the ethanolamine and choline glycerophosphatides were composed primarily of mixtures of 16:0, 18:1 and 18:0 moieties. These same three groups are also the major alkyl chains found in the PAF produced by stimulated rabbit and human neutrophils (Mueller et al. 1984). The largest amount of alkylacyl-GPC was found in pork, in which it was equivalent to ~700 mg/kg meat. Whether and to what extent these levels of dietary ether lipids would affect the production of, and subsequent biological

responses induced by, PAF in humans are presently unknown. However, our analyses demonstrate that ether-linked glycerolipids occur in the meat, fish and poultry consumed in the American diet at levels sufficient to justify the continuation of our animal studies designed to assess the role of dietary ether-linked glycerolipid supplementation on the production of PAF and on associated biological alterations (e.g., blood pressure and inflammatory responses).

The phospholipid fractions represented from 10% (pork) to 25% (turkey) of the total lipid weight in the foodstuffs analyzed. Because the phospholipids, and especially the ether-linked subclasses, contain much more unsaturated acyl groupings than the triacylglycerols, they would be expected to be major dietary sources of not only ether-linked lipids but also polyunsaturated fatty acids. It has already been demonstrated that cellular polyunsaturated acyl groups are required for maximum generation of PAF by cells (Pickett and Ramesha 1987, Ramesha and Pickett 1986, Suga et al. 1990), and the polyunsaturated fatty acids released from the phospholipids by digestive processes would provide another dietary source of these important acyl groups. Therefore, the relationship between dietary ether-linked glycerolipids and polyunsaturated fatty acids, relative to the potent synergistic biological metabolites both can generate, is important to consider in nutritional approaches to treating diseases involving PAF and eicosanoid mediators.

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