Effect of fish oil supplementation on the composition of molecular species of choline and ethanolamine glycerophospholipids in ruminant muscle

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Abstract Choline glycerophospholipids and ethanolamine glycerophospholipids of ruminant skeletal muscle contain approximately 40% and 65% plasmalogen, respectively. In the 1,2-diacyl-*sn*-glycero-3-phosphocholine (diacyl CPG), 16:0-18:2(n-6) and 16:0-18:1(n-9) accounted for about 50% of the total molecular species; in the 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphocholine (alkenyl CPG), 16:0-18:2(n-6) was the predominant species. Fish oil supplementation resulted in a sixfold increase in the proportion of 16:0-20:5(n-3) and a two- to threefold increase in the proportion of 18:1-20:5(n-3) and 16:0-22:6(n-3) in the diacyl CPG, and there was a 40% decrease in the proportion of 16:0-18:1(n-9). In the alkenyl CPG, fish oil supplementation increased the proportion of molecular species containing C_{20} and C_{22} polyenoic fatty acids from 34% to 64%; in both sheep and cattle, the proportion of 16:0-20:5(n-3) was greater than any other molecular species. In contrast to the diacyl CPG, there was also an increase in the proportion of 18:0-20:5(n-3) in the alkenyl CPG. In the 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (diacyl EPG), 18:0-20:4(n-6) represented about 30% of the molecular species and this was reduced to less than 20% by fish oil supplementation. In the 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphoethanolamine (alkenyl EPG), the molecular species containing C_{20} and C_{22} polyenoic fatty acids in the *sn*-2 position increased from 55% to 78% with fish oil supplementation and 18:0-20:5(n-3) was the predominant molecular species; there were reductions in the proportions of 16:0-18:2(n-6), and 18:0-18:2(n-6) but 18:0-20:4(n-6) was unchanged. Fish oil supplementation induced different patterns of fatty acid incorporation and substitution in the *sn*-2 position of the diacyl and alkenyl CPG and EPG of skeletal muscle in ruminants and this is indicative of selective acyl remodelling in these two phospholipids.—**Scott, T. W., J. R. Ashes, E. Fleck, and S. K. Gulati.** Effect of fish oil supplementation on the composition of molecular species of choline and ethanolamine glycerophospholipids in ruminant muscle. *J. Lipid Res.* 1993. **34:** 827-835.

Supplementary key words diacyl and alkenyl phospholipids • eicosapentaenoic and docosahexaenoic fatty acids

Recently we have demonstrated that the n-3 fatty acids of fish oil are not hydrogenated in the digestive tract of ruminants; however, they are absorbed and incorporated into the phospholipids, but not triacylglycerols, of muscle (1). In the total phospholipids, the incorporated eicosapentaenoic 20:5(n-3) and docosahexaenoic 22:6(n-3) acids substituted primarily for oleic 18:1(n-9) and/or linoleic 18:2(n-6), and there was no consistent change in the proportion of arachidonic 20:4(n-6) acid (1). Examination of the fatty acid profiles of total phospholipids does not reveal what changes may be occurring in individual phospholipids or indeed in the distribution of the individual molecular species that characterize specific glycerophospholipids (2). The profiles of muscle phospholipids are further complicated because of the relatively high proportions of choline and ethanolamine plasmalogens as well as the diacyl glycerophospholipids (3-7). For example, in canine myocardial sarcoplasmic reticulum, the plasmalogens account for 53% of the total phospholipids (7); in this tissue the ethanolamine glycerophospholipids contain 73% plasmalogen and these are predominantly comprised of molecular species with C_{18} vinyl ethers at the *sn*-1 position and 20:4(n-6) at the *sn*-2 position (6, 7). In ruminants, earlier studies suggested that the phospholipids of heart muscle contain approximately 30-35% plasmalogen and about two-thirds of this was in the choline form (3, 4); recent analysis of beef ribeye muscle has also found that 34% of the phospholipid was present as plasmalogens and the choline subclass accounted for 53% of the total (5).

The aims of the present investigations were to characterize the molecular species of the choline and ethanolamine phospholipids in skeletal muscle of ruminants and examine what remodelling occurs as a result of feeding fish oil supplements containing 17% 20:5(n-3) and 11% 22:6(n-3).

Abbreviations: CPG, choline glycerophospholipids; EPG, ethanolamine glycerophospholipids; diacyl CPG, 1,2-diacyl-*sn*-glycero-3-phosphocholine; alkenyl CPG, 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphocholine; diacyl EPG, 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine; alkenyl EPG, 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphoethanolamine; TLC, thin-layer chromatography; DG, diradylglycerols; HPLC, high performance liquid chromatography; GLC, gas-liquid chromatography.

MATERIALS AND METHODS

Materials

Phospholipase C (*Bacillus cereus* Type XIII), benzoic anhydride, and 4-dimethylaminopyridine were purchased from Sigma (St. Louis, MO). Phospholipids and diacylglycerol standards were obtained from Serdary, London, Ontario. Thin-layer plates were from E. Merck (Darmstadt, Germany).

Animals and diets

Merino wethers weighing approximately 35 kg and European × British crossbred steers were fed a basal diet comprised (w/w) of 30% maize, 30% wheaten chaff, 20% lupin, 15% wheat screenings, 2.5% by weight calcium carbonate, and 2.5% sodium chloride. Two sheep and one steer were fed the basal ration and four sheep and two steers were supplemented with 25% fish oil ("Maxepa", Scherer Pty Ltd, Melbourne, Australia), that had been encapsulated in a matrix of formaldehyde-treated protein to prevent loss of appetite (8). The animals were slaughtered after 5 weeks of feeding and *Longissimus dorsi* muscles were sampled and frozen prior to analysis.

Lipid extraction, phospholipid separation and fatty acid analysis

Muscle lipids were extracted with chloroform–methanol 2:1(v/v) using procedures described elsewhere (1, 9); all solvents contained 0.01% w/v butylated hydroxytoluene. Phospholipids and neutral lipids were separated by thin-layer chromatography (TLC) on silica gel G plates using hexane–diethyl ether–acetic acid 80:15:1 (by vol). Lipids were visualized by spraying the plate with 2,7-dichlorofluorescein (0.02% w/v in ethanol) and exposure to ultraviolet light. Areas of gel corresponding to the phospholipids were scraped off the plate and the lipid was extracted (10). Individual phospholipids were separated by TLC using procedures previously described (11); the areas of gel containing the choline and ethanolamine phospholipids (both diacyl and plasmalogens) were removed and the lipid was extracted (10). A portion of this extract was used to prepare fatty acid methyl esters (12), and analyzed by gas chromatography (Varian model 3400) using a 25 m × 0.25 mm fused silica capillary column (BP × 70, SGE Pty Ltd, Melbourne, Australia). Fatty acid methyl esters and the dimethyl acetals were identified by comparison of retention times with standard mixtures. An aliquot was also used to determine the amount of phospholipid phosphorus (13) and the remainder was used for analysis of molecular species.

Separation of molecular species

Individual phospholipids (1–2 mg) were hydrolyzed with phospholipase C using the procedures outlined by Louie, Wiegand, and Anderson (14) and the released

TABLE 1. Composition of the molecular species of 1,2-diacyl-*sn*-glycero-3-phosphocholine of ruminant muscle (mol %)

Peak No.	Molecular Species	Sheep		Cattle		
		Control	Fish Oil	Control	Fish Oil	
					(i)	(ii)
1	18:1–20:5	0.20 ± 0.04	1.25 ± 0.14[a]	0.20	0.60	1.10
2	16:0–20:5	1.03 ± 0.28	7.15 ± 1.69[a]	0.80	6.60	8.80
3	16:0–22:6	1.53 ± 0.10	4.15 ± 0.46[a]	0.70	1.50	2.60
4	18:1–22:5	1.03 ± 0.02	2.05 ± 0.06[a]	0.90	1.10	1.10
5	18:1–20:4	2.47 ± 0.14	2.98 ± 0.27	3.20	4.50	4.70
6	16:0–22:5	2.17 ± 0.24	2.65 ± 0.13	1.40	1.50	2.10
7	16:0–20:4	3.90 ± 0.43	5.18 ± 0.54	2.10	3.40	4.70
8	18:0–20:5	1.00 ± 0.13	1.38 ± 0.28	1.90	1.50	2.30
9[b]	18:1–18:2	4.47 ± 0.09	4.88 ± 0.33	3.40	2.10	3.50
10[b]	18:0–22:6	ND	ND	ND	ND	ND
11	16:0–18:2	23.83 ± 0.38	30.68 ± 1.95[c]	19.50	24.90	27.50
12	16:0–22:4	4.23 ± 0.31	2.78 ± 0.10[c]	2.90	3.40	2.90
13	18:0–22:5	1.83 ± 0.41	1.90 ± 0.12	1.50	1.00	1.60
14	18:0–20:4	0.87 ± 0.17	0.63 ± 0.02			
15	18:1–18:1	3.70 ± 0.14	2.48 ± 0.24[a]	4.60	2.00	2.30
16[b]	16:0–18:1	25.57 ± 0.88	15.48 ± 0.97[a]	34.40	24.40	21.30
17[b]	18:0–18:2	7.00 ± 0.16	5.68 ± 0.32[a]	5.10	4.00	3.50
18	18:0–18:1	5.03 ± 0.15	2.48 ± 0.39[a]	5.60	3.00	1.90
19	18:0–16:0	0.13 ± 0.04	0.33 ± 0.18	0.70	0.90	0.40

Composition expressed as mean (mol %) ± SEM for duplicate analysis of muscle samples from two control and four fish oil-supplemented sheep; mol % for duplicate analysis of a control and two fish oil-supplemented cattle; ND, not detected.

[a] $P < 0.01$ when compared to control by F test.

[b] These species tend to co-elute using acetonitrile–2-propanol 80:20 (v/v) and were resolved in methanol–2-propanol 95:5 (v/v) using procedures outlined in Methods.

[c] $P < 0.05$ when compared to control by F test.

1,2-diradylglycerols (DG) were extracted to prepare benzoate derivatives. This was done using a modification (14) of the method described by Blank et al. (15). The DGs were dissolved in 0.3 ml of benzene containing benzoic anhydride (10 mg) and 4-dimethylaminopyridine (4 mg) and allowed to react for 2 h. The benzoate derivatives were extracted three times with 2 ml of hexane and separated into the three subclasses viz., diacyl, alkylacyl, and alkenylacyl by TLC on silica gel G in a solvent system of toluene–hexane–diethyl ether 50:45:5 (by vol). The diradylglycero-benzoates were visualized under ultraviolet light as described above, and relative RFs were diacyl 0.26, alkylacyl 0.37, and alkenylacyl 0.46. The areas of gel corresponding to these derivatives were removed and extracted (10). The DGs were evaporated to dryness, redissolved in acetonitrile–2-propanol 80:20 (v/v), and read at 230 nm to determine the distribution of the three subclasses in the ethanolamine and choline phospholipids (15).

The molecular species of each of the diacyl and alkenylacyl derivatives were separated using a Waters HPLC system comprised of a 501 pump, a 484 UV detector (operating at 230 nm), and a 740 data module. An SGE ODS 2-8/5 column (5 μm, 25 cm × 4 mm i.d.) was used at an operating temperature of 25°C. Samples of the benzoate derivatives were run in two solvent systems, viz acetonitrile–2-propanol 80:20 (v/v) and methanol–2-propanol 95:5 (v/v) (16). The molecular species were separated by isocratic elution at a flow rate of 1.0 ml/min and 0.8 ml/min, respectively, and 12:0–12:0 was added as an internal standard for each molecular species. Identification of the individual molecular species was achieved by a combination of HPLC analysis of standards, graphing the relative retention times using the methods outlined by Patton, Fasulo, and Robins (17) and Nakagawa and Horrocks (18), and GLC analysis of the fatty acid methyl esters of the peaks corresponding to the eluted species.

RESULTS

Phospholipid distribution and fatty acid profile

Choline glycerophospholipid (CPG) and ethanolamine glycerophospholipid (EPG) accounted for approximately 48% and 28%, respectively, of the total muscle phospholipids in sheep and cattle. In the CPG extracted from sheep muscle, the diacyl subclass accounted for 58.6 ± 2.7% of the lipid, and the contents of the alkylacyl and alkenylacyl subclasses were 17.6 ± 2.2% and 23.8 ± 1.9%, respectively. In cattle the values were

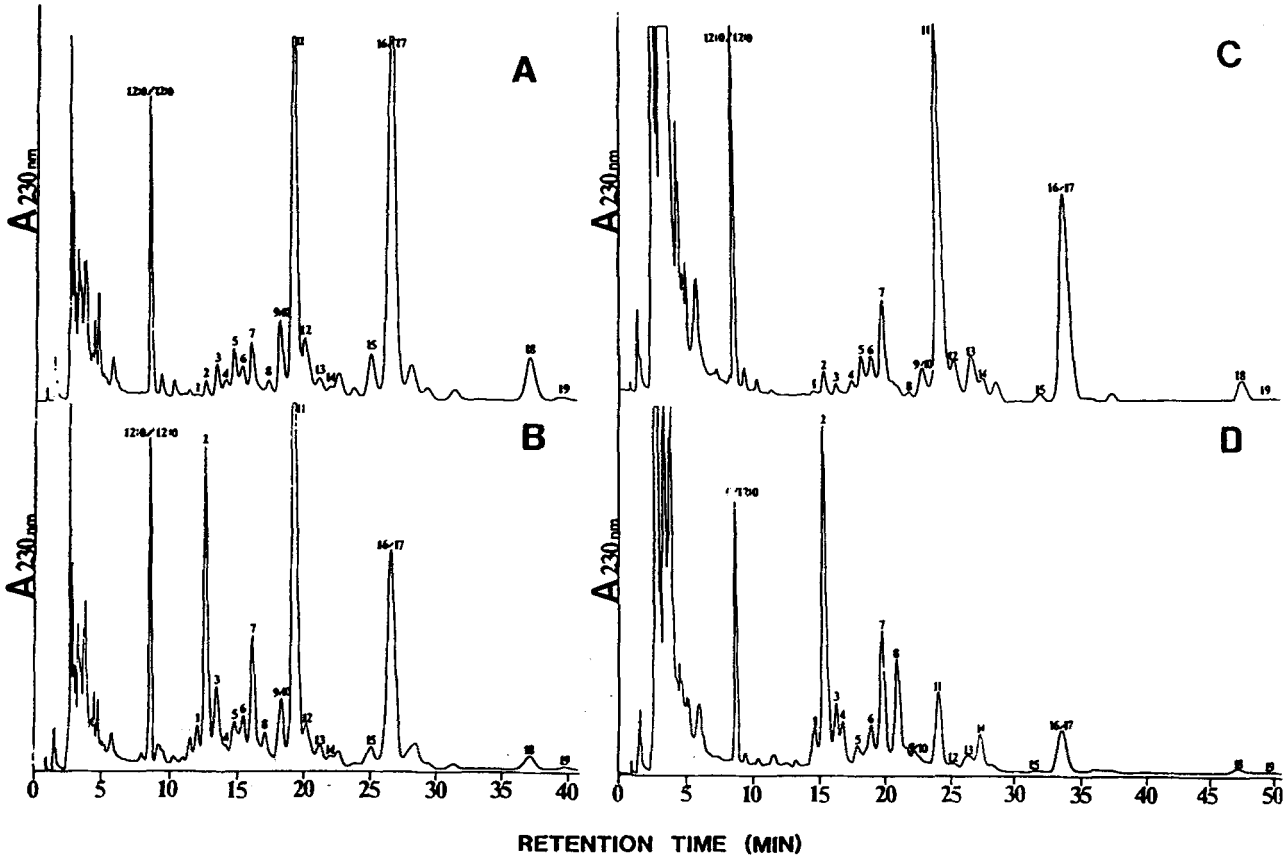

Fig. 1. Effect of fish oil supplementation on the HPLC profiles of molecular species of choline glycerophospholipids (CPG) of sheep skeletal muscle. A, Control: diacyl CPG; B, fish oil: diacyl CPG; C, control: alkenylacyl CPG; D, fish oil: alkenylacyl CPG. Refer to Table 1 for peak identification.

45.4 ± 4%, 23.2 ± 6.5%, and 31.4 ± 4.1% for the diacyl, alkylacyl, and alkenylacyl choline subclasses. In the EPG from sheep muscle, the alkenylacyl subclass was the major lipid and accounted for 43.8 ± 3.1%; the proportions of alkylacyl and diacyl EPG were 19.8 ± 3.2% and 36.5 ± 2.8%, respectively. A similar pattern was observed in the EPG from cattle muscle and the values were 48.6 ± 1.4%, 15.5 ± 0.5%, and 35.8 ± 1.0%, respectively, for the alkenylacyl, alkylacyl, and diacyl EPG.

The feeding of fish oil supplements to sheep and cattle increased the proportion of 20:5(n-3) and 22:6(n-3) in the CPG and EPG isolated from skeletal muscle. In the CPG from sheep, 20:5(n-3) increased from 2.1% to 8.0% and 22:6(n-3) from 0.8% to 1.7%. In cattle the values increased from 1.3% to 12% and from 0.1% to 1.6%, respectively, for 20:5(n-3) and 22:6(n-3). In the sheep EPG, fish oil supplementation increased the proportions of 20:5(n-3) from 3.3% to 7.1% and 22:6(n-3) from 1.2% to 2.5%; in cattle the increases were from 4.7% to 10.9% for 20:5(n-3) and from 0.5% to 2.2% for 22:6(n-3).

Composition of molecular species and effect of fish oil supplementation

Table 1 and **Fig. 1A** show that the 16:0-18:2(n-6) and 16:0-18:1(n-9) were the predominant molecular species in the diacyl CPG; in unsupplemented sheep they accounted for approximately 49% of the total and were in a 1:1 ratio. In the diacyl CPG of cattle these two species represented greater than 50% of the total and the proportion of 16:0-18:1(n-9) appeared to be higher than 16:0-18:2(n-6) (Table 1). Fish oil supplementation resulted in a sixfold increase in the proportion of 16:0-20:5(n-3) and a two- to threefold increase in the proportion of 18:1-22:5(n-3), 16:0-22:6(n-3) and 18:1-20:5(n-3) in the diacyl CPG from muscle of both cattle and sheep; there were also increases in the proportion of 16:0-18:2(n-6), but no change in the 18:0-20:5(n-3) species (Table 1, Fig. 1B). The proportion of 16:0-18:1(n-9) was reduced by 40% and there were also reductions in 16:0-22:4(n-6), 18:1-18:1(n-9), 18:0-18:1(n-9), and 18:0-18:2(n-6) in the diacyl CPG from muscle of both sheep and cattle (Table 1, Fig. 1B).

The distribution of the molecular species in the alkenyl CPG from unsupplemented animals is shown in **Table 2** and Fig. 1C. In both sheep and cattle the 16:0-18:2(n-6) was the dominant molecular species and accounted for 30% and 27%, respectively. The alkenyl CPG contained molecular species with more C_{20} and C_{22} polyenoic fatty acids than the diacyl CPG, the values for sheep being 33.8% and 20.2%, respectively, and these were increased to 64.3% and 32.3% on feeding fish oil (cf Tables 1 and 2). Fish oil supplementation caused a three- to sevenfold increase in the proportions of 18:0-20:5(n-3), 16:0-20:5(n-3), 18:1-20:5(n-3); for example, 16:0-20:5(n-3) increased

TABLE 2. Composition of the molecular species of 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphocholine of ruminant muscle (mol %)

Peak No.	Molecular Species	Sheep		Cattle		
		Control	Fish Oil	Control	Fish Oil	
					(i)	(ii)
1	18:1-20:5	0.5 ± 0.08	2.38 ± 0.25[a]	0.50	5.70	1.80
2	16:0-20:5	2.33 ± 0.64	17.05 ± 2.44[a]	3.00	20.20	22.30
3	16:0-22:6	1.07 ± 0.03	3.93 ± 0.27[a]	1.10	2.30	3.00
4	18:1-22:5	1.67 ± 0.22	2.85 ± 0.34	1.30	2.40	2.80
5	18:1-20:4	3.00 ± 0.17	2.73 ± 0.45	4.70	3.90	5.60
6	16:0-22:5	3.87 ± 0.42	6.13 ± 0.59	3.40	2.60	3.70
7	16:0-20:4	9.13 ± 0.12	11.48 ± 0.19[a]	7.20	7.90	9.20
8	18:0-20:5	0.97 ± 0.27	8.18 ± 1.29[a]	2.80	7.70	8.70
9[b]	18:1-18:2	3.07 ± 0.07	3.48 ± 0.10[c]	2.90	1.90	3.00
10[b]	18:0-22:6	ND	ND	ND	ND	ND
11	16:0-18:2	30.00 ± 2.48	15.63 ± 2.07[c]	26.70	13.50	15.50
12	16:0-22:4	3.57 ± 0.15	1.60 ± 0.15[a]	2.50	1.80	1.70
13	18:0-22:5	5.17 ± 0.14	3.60 ± 0.32[c]	6.80	2.30	2.60
14	18:0-20:4	2.43 ± 0.31	4.33 ± 0.28[c]		1.80	2.40
15	18:1-18:1	1.13 ± 0.22	0.55 ± 0.06	1.70	0.70	0.70
16[b]	16:0-18:1	13.30 ± 1.23	3.90 ± 0.51[a]	18.90	8.00	6.20
17[b]	18:0-18:2	10.07 ± 0.20	6.28 ± 0.86[a]	6.60	3.70	4.40
18	18:0-18:1	2.50 ± 0.42	1.35 ± 0.24	3.00	2.80	1.40
19	18:0-16:0	0.13 ± 0.03	0.30 ± 0.17	0.20	0.40	0.10

Composition expressed as mean (mol %) ± SEM for duplicate analysis of muscle samples from two control and four fish oil-supplemented sheep; mol % for duplicate analysis of a control and two fish oil-supplemented cattle; ND, not detected.

[a] $P < 0.01$ when compared to control by F test.

[b] These species tend to co-elute using acetonitrile-2-propanol 80:20 (v/v) and were resolved in methanol-2-propanol 95:5 (v/v) using procedured outlined in Methods.

[c] $P < 0.05$ when compared to control by F test.

TABLE 3. Composition of the molecular species of 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine of ruminant muscle (mol %)

Peak No.	Molecular Species	Sheep		Cattle		
		Control	Fish Oil	Control	Fish Oil	
					(i)	(ii)
1	18:1-20:5	0.93 ± 0.14	2.43 ± 0.23[a]	0.60	1.40	2.10
2	16:0-20:5	0.87 ± 0.09	3.82 ± 0.25[b]	0.80	3.90	4.70
3	16:0-22:6	2.20 ± 0.04	4.00 ± 1.26	2.30	1.90	3.00
4	18:1-22:5	1.07 ± 0.04	1.20 ± 0.15	3.30	3.50	0.90
5	18:1-20:4	1.97 ± 0.19	1.63 ± 0.10	1.50	1.70	1.80
6	16:0-22:5	4.40 ± 0.25	4.90 ± 0.23	4.10	4.90	6.50
7	16:0-20:4	4.73 ± 0.38	6.00 ± 0.52	1.00	1.30	1.60
8	18:0-20:5	4.73 ± 0.35	10.10 ± 0.67[a]	7.80	16.30	16.50
9[c]	18:1-18:2	3.83 ± 0.27	4.03 ± 1.03	2.30	1.50	1.60
10[c]	18:0-22:6	6.67 ± 0.07	6.83 ± 1.28	3.70	4.60	4.90
11	16:0-18:2	4.30 ± 0.35	6.28 ± 0.63[d]	3.40	4.70	4.90
12	16:0-22:4	2.80 ± 0.02	2.53 ± 0.25	2.90	3.00	2.90
13	18:0-22:5	2.43 ± 0.36	2.30 ± 0.08	4.30	2.90	3.60
14	18:0-20:4	30.13 ± 0.41	19.65 ± 0.67[b]	29.30	13.10	18.10
15	18:1-18:1	2.97 ± 0.12	1.78 ± 0.11[a]	2.70	1.90	1.70
16[c]	16:0-18:1	1.07 ± 0.46	3.60 ± 1.10	4.10	5.90	3.90
17[c]	18:0-18:2	11.60 ± 0.20	10.33 ± 1.58	13.60	13.30	10.90
18	18:0-18:1	5.13 ± 0.15	3.85 ± 0.21[a]	7.20	6.20	4.80
19	18:0-16:0	0.67 ± 0.09	0.23 ± 0.06[d]	0.40	0.30	0.30

Composition expressed as mean (mol %) ± SEM for duplicate analysis of muscle samples from two control and four fish oil-supplemented sheep; mol % for duplicate analysis of a control and two fish oil-supplemented cattle.

[a] $P < 0.01$ when compared to control by F test.

[b] $P < 0.001$ when compared to control by F test.

[c] These species tend to co-elute using acetonitrile-2-propanol 80:20 (v/v) and were resolved in methanol-2-propanol 95:5 (v/v) using procedures outlined in Methods.

[d] $P < 0.05$ when compared to control by F test.

from about 2.3% to 17.1% in sheep and 3% to 21% in cattle. There were also increases in the proportions of 16:0-22:6(n-3), 16:0-20:4(n-6), 18:0-20:4(n-6), and 18:1-18:2(n-6) (Table 2, Fig. 1D). In contrast to the diacyl CPG, the 16:0-18:2(n-6) species in the alkenyl CPG of sheep and cattle was reduced by 50% on feeding fish oil. There were also reductions in the proportions of 16:0-22:4(n-6), 16:0-18:1(n-9), and 18:0-18:2(n-6) (Table 2, cf. Fig. 1C and 1D).

Table 3 and **Fig. 2A** show that 18:0-20:4(n-6) and 18:0-18:2(n-6) accounted for about 30% and 12%, respectively, of the molecular species that were present in the diacyl EPG of muscle of unsupplemented sheep and cattle; fish oil supplementation caused a two- to fourfold increase in the proportion of 18:0-20:5(n-3), 16:0-20:5(n-3), and 18:1-20:5(n-3). The 18:0-20:5(n-3) increased from 4.7% to 10.1% in sheep and 7.8% to 16.4% in cattle (Table 3, Fig. 2B). The most significant decrease occurred in the proportion of 18:0-20:4(n-6); it was reduced by about 30-40% in both sheep and cattle. There were reductions in other minor species including 18:0-18:1(n-9) and 18:1-18:1(n-9), but the proportion of 18:0-18:2(n-6) did not change (Table 3, Fig. 2B).

The distribution of the molecular species in the alkenyl EPG from muscle of sheep and cattle is shown in **Table 4,** Fig. 2C. In comparison to the diacyl EPG, the 18:0-20:4(n-6) accounted for about 15% and 11% of the total in sheep and cattle, respectively (Table 4); the next three major species were 18:0-18:2(n-6) (14.5%), 16:0-18:2(n-6) (10.6%), and 16:0-20:4(n-6) (10.4%) in sheep, and a similar pattern occurred in cattle (Table 4). The alkenyl EPG was comprised of molecular species containing about 55% of C_{20} and C_{22} polyenoic acids and this was increased to 78% by feeding fish oil (Table 4). The proportion of 18:0-20:5(n-3) increased significantly from 5.5% to 19% in sheep and from 7.3% to 22% in cattle; similar increases were observed in 16:0-20:5(n-3), 18:1-20:5(n-3), 16:0-22:6(n-3) and also in the 18:1-18:2(n-6) species (Table 4, Fig. 2D). In contrast to the reductions observed in the proportion of 18:0-20:4(n-6) in the diacyl EPG, there was no change in this species in the alkenyl EPG (Table 4, cf. Fig. 2C and 2D). Major decreases were observed in the proportions of 16:0-18:2(n-6) and 18:0-18:2(n-6) in the alkenyl EPG from muscle of both sheep and cattle fed the fish oil supplements; reductions also occurred in 18:0-22:5(n-3), 18:0-18:1(n-9), and 18:1-18:1(n-9) (Table 4, cf Fig. 2C and 2D).

Fig. 3 summarizes the remodelling that occurred in the major molecular species of the diacyl and alkenylacyl CPG and EPG of sheep muscle after feeding fish oil supplements. In both the diacyl CPG and alkenylacyl CPG major changes occurred in the molecular species containing 18:1 (i.e., 16:0-18:1(n-9) + 18:0-18:1(n-9)). In the di-

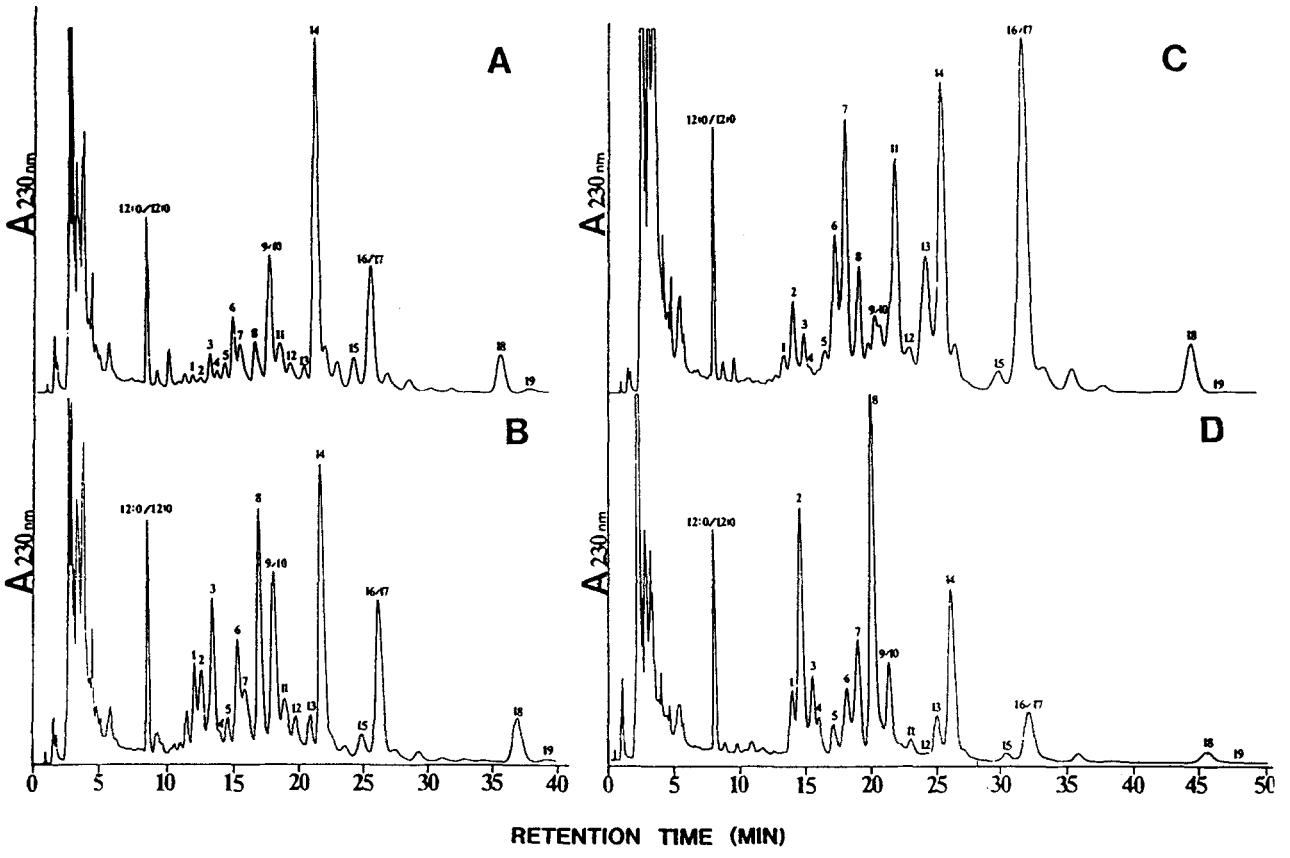

Fig. 2. Effect of fish oil supplementation on the HPLC profiles of molecular species of ethanolamine glycerophospholipids (EPG) of sheep skeletal muscle. A, Control: diacyl EPG; B, fish oil: diacyl EPG; C, control: alkenylacyl EPG; D, fish oil: alkenylacyl EPG. Refer to Table 1 for peak identification.

acyl CPG the molecular species containing 18:2 did not change but these species were significantly reduced in the alkenyl CPG. This reduction parallels the larger increases observed in the 20:5(n-3) content of the molecular species from this plasmalogen (Fig. 3). In the diacyl EPG the molecular species containing 20:4(n-6) were reduced by fish oil supplementation; however, these species were preserved in the alkenyl EPG (Fig. 3). The molecular species containing 18:2 (i.e., 16:0-18:2(n-6), 18:0-18:2(n-6)) were reduced in the alkenyl EPG and these changes reflect the increase in 20:5(n-3) content of the 16:0 and 18:0 molecular species in the plasmalogen (Fig. 3).

DISCUSSION

The present studies demonstrate that the choline and ethanolamine glycerophospholipids of skeletal muscle from sheep and cattle contain significant proportions of plasmalogens; this confirms recent studies for beef (5) and is similar to that reported for other species (5-7). For example, more than 60% of the EPG were in the ether form and of this about two-thirds was present as the alkenyl form. This pattern resembles that found in canine skeletal muscle and myocardial sarcolemma (6, 7). The predominant molecular species in both the diacyl and alkenyl CPG from unsupplemented animals contained 16:0 in the *sn*-1 position and 18:1(n-9) or 18:2(n-6) in the *sn*-2 position and the distribution was similar to that occurring in the rabbit (19) and canine (6). The high proportion of 20:4(n-6) that occurs in the *sn*-2 position of the molecular species of diacyl EPG from ruminant muscle is similar to chicken (5) but is different from that reported for other species, where there is either more 22:6(n-3), as occurs in fish (5), or alternatively the 18:0 combines with a number of other fatty acids (19). In ruminants the proportions of 16:0-20:4(n-6) and 16:0-18:2(n-6) in both the alkenyl CPG and EPG are higher than in their corresponding diacyl phospholipids; the reasons for this difference are unknown, although it has been suggested that plasmalogens containing high proportions of tetraenoic fatty acids may influence calcium translocation in sarcoplasmic reticulum (7). There is also evidence to suggest that membranes, which are enriched in plasmalogens, exhibit different molecular dynamics in comparison to the bilayers that contain diacylphospholipids, and it is likely that these differences would be influenced by the composition of the fatty acid in the *sn*-2 position (20).

TABLE 4. Composition of the molecular species of 2-acyl-1(1-alkenyl)-*sn*-glycero-3-phosphoethanolamine of ruminant muscle (mol %)

Peak No.	Molecular Species	Sheep		Cattle		
		Control	Fish Oil	Control	Fish Oil	
					(i)	(ii)
1	18:1-20:5	1.12 ± 0.08	2.54 ± 0.17[a]	1.00	3.40	3.30
2	16:0-20:5	3.33 ± 0.38	11.05 ± 0.80[b]	4.50	13.40	14.30
3	16:0-22:6	1.90 ± 0.10	4.10 ± 0.20[a]	0.70	2.80	2.70
4	18:1-22:5	0.67 ± 0.25	1.79 ± 0.08[c]	0.90	2.30	2.20
5	18:0-20:4	1.83 ± 0.04	2.05 ± 0.04[c]	3.00	2.20	2.80
6	16:0-22:5	6.10 ± 0.31	5.30 ± 0.23	7.90	4.20	4.90
7	16:0-20:4	10.40 ± 0.41	9.35 ± 0.35	8.10	6.30	6.60
8	18:0-20:5	5.47 ± 0.70	18.95 ± 1.22[b]	7.30	21.10	22.40
9[d]	18:1-18:2	4.70 ± 0.34	7.05 ± 0.34[a]	3.30	5.30	5.40
10[d]	18:0-22:6	ND	ND	ND	ND	ND
11	16:0-18:2	10.53 ± 0.72	2.53 ± 0.60[b]	11.30	4.00	4.30
12	16:0-22:4	1.90 ± 0.10	2.05 ± 0.80	3.40	2.40	2.10
13	18:0-22:5	7.30 ± 0.20	5.55 ± 0.41[c]	8.10	3.40	4.20
14	18:0-20:4	14.83 ± 0.80	15.18 ± 0.54	10.90	7.20	10.20
15	18:1-18:1	1.27 ± 0.09	0.85 ± 0.06[a]	2.00	1.30	0.90
16[d]	16:0-18:1	4.23 ± 0.33	1.90 ± 0.06[a]	8.20	2.30	3.30
17[d]	18:0-18:2	14.47 ± 1.08	5.78 ± 0.64[a]	12.20	6.90	5.70
18	18:0-18:1	3.40 ± 0.08	0.93 ± 0.39[a]	4.60	3.10	2.30
19	18:0-16:0	0.13 ± 0.02	0.80 ± 0.35	0.20	0.80	0.40

Composition expressed as mean (mol %) ± SEM for duplicate assays of muscle samples from two control and four fish oil-supplemented sheep; mol % for duplicate analysis of a control and two fish oil-supplemented cattle; ND, not detected.

[a] $P < 0.01$ when compared to control by F test.

[b] $P < 0.001$ when compared to control by F test.

[c] $P < 0.05$ when compared to control by F test.

[d] These species tend to co-elute using acetonitrile-2-propanol 80:20 (v/v) and were resolved in methanol-2-propanol 95:5 (v/v) using procedures outlined in Methods.

Feeding of fish oil supplements to ruminants increased the proportion of 20:5(n-3) and 22:6(n-3) into the CPG and EPG of skeletal muscle and this is reflected by the significant changes that occurred in the molecular species. In the diacyl CPG the incorporated 20:5(n-3) and 22:6(n-3) substituted for 18:1(n-9) in the *sn*-2 position. This substitution occurred primarily where 16:0 occupies the *sn*-1 position and is indicative of the influence exerted by this acyl chain on the pattern of remodelling. However, this specificity does not exist in the alkenyl CPG and the 20:5(n-3) substituted for 18:1(n-9) and 18:2(n-6) in the *sn*-2 position and combined with both 16:0 and 18:0 at the *sn*-1 vinyl ether position; this results in a much higher proportion of 20:5(n-3) in the plasmalogen species compared to its diacyl counterpart. This difference in the pattern of substitution between the diacyl and alkenyl CPG suggests that the pathways of remodelling, whether via the acyltransferase (21) or transacylase pathways (22), have different specificities. Recent evidence on the differential turnover of polyunsaturated fatty acids in plasmalogen and diacyl glycerophospholipids in P388D$_1$ cells (23) and cardiac myocytes (24) support this suggestion. Further evidence for differential incorporation of polyunsaturated fatty acids in diacyl and alkenyl phospholipids is demonstrated by the pattern of substitution that we found in the EPG of skeletal muscle from ruminants receiving fish oil supplement. In the diacyl EPG, 20:5(n-3) substituted primarily for 20:4(n-6) at the *sn*-2 position and combined with both 16:0 and 18:0 in the *sn*-1 position, whereas in the alkenyl EPG, 20:4(n-6) was preserved at the *sn*-2 position, and 18:2(n-6) and 18:1(n-9) were depleted. This preservation was different from that observed in P388D$_1$ cells, a macrophage-like cell supplemented with 22:6(n-3) (23), or rat neutrophils (25) and peritoneal macrophages isolated from mice (26), where the feeding of fish oil resulted in a depletion of 20:4(n-6) from the *sn*-2 position in both the diacyl and alkenyl EPG. In human platelets, fish oil supplementation reduced the proportion of 20:4(n-6) in the total alkenyl EPG but not the diacyl EPG (27); however, individual molecular species were not determined.

The pathways of phospholipid remodelling have not been examined in ruminant muscle and usually the amount of C_{18} polyunsaturated fatty acids that are available for chain elongation and/or incorporation are low because of the extensive biohydrogenation of dietary lipids in the alimentary tract (28). Recently we demonstrated that C_{20} and C_{22} polyenoic fatty acids are not hydrogenated and are readily incorporated into muscle phospholipids (1). The results described herein show that the 20:5(n-3) and 22:6(n-3) content of the molecular species of the alkenyl phospholipids from supplemented animals is higher than the diacyl subclass, and as this in-

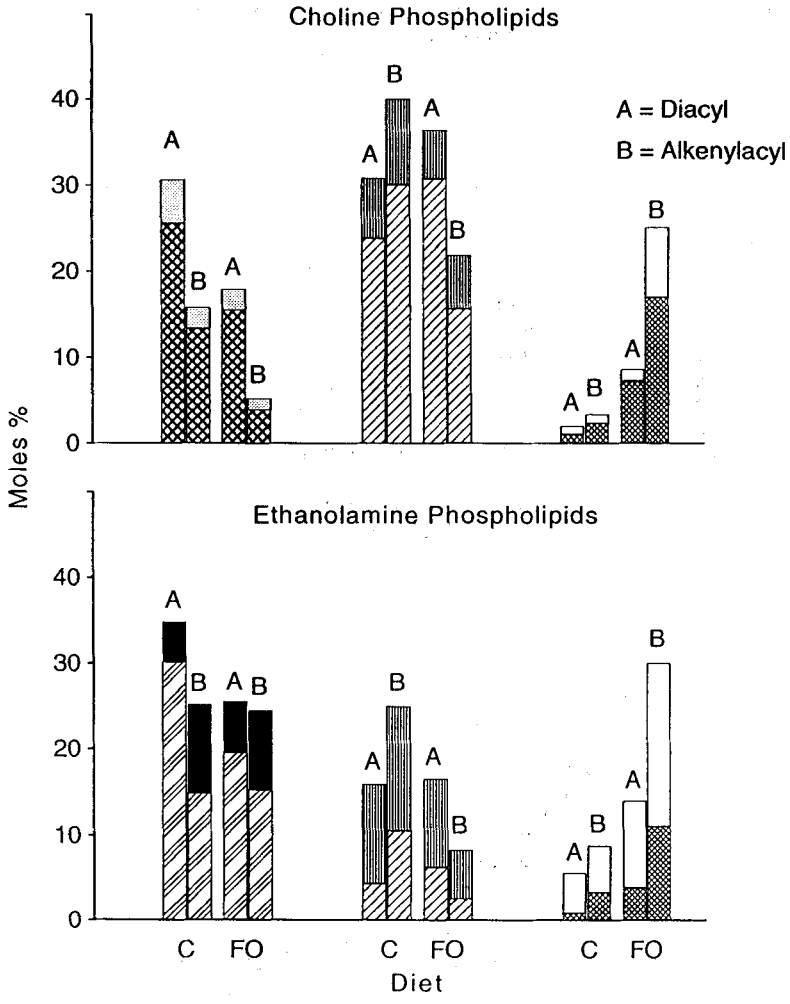

Fig. 3. Changes in the distribution of the major molecular species in choline and ethanolamine glycerophospholipids of sheep muscle; C, control; FO, fish oil; (▨) 18:0-18:1, (▩) 16:0-18:1; (▨) 16:0-18:2; (▥) 18:0-18:2; (■) 16:0-20:5; (□) 18:0-20:5; (▨) 18:0-20:4; (■) 16:0-20:4.

corporation does not reduce the proportion of 20:4(n-6) in the *sn*-2 position (see above), it may suggest that ruminants preferentially conserve C_{20} polyenoic fatty acids in specific membrane phospholipids that have antioxidant properties (29). The pattern of substitution that occurs in the muscle glycerophospholipids as a result of fish oil supplementation could have important physiological implications with respect to the pattern of eicosanoid biosynthesis (26, 30) and the activation of different forms of protein kinase C by diacyl- and/or ether-linked diradylglycerols during signal transduction pathways (31, 32).

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