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ORIGINAL ARTICLE

Oral Docosapentaenoic Acid (22:5n-3) Is Differentially Incorporated into Phospholipid Pools and Differentially Metabolized to Eicosapentaenoic Acid in Tissues from Young Rats

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Abstract The present study assessed the effect of oral supplementation with docosapentaenoic acid (DPA, 22:5n-3) on the levels of serum and tissue lipid classes and their fatty acid compositions including individual phospholipid types in rat liver, heart, and kidney. Sprague–Dawley rats received daily oral gavage over 10 days as corn oil without (controls) or with purified DPA in free fatty acid form (21.2 mg/day). The DPA group exhibited significantly lower serum lipid concentrations. The concentrations in μmol/100 g serum or μmol/g tissue of DPA in the total lipid (TL) were higher by 2.3-, 2.4-, 10.9-, and 5.1-fold in the DPA group of serum, liver, heart, and kidney, respectively, with the phospholipids (PL) being the major DPA reservoir (45.2–52.1% of the DPA in the TL). No significant differences in DHA (22:6n-3) amounts in TL appeared. The highest relative mol% values as DPA were in heart tissue (means of 11.1% in PL and 16.2% in phosphatidylinositol) and lowest in kidney. The EPA (20:5n-3) concentrations were markedly higher in the DPA group and most pronounced in the kidney (5.1 times higher in the TL as compared to controls) relative to liver and heart yielding an estimated apparent % conversion of DPA to EPA of 67% and EPA:DPA ratios reaching 5.74 in kidney phosphatidylethanolamine. The serum lipid-lowering potential of dietary DPA and its impact in the kidney with the derived EPA warrants investigation.

Keywords Docosapentaenoic acid (DPA 22:5n-3) · Rat serum · Cholesteryl esters · Triacylglycerol(s) · Liver · Heart · Kidney · Apparent retroconversion · Eicosapentaenoic acid (EPA 20:5n-3) · Individual phospholipids

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Abbreviations

ARA	Arachidonic acid 20:4n-6
ACAT	Acyl-CoA:cholesterol acyltransferase
CE	Cholesteryl esters
CerPCho	Sphingomyelin
DHA	Docosahexaenoic acid 22:6n-3
DPA	Docosapentaenoic acid 22:5n-3
EPA	Eicosapentaenoic acid 20:5n-3
LCAT	Lecithin:cholesterol acyltransferase
NEFA	Non-esterified fatty acids
PL	Phospholipid(s)
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdIns	Phosphatidylinositol
PtdSer	Phosphatidylserine
PUFA	Polyunsaturated fatty acid
TAG	Triacylglycerol(s)
TC	Total cholesterol
TL	Total lipid(s)
TLC	Thin-layer chromatography

Introduction

Docosapentaenoic acid (DPA, 22:5n-3) as present in seal meat, whale meat/blubber, plus some fish/fish oils to a lesser extent is a significant component of the long-chain omega-3 fatty acids found in the Inuit diet [1]. While eicosapentaenoic acid (EPA, 20:5n-3) plus docosahexaenoic acid (DHA, 22:6n-3) are credited for the apparent cardioprotective effects of diets rich in marine fats [2],

higher levels of DPA in the circulation have been associated with a lower risk for coronary heart disease in population studies [3]. Furthermore, DPA has shown the ability to inhibit human platelet aggregation in vitro and to suppress thromboxane formation [4]. Intervention trials using seal oil containing DPA in addition to EPA/DHA have exhibited beneficial effects on selected cardiovascular disease risk factors in healthy volunteers [5, 6]. The much lower intakes of DPA in the diets of populations who consume small amounts of fish are derived mostly from meat plus poultry [7].

Very recently, supplementation with DPA in rats has indicated that it is both elevated and partly retroconverted to EPA in liver, adipose, heart, and skeletal muscle based on total lipid analyses along with data suggesting that dietary DPA can be converted to DHA in the liver [8]. We have extended the previous work herein by measuring the effect of DPA-supplementation in the rat on the levels of various serum and tissue lipids and their fatty acid profiles as well as the fatty acid compositions of various lipid fractions including individual phospholipids in liver, heart, and kidney. This present study differs from previous work [8] which was restricted to total lipid analyses only in selected tissues and excluded the serum and kidney. The present findings reveal dramatic differences in the resulting fatty acid compositions between individual lipid/phospholipid classes upon DPA supplementation and indicate a particularly high capacity for the retroconversion of DPA to EPA in the kidney.

Experimental Procedure

Animals and Study Design

This study was approved by Animal Care Services, Office of Research, University of Guelph (Animal Utilization Protocol No. 96R093). Seventeen male Sprague–Dawley weanling rats (Charles River Canada, St. Constant, P.Q.) having an average body weight of 49.6 ± 0.6 g and approximately 21 days of age were randomly housed in stainless steel cages with 12 h light–dark cycle and a constant room temperature of 25 °C. All animals were fed (ad libitum) Purina Laboratory Chow (Purina Mills Inc., St. Louis, MO), devoid of long-chain n-3 fatty acids including DPA, and given either 500 μL per day of corn oil alone (control group; Mazola Corn Oil, Corn Products Inc., St. Louis, MO) or an equal volume of corn oil containing a small amount of purified DPA [Nu-Chek Prep Inc., Elysian, MN, U-101-A (free fatty acid form), >99% DPA devoid of 20:5n-3 and 22:6n-3] via oral gavage. The fatty acid composition of the two oral gavages, namely corn oil versus corn oil-DPA, contained mostly oleic acid (28.9 and 26.5 wt%) and linoleic acid (55.0 and 53.4 wt%) with DPA representing 0 and 4.24 wt%, respectively, of total fatty acids. The oral gavages were administered for a period of 10 days after which time the animals were sacrificed and various tissues (plus serum via blood centrifugation) were taken for analyses. The dietary conditions and compositions (including oral gavage) for the DPA group (DPA-supplemented rats) were estimated to provide approximately 2.5% of the total fat intake (oral gavage plus dietary chow) as DPA which is in the general range as reported historically for the Greenland Inuit population [9]. In absolute amounts, the DPA dose was 21.2 mg/day which is higher than Inuit intakes on a body weight basis.

Lipid and Fatty Acid Analyses

Total serum cholesterol levels were determined by a microcolorimetric method [10]. The individual lipid classes were separated by thin-layer chromatographic (TLC) procedures following lipid extraction using methods similar to those described [11, 12]. The TLC plate developed in the neutral lipid system provided for the separation of triacylglycerols (TAG), total phospholipids (PL), non-esterified fatty acids (NEFA), and cholesteryl esters (CE) whereas development in the polar lipid system provided isolations of the individual phospholipids including phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), phosphatidylinositol (PtdIns), phosphatidylserine (PtdSer), and sphingomyelin (CerPCho) as described [11, 12]. The addition of known amounts of an internal standard (odd-carbon fatty acid) to the total lipid (TL) extracts and the isolated lipid fractions provided for determination of both the individual and total fatty acid amounts plus the relative wt% of total fatty acids following transmethylation and gas–liquid chromatographic analyses [11, 12].

Statistical Analyses

The experimental data were analyzed for statistical significance by Student's t-test [13].

Results

The initial and final body weights (following 10 days of oral gavage treatment) for all animals ($n = 17$) were 49.4 ± 0.4 (mean ± SE) and 102.2 ± 2.4 g, respectively. Weights of liver, heart, and kidney were 5.13 ± 0.17, 0.47 ± 0.01, and 0.57 ± 0.02 g, respectively. Total serum fatty acid amounts in the TL of the DPA-supplemented rats were significantly lower (by 15.7% overall) relative to controls (Table 1). Moderately lower levels were also found for the PL (by 13.4%) and the CE (by 15.9%)

Table 1 Concentrations (μmol/g serum) of total fatty acids in serum lipids from control and DPA-supplemented rats

Lipid class	Control	DPA
Total lipid (TL)	4.71 ± 0.25	3.97 ± 0.13[a]
Total phospholipids (PL)	2.02 ± 0.05	1.75 ± 0.05[b]
Triacylglycerols (TAG)	0.93 ± 0.13	0.73 ± 0.12
Non-esterified fatty acids (NEFA)	0.24 ± 0.02	0.27 ± 0.02
Cholesteryl esters (CE)	1.13 ± 0.06	0.95 ± 0.06[a]
Total cholesterol (TC), μmol sterol/g serum	2.17 ± 0.07	1.91 ± 0.04[b]

Data are mean values ± SE for 8 rats from each group

Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$

whereas this was not statistically significant in the case of the TAG where the mean level was 21.5% lower as compared to the controls. Total serum cholesterol levels were moderately lower (by 12.0%) and statistically significant ($P < 0.01$) for the DPA group relative to controls (Table 1). The absolute amount of DPA in TL (μmol/100 g serum) increased by 2.28-fold in the DPA-supplemented rats relative to controls (Fig. 1) with considerable increases apparent in all the individual lipid classes. The single major reservoir of DPA following DPA supplementation was in the PL at a mean of 3.66 μmol/100 g serum (49.2% of DPA in TL) with the TAG, NEFA, and CE contributing 32.0, 13.1, and 5.3%, respectively. The corresponding concentrations (μmol/100 g serum) for EPA in the TL of the control versus DPA groups were 5.65 ± 0.50 and 12.13 ± 0.69, respectively, with a P value of <0.001. Interestingly, the single major reservoir of EPA was in the serum CE at 6.55 ± 0.46 μmol/100 g serum which represented 54.0% of the EPA in the TL for the DPA group. The absolute levels (μmol/100 g serum) of DHA in serum TL (Fig. 2) were not significantly different with values of

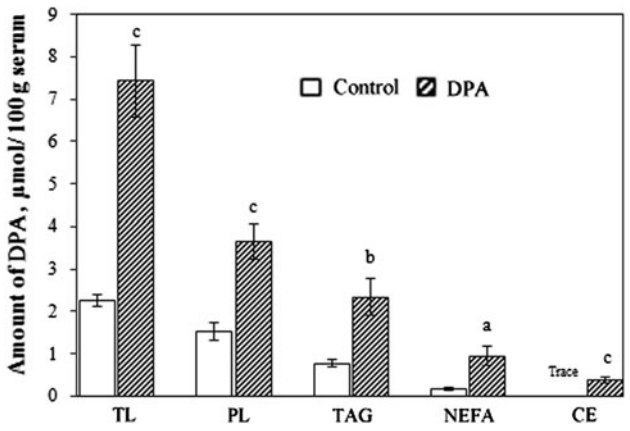

Fig. 1 Concentrations of DPA (22:5n-3) in serum lipids from control and DPA-supplemented rats. Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$

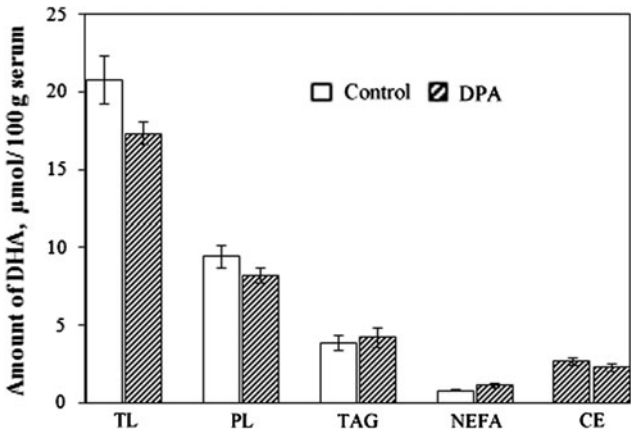

Fig. 2 Concentrations of DHA (22:6n-3) in serum lipids from control and DPA-supplemented rats. No statistically significant differences were found between the DPA versus control group

20.79 ± 1.55 and 17.38 ± 0.76 for the control and DPA groups, respectively. Significantly lower concentrations ($P < 0.001$) of ARA but not 18:2n-6 were found in the TL of the DPA group (48.70 ± 4.70) relative to the controls (87.88 ± 3.74).

The relative mol% of total fatty acids as DPA following supplementation was moderately higher in the TAG fraction as compared to the serum PL with the lowest level being in the CE (Table 2). A moderately higher level of DHA ($P < 0.05$) was found only in the TG upon DPA supplementation although the absolute amounts of DHA (in μmol/g serum) were not significantly different between the control and DPA group for any of the lipid fractions (data not shown). Significantly higher levels of EPA (Table 2) were found in the DPA group—particularly in the TAG and CE fractions. Markedly lower levels of arachidonic acid (ARA, 20:4n-6) but not linoleic acid (18:2n-6) were found in the TAG, PL, and CE within the DPA group relative to controls.

The total fatty acid amounts in TAG were considerably lower in the liver TAG of the DPA group relative to control (1.49 ± 0.28 vs. 4.07 ± 0.94, μmol/g tissue) and in the kidney TAG (2.30 ± 0.25 vs. 4.93 ± 0.56) whereas no statistically significant differences were found in the heart TAG or in the PL for any of the three tissues. The absolute concentrations of DPA in the total lipids of liver, heart, and kidney were dramatically higher (by 2.4-, 10.8-, and 5.1-fold, respectively) in the DPA group relative to controls without any statistically significant differences in the DHA contents (Table 3). The tissue concentrations of DPA in the PL fraction for the DPA group contributed 1.26 ± 0.11 (mean ± SE), 1.17 ± 0.05, and 0.54 ± 0.03 μmol/g tissue which represented 52.1, 45.2, and 51.9% of the DPA amounts in TL for liver, heart, and kidney, respectively. EPA levels were higher in the DPA group by 3.9-, 2.2-, and 4.1-fold, respectively. Considerably lower levels of ARA

Table 2 Fatty acid compositions of serum lipids from control and DPA-supplemented rats (mol% of total fatty acids)

Fatty acids	TAG		Total PL		NEFA		CE	
	Control	DPA	Control	DPA	Control	DPA	Control	DPA
16:0	25.50 ± 0.59	25.34 ± 1.32	25.64 ± 0.43	27.40 ± 0.45	26.69 ± 1.01	25.31 ± 1.05	9.00 ± 1.10	10.12 ± 1.75
18:0	5.83 ± 0.60	6.21 ± 0.60	24.53 ± 0.63	23.46 ± 0.51	9.06 ± 0.42	11.45 ± 0.84	0.21 ± 0.14	0.55 ± 0.24
18:1	36.23 ± 0.66	31.51 ± 2.28	7.86 ± 0.14	8.03 ± 0.31	27.63 ± 1.23	23.09 ± 1.31	8.48 ± 0.45	13.55 ± 0.75[c]
18:2n-6	20.59 ± 0.46	20.06 ± 0.51	20.61 ± 0.75	23.01 ± 1.17	21.37 ± 0.52	22.81 ± 1.13	27.03 ± 0.82	32.56 ± 1.50[b]
18:3n-3	0.83 ± 0.05	0.76 ± 0.08	0.01 ± 0.01	0.02 ± 0.02	0.96 ± 0.06	0.75 ± 0.14	0.02 ± 0.02	0.08 ± 0.04
20:4n-6 (ARA)	3.51 ± 0.38	2.02 ± 0.36[a]	14.47 ± 0.40	9.48 ± 0.42[c]	8.89 ± 0.56	6.83 ± 1.82	50.22 ± 1.22	32.57 ± 1.64[c]
20:5n-3 (EPA)	1.29 ± 0.13	3.85 ± 1.01[a]	0.66 ± 0.26	1.10 ± 0.10	0.63 ± 0.06	2.08 ± 0.25[c]	2.60 ± 0.21	6.96 ± 0.53[c]
22:4n-6	0.39 ± 0.04	0.03 ± 0.02[c]	0.47 ± 0.03	0.39 ± 0.04	0.45 ± 0.05	Trace[c]	Trace	Trace
22:5n-6	0.34 ± 0.02	0.01 ± 0.01[c]	0.30 ± 0.02	0.03 ± 0.02[c]	0.46 ± 0.05	Trace[c]	Trace	Trace
22:5n-3 (DPA)	0.93 ± 0.10	3.78 ± 0.82[a]	0.83 ± 0.13	2.18 ± 0.22[c]	0.58 ± 0.09	3.41 ± 0.63[b]	Trace	0.41 ± 0.07[c]
22:6n-3 (DHA)	4.57 ± 0.42	6.43 ± 0.64[a]	4.63 ± 0.48	4.90 ± 0.30	3.28 ± 0.27	4.28 ± 0.38	2.43 ± 0.16	3.20 ± 0.48

Data are mean values ± SE for each group

Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$

Table 3 Concentrations (μmol/g) selected fatty acids in total lipids of liver, heart, and kidney from control and DPA-supplemented rats

Fatty acids	Liver		Heart		Kidney	
	Control	DPA	Control	DPA	Control	DPA
DPA (22:5n-3)	0.72 ± 0.08	2.42 ± 0.30[a]	0.22 ± 0.02	2.59 ± 0.20[b]	0.17 ± 0.03	1.04 ± 0.09[b]
DHA (22:6n-3)	7.01 ± 0.57	6.69 ± 0.63	2.01 ± 0.21	1.92 ± 0.09	1.21 ± 0.10	1.18 ± 0.06
EPA (20:5n-3)	0.31 ± 0.06	1.51 ± 0.19[a]	0.06 ± 0.00	0.19 ± 0.02[b]	0.43 ± 0.02	2.20 ± 0.08[b]
ARA (20:4n-6)	12.80 ± 1.00	7.83 ± 0.30[a]	3.13 ± 0.23	3.00 ± 0.22	8.01 ± 0.44	5.43 ± 0.24[a]

Data are mean values ± SE for each group

Statistical significance (DPA vs. control): [a]$P < 0.01$, [b]$P < 0.001$

were found in the liver and kidney upon DPA supplementation relative to controls with no significant differences appearing in heart tissue. No significant differences in the absolute amounts of 18:2n-6 were found in any tissue between the DPA and control groups (data not shown). The relative mol% of total fatty acids as DPA was found to be significantly higher in the TL, PL, and TAG for all three tissues in the DPA group (Tables 4, 5, 6). The DPA rose to similar levels (mean values of 2.93–3.62 mol%) in liver TL, PL, and TAG upon DPA supplementation with the highest level by far of DPA in the PL of all tissues being in the heart (11.09 mol%). In the case of TAG, the highest level of DPA following DPA-supplementation was in the kidney (5.33 mol%) followed by the heart and liver. The DHA levels as mol% were significantly higher in the DPA group relative to controls in the TAG but not the PL of all three tissues. In heart tissue, the DHA level was actually significantly lower in the PL fraction of the DPA group. Following DPA supplementation, the mol% of EPA was considerably higher in the PL as compared to the TAG fraction for all tissues and, in the case of the kidney, the mol% as DPA markedly surpassed that of DPA itself. In all three tissues, the PL exhibited a significant lowering of the mol% as ARA in the DPA group which was even more pronounced in the case of 22:5n-6.

The relative mass distribution (based on μmol/g tissue) for the esterified DPA (DPA group) found in association with the individual phospholipid types in the three tissues (Fig. 3 a–c) indicates that the major reservoir of DPA was almost equally distributed between PtdCho (39.3–46.4% of total) and PtdEtn (38.0–44.7% of total). The highest mol% of fatty acids as DPA for each individual phospholipid (Table 7) was found in heart tissue (relative to liver and kidney). In contrast, the levels of EPA for all individual phospholipids were considerably higher in kidney compared to the corresponding phospholipids in the other

Table 4 Fatty acid compositions of lipid classes from livers of control and DPA-supplemented rats (mol% of total fatty acids)

Fatty acids	TL		Total PL		TAG	
	Control	DPA	Control	DPA	Control	DPA
16:0	23.97 ± 0.75	23.80 ± 0.68	19.99 ± 0.40	21.16 ± 0.80	37.09 ± 0.85	38.13 ± 1.04
18:0	17.51 ± 0.28	18.94 ± 0.90	26.27 ± 0.43	24.61 ± 0.52[a]	2.01 ± 0.21	3.03 ± 0.89
18:1	19.33 ± 0.99	15.15 ± 1.44[a]	7.01 ± 0.06	7.67 ± 0.44	43.45 ± 0.71	37.63 ± 3.05
18:2n-6	12.83 ± 0.30	13.95 ± 0.41	9.20 ± 0.30	12.44 ± 0.26[c]	14.11 ± 0.51	13.85 ± 1.20
18:3n-3	0.12 ± 0.02	0.06 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.14 ± 0.06	0.14 ± 0.06
20:4n-6 (ARA)	15.64 ± 0.40	11.99 ± 0.77[b]	23.94 ± 0.25	16.62 ± 0.51[c]	0.60 ± 0.12	0.55 ± 0.12
20:5n-3 (EPA)	0.38 ± 0.06	2.24 ± 0.19[c]	0.38 ± 0.05	2.40 ± 0.21[c]	0.27 ± 0.18	0.69 ± 0.32
22:4n-6	0.46 ± 0.02	0.26 ± 0.04[b]	0.36 ± 0.05	0.33 ± 0.07	0.40 ± 0.06	0.03 ± 0.02[b]
22:5n-6	0.33 ± 0.03	Trace[c]	0.28 ± 0.06	Trace[b]	0.23 ± 0.06	Trace[a]
22:5n-3 (DPA)	0.88 ± 0.06	3.62 ± 0.32[c]	0.11 ± 0.11	3.10 ± 0.25[c]	0.32 ± 0.04	2.93 ± 0.52[b]
22:6n-3 (DHA)	8.56 ± 0.29	9.98 ± 0.31[a]	12.44 ± 0.38	11.66 ± 0.33	1.37 ± 0.19	3.02 ± 0.54[a]

Data are mean values ± SE for each group

Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$

Table 5 Fatty acid compositions of lipid classes from hearts of control and DPA-supplemented rats (mol% of total fatty acids)

Fatty acids	TL		Total PL		TAG		NEFA	
	Control	DPA	Control	DPA	Control	DPA	Control	DPA
16:0	18.41 ± 0.87	18.26 ± 0.35	14.77 ± 0.33	15.06 ± 0.45	28.70 ± 0.44	32.44 ± 1.01[a]	20.74 ± 0.51	22.42 ± 1.46
18:0	22.48 ± 0.21	21.87 ± 0.53	24 .66 ± 0.12	25.41 ± 0.22[a]	7.72 ± 0.46	7.97 ± 0.32	15.03 ± 0.23	15.15 ± 0.64
18:1	12.63 ± 0.39	11.66 ± 0.50	8.11 ± 0.22	7.86 ± 0.27	36.04 ± 0.53	30.67 ± 0.43[c]	19.62 ± 0.57	17.20 ± 0.65[a]
18:2 n-6	19.25 ± 0.49	16.47 ± 0.40[b]	18.52 ± 0.60	15.44 ± 0.55[b]	24.92 ± 0.38	21.56 ± 0.57[c]	21.38 ± 0.44	18.90 ± 0.59[b]
18:3n-3	0.19 ± 0.03	0.17 ± 0.02	0.01 ± 0.00	Trace	0.81 ± 0.06	0.82 ± 0.03	0.42 ± 0.04	0.32 ± 0.04
20:4n-6 (ARA)	15.04 ± 0.68	12.14 ± 0.60[b]	17.82 ± 0.33	14.45 ± 0.41[c]	0.71 ± 0.02	0.38 ± 0.02[c]	12.21 ± 0.49	8.46 ± 0.31[c]
20:5n-3 (EPA)	0.29 ± 0.01	0.77 ± 0.04[c]	0.29 ± 0.01	0.78 ± 0.04[c]	0.12 ± 0.01	0.46 ± 0.04[c]	0.39 ± 0.01	1.03 ± 0.04[c]
22:4n-6	0.61 ± 0.03	0.30 ± 0.04[c]	0.75 ± 0.03	0.35 ± 0.01[c]	0.11 ± 0.03	0.04 ± 0.02	0.53 ± 0.02	0.16 ± 0.02[c]
22:5n-6	0.34 ± 0.03	0.13 ± 0.02[c]	0.38 ± 0.03	0.15 ± 0.01[c]	0.04 ± 0.02	0.02 ± 0.01	0.26 ± 0.02	0.08 ± 0.00[c]
22:5n-3 (DPA)	1.05 ± 0.07	10.40 ± 0.28[c]	1.18 ± 0.05	11.09 ± 0.05[c]	0.14 ± 0.01	4.74 ± 0.50[c]	1.03 ± 0.07	9.98 ± 0.96[c]
22:6n-3 (DHA)	9.70 ± 0.94	7.83 ± 0.29	13.51 ± 0.56	9.41 ± 0.34[c]	0.69 ± 0.03	0.90 ± 0.05[b]	8.40 ± 0.58	6.29 ± 0.38[a]

Data are mean values ± SE for each group

Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$

tissues (Table 7). The highest EPA:DPA molar ratios for all individual phospholipids were found in the kidney ranging from 1.03 in PtdIns to 5.74 in PtdEtn. The corresponding ratios across individual phospholipids were lowest in heart tissue (0.03–0.08) with intermediary ratios being found in the liver (0.15–0.91).

Discussion

Previous studies involving the feeding of dietary seal oil to human subjects have demonstrated a significant elevation in the level of DPA in the circulating lipid fractions [5, 6] as well as a significant rise in DPA concentrations in tissue lipid in animal studies [14]. Such effects cannot be directly attributed to the consumption of DPA since it represents approximately 5% of the fatty acids in seal oil with the higher level of EPA having the capacity to generate considerable amounts of DPA via chain elongation [15, 16]. In addition, previous studies in humans on the effects of dietary seal on circulating lipids [5, 6] cannot be attributed to the effects of DPA specifically since EPA plus DHA are more predominant components of such oils. In their newly released review, Kaur et al. [17] have updated the current knowledge available on the metabolism and the biological effects of DPA. The recently published study by Kaur et al. [8] evaluated the effect of purified dietary DPA directly in rats on the fatty acid compositions of liver, adipose, heart, skeletal muscle, and brain. The present study has measured changes in plasma and tissue lipid levels and fatty acid

Table 6 Fatty acid compositions of lipid classes from kidneys of control and DPA-supplemented rats (mol% of total fatty acids)

Fatty acids	TL		Total PL		TAG	
	Control	DPA	Control	DPA	Control	DPA
16:0	26.78 ± 0.72	25.61 ± 0.34	24.26 ± 0.62	24.62 ± 0.60	32.35 ± 1.44	29.15 ± 1.03
18:0	17.10 ± 0.09	17.90 ± 0.09[b]	20.19 ± 0.49	19.98 ± 0.33	8.12 ± 1.00	7.92 ± 0.89
18:1	15.19 ± 0.39	13.52 ± 0.35[b]	11.14 ± 0.15	11.19 ± 0.09	32.81 ± 0.63	31.16 ± 0.82
18:2n-6	16.93 ± 0.23	17.30 ± 0.23	16.34 ± 0.33	17.34 ± 0.45	24.51 ± 0.75	23.32 ± 0.79
18:3n-3	0.03 ± 0.01	0.24 ± 0.20	0.01 ± 0.01	0.01 ± 0.01	0.64 ± 0.05	0.66 ± 0.11
20:4n-6 (ARA)	18.98 ± 0.44	13.88 ± 0.30[c]	23.01 ± 0.42	15.87 ± 0.46[c]	0.62 ± 0.08	0.60 ± 0.12
20:5n-3 (EPA)	1.02 ± 0.04	5.64 ± 0.25[c]	1.17 ± 0.04	6.05 ± 0.30[c]	0.06 ± 0.03	0.40 ± 0.04[c]
22:4n-6	0.59 ± 0.04	0.26 ± 0.05[c]	0.55 ± 0.08	0.24 ± 0.04[a]	0.22 ± 0.04	0.13 ± 0.04
22:5n-6	0.10 ± 0.04	Trace[a]	0.05 ± 0.05	Trace	0.01 ± 0.01	Trace
22:5n-3 (DPA)	0.38 ± 0.06	2.64 ± 0.18[c]	0.28 ± 0.06	1.78 ± 0.06[c]	0.11 ± 0.02	5.33 ± 0.50[c]
22:6n-3 (DHA)	2.88 ± 0.19	3.02 ± 0.12	3.00 ± 0.13	2.92 ± 0.15	0.55 ± 0.04	1.31 ± 0.12[c]

Data are mean values ± SE for each group

Statistical significance (DPA vs. control): [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$

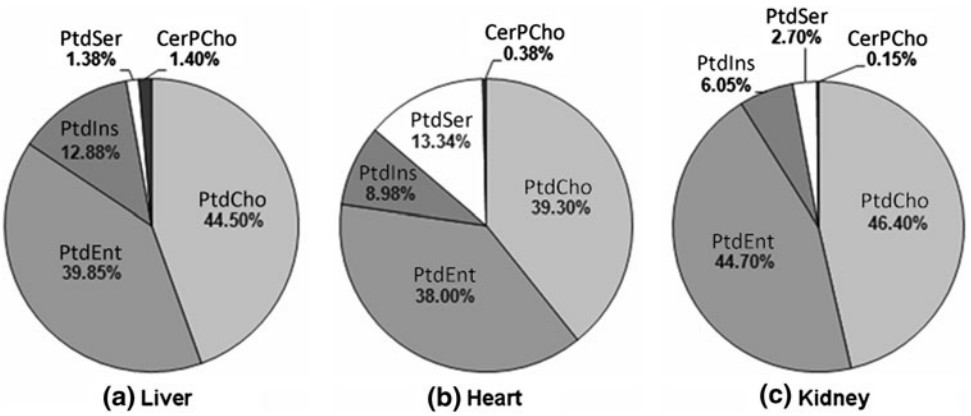

Fig. 3 The relative % distribution (based on μmol/g tissue) for the esterified DPA (DPA group) found in association with the individual phospholipid types in the liver, heart, and kidney

compositions resulting from DPA ingestion including an extensive analyses of the resulting fatty acid alterations in the liver, heart, and the kidney and individual phospholipid types therein.

The moderately lower levels of total lipid (based on total fatty acid concentrations) in the serum lipid of the DPA group (Table 1) was accounted for by significantly lower levels of the summed fatty acid as PL and CE while the lower levels as TAG did not reach statistical significance. Such measurements of circulating lipids were not reported in the recent publication by Kaur et al. [8]. Moderately lower levels of total serum cholesterol were also found in the present study as was found for plasma cholesterol levels in rats following dietary treatment with concentrated DHA [16]. In the circulation, serum PL was the single predominant reservoir of esterified DPA whereas the TAG fraction showed a moderately greater enrichment (as mol% of total fatty acids) in DPA as compared to PL (Table 2). Interestingly, the very low level of accumulation of DPA in serum CE (0.41%) upon supplementation (Table 2) resulted in the DPA:ARA molar ratio in serum CE to be only a small fraction of that in the corresponding PL (0.012 vs. 0.23). Since serum CE is derived from LCAT (lecithin:cholesterol acyltransferase) activity [18, 19] and tissue (liver) ACAT (acyl-CoA:cholesterol acyltransferase) activity [20], such esterification reactions appear to discriminate against substrates containing DPA. Previous human studies on plasma LCAT have suggested a preferential utilization of EPA-containing species of phospholipid (lecithin) relative to DHA species [21] which appears in keeping with the much higher levels of EPA (relative to DHA) in the serum CE relative to the PL of the DPA supplemented rats (Table 2). It is not yet known if the relative participation of DPA species in the LCAT reaction may influence its apparent role in reducing atherosclerosis [19]. It is noted that higher intakes of DPA in humans and higher levels of DPA in the circulation have been associated with protection against carotid atherosclerosis [22] and coronary heart disease [3].

Table 7 Long-chain polyunsaturated fatty acid compositions (mol% of total fatty acids) and EPA:DPA ratios of individual phospholipids from tissues of DPA-supplemented rats

Fatty acid	20:4n-6 (ARA)	20:5n-3 (EPA)	22:4n-6	22:5n-6	22:5n-3 (DPA)	22:6n-3 (DHA)	EPA:DPA Ratio
Liver							
PtdCho	14.41 ± 0.70	2.44 ± 0.24	0.02 ± 0.01	0.02 ± 0.01	2.69 ± 0.21	10.55 ± 0.45	0.91
PtdEtn	17.17 ± 0.61	3.41 ± 0.29	0.09 ± 0.02	0.01 ± 0.01	4.48 ± 0.35	18.24 ± 0.65	0.76
PtdIns	34.49 ± 0.51	0.68 ± 0.08	0.33 ± 0.03	0.12 ± 0.03	4.41 ± 0.48	5.10 ± 0.11	0.15
PtdSer	14.87 ± 0.75	2.37 ± 0.27	0.30 ± 0.09	Trace	3.49 ± 0.38	12.97 ± 0.78	0.68
Heart							
PtdCho	17.31 ± 0.56	0.76 ± 0.04	0.07 ± 0.03	0.03 ± 0.01	10.49 ± 0.43	6.07 ± 0.31	0.07
PtdEtn	15.13 ± 0.24	1.17 ± 0.06	0.23 ± 0.02	0.17 ± 0.01	14.11 ± 0.47	19.80 ± 0.52	0.08
PtdIns	23.60 ± 0.65	0.73 ± 0.06	0.08 ± 0.02	0.01 ± 0.01	16.23 ± 0.33	3.81 ± 0.44	0.04
PtdSer	4.43 ± 0.20	0.40 ± 0.02	0.93 ± 0.06	0.38 ± 0.03	13.32 ± 0.32	12.36 ± 0.43	0.03
Kidney							
PtdCho	8.25 ± 0.24	3.77 ± 0.28	0.03 ± 0.01	0.01 ± 0.00	1.73 ± 0.15	3.94 ± 0.13	2.18
PtdEtn	25.90 ± 0.83	10.84 ± 0.40	0.17 ± 0.01	Trace	1.89 ± 0.09	3.46 ± 0.13	5.74
PtdIns	31.64 ± 0.48	2.61 ± 0.22	0.25 ± 0.04	0.08 ± 0.01	2.54 ± 0.15	3.54 ± 0.27	1.03
PtdSer	19.18 ± 0.45	8.86 ± 0.38	0.45 ± 0.03	0.45 ± 0.03	3.20 ± 0.07	1.45 ± 0.06	2.77

Data are mean values ± SE for each group or mean values only (EPA:DPA ratio)

The considerably lower concentrations of TAG in the liver (by 64%) and kidney (by 53%) in the DPA-supplemented rats as found herein are consistent with the TAG-lowering effect of fish oils containing EPA plus DHA in these same tissues as reported [11, 23]. Potential mechanisms for such a suppression in tissue TAG levels with dietary DPA likely involve a diminution in lipogenic enzyme activities and corresponding gene expression, a significant suppression in 1,2-diacylglycerol conversion to TAG via CoA:1,2-diacylglycerol acyltransferase, and/or increased fatty acid oxidation [11].

In addition to the markedly higher concentrations of DPA in the TL and individual lipid types relative to controls in the DPA group, considerably higher levels of EPA were also found in the TL of serum, liver, heart, and kidney along with considerable heterogeneity in the magnitude of such elevations across the various lipid classes (Fig. 1; Tables 3, 4, 5, 6). The DPA concentration in liver averaged 2.42 μmol/g tissue (Table 3) which was approximately 20% lower than that reported upon DPA supplementation in the recent paper by Kaur et al. [8] which likely reflects the lower dose in our study (21.2 vs. 50 mg/day) and differences in animal body weights. We found a moderately higher rise in concentration of DPA in heart as compared to liver as observed by others [8] which may reflect a preferential uptake of DPA by cardiac tissue. In their study, Kaur et al. [8] observed increased concentrations of EPA, in addition to DPA, in liver, heart, and skeletal muscle which they attributed to the process of DPA retroconversion into EPA in vivo as described by others and attributed to involve the peroxisomal acyl-CoA oxidation [24, 25]. The former authors [8] estimated the extent of apparent retroconversion of DPA to EPA as [ΔEPA × 100/Δ(DPA + EPA)] and reported such to be 28% in liver and 4% in heart. Using such an approach from our data (Table 3), our corresponding estimates are generally similar to the aforementioned and amount to 41 and 5%, respectively. The moderately higher estimated percentages from our data may be due in part to the longer (10 day) supplementation regimen in our research as compared to 7 days by the other group [8]. It is particularly interesting that the estimated % conversion of DPA to EPA based on our kidney data (Table 3) is 67% and dramatically higher than that reported for all other tissues by us herein plus the previous work [8]. It is possible that the kidney may have an unique affinity for the uptake of EPA; however, this appears unlikely since it has been observed that the feeding of purified EPA to rats resulted in a lower mean EPA % in the kidney as compared to liver lipids [16]. It is likely that the kidney has a particularly high capacity for the retroconversion of DPA to EPA which accounts for the predominance of EPA over DPA following DPA supplementation in the total lipid, total phospholipid, and major phospholipid fractions (Tables 3, 6, 7).

The significantly lower percentages of fatty acids as ARA in the PL of all tissues with DPA supplementation (Tables 4, 5, 6), and as DHA in heart PL, likely represents competition from DPA and the derived EPA for esterification via acyltransferase activities. The relative cellular availability and preferential utilization of DPA versus other long-chain n-3 and n-6 fatty acids plus its enzymic competition with such for entry into individual phospholipid

types via various de novo biosynthetic, deacylation-reacylation, and transacylation reactions [26, 27] likely contribute to the fatty acid profiles in PtdCho, PtdEtn, PtdIns, and PtdSer following DPA ingestion (Table 7). The particularly high enrichment of heart PtdIns, PtdEtn, and PtdSer with DPA upon supplementation (Table 7) may possibly arise from a preferential utilization of DPA-CoA by the corresponding acyltransferase reactions and/or other reactions in the biosynthetic pathways. The marked differences in such compositions indicate that much heterogeneity exists in the regulation of DPA levels along with other long-chain polyunsaturates (PUFA) between individual phospholipid types and tissues. The extent to which these compositions influence the formation and functioning of bioactive products resulting from DPA such as hydroxylated derivatives via lipoxygenase activities [28] including cellular mediators from ARA, EPA, and DHA [29] upon their enzymic release from these PL reservoirs remains to be studied.

The observed serum lipid-lowering potential of DPA and other physiological effects will need evaluation in human trials as concentrates of DPA (devoid of EPA/DHA) become available in bulk amounts. Our current results strongly suggest a particularly high capacity for the apparent retroconversion of DPA to EPA in the kidney which is worthy of further investigation with respect to mechanisms and potential significance to renal functioning in health and disease.

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