Biochem. J. (1992) 287, 237–240 (Printed in Great Britain)

Phospholipid molecular species composition of mouse liver nuclei

Influence of dietary $n-3$ fatty acid ethyl esters

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The effect of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) ethyl esters on the individual molecular species composition of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was determined in mouse liver nuclei. After a 10 day feeding period, there was a depletion of the *sn*-2 position of $n-6$ polyunsaturated fatty acids (PUFA) and substitution with $n-3$ PUFA. EPA feeding significantly increased ($P < 0.05$) diacyl PC and PE 16:0–20:5,$n-3$, 16:0–22:6,$n-3$, 18:0–20:5,$n-3$ and 18:0–22:6,$n-3$ relative to control (safflower oil ethyl ester fed) animals. In comparison, DHA feeding significantly increased ($P < 0.05$) 22:6 $n-3$-containing species, specifically 18:1–22:6,$n-3$, 16:0–22:6,$n-3$ and 18:0–22:6,$n-3$ in PC, and 18:1–22:6,$n-3$, 16:0–22:6,$n-3$ and 18:0–22:6,$n-3$ in PE. In addition, the presence of 18:0–20:5,$n-3$ PC in the nuclei of DHA-fed rats and of 18:2–20:5,$n-3$, 18:1–20:5,$n-3$ and 18:0–20:5,$n-3$ in nuclear PE indicate the incorporation of DHA retroconversion (22:6,$n-3 \rightarrow$20:5,$n-3$) products. These results indicate both EPA and DHA are extensively incorporated into nuclear phospholipids, and therefore could potentially influence gene function.

INTRODUCTION

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the most abundant lipids in nuclei [1]. Various studies have suggested the involvement of a phospholipid component, primarily phosphatidylinositol, in key aspects of nuclear function [2–8]. Unfortunately, clarification of the role of nuclear phospholipids and their modulation by the diet has not received much attention. Although the fatty acid composition of liver nuclei has been reported [4,5,9–11], only a single study to date has examined the effect of fish oil supplementation on nuclear fatty acid composition [9]. This is significant because the consumption of dietary fish oil, containing $n-3$ polyunsaturated fatty acids (PUFA), may have important preventive and therapeutic medical benefits [12–14].

It is becoming increasingly apparent that the characterization of phospholipids according to specific *sn*-1 and *sn*-2 aliphatic moieties, i.e. molecular species, is required to elucidate enzyme specificities involved in phospholipid metabolism [15,16]. For example, there is evidence that certain phospholipid remodelling enzymes selectively discriminate between substrates based on the *sn*-1 covalent linkage and composition of the *sn*-1 and *sn*-2 moieties [15,17]. To date, however, no attempt has been made to separately determine the specific incorporation of the fish oil-derived eicosapentaenoic acid (EPA; $C_{20:5,n-3}$) and docosahexaenoic acid (DHA; $C_{22:6,n-3}$) into nuclear phospholipid molecular species. Therefore the purpose of the present study was: (i) to characterize the mol % composition of mouse liver nuclear PC and PE molecular species; and (ii) to determine the individual effects of EPA and DHA alimentation on PC and PE composition.

MATERIALS AND METHODS

Materials

Reagents for nuclei preparation and enzyme assays were purchased from Sigma (St. Louis, MO, U.S.A.). All dietary components, except the dietary lipids, were purchased from U.S. Biochemicals (Cleveland, OH, U.S.A.). All solvents were of h.p.l.c. grade.

Animals and diets

Female C57BL/6 mice (Frederick Cancer Research Facility, Frederick, MD, U.S.A.) weighing 15–18 g were randomly divided into three groups of eight mice each. Mice were fed, *ad libitum*, on one of three semi-purified diets that were adequate in all nutrients [15]. The diets varied only in the type of lipid source fed, i.e. either 3% (by wt.) safflower oil ethyl esters (SAF) containing greater than 60% $C_{18:2,n-6}$ (Table 1), or 2% SAF plus either 1% EPA or DHA ethyl ester. The EPA and DHA ethyl esters were greater than 97% pure as determined by gas chromatography [18] (results not shown). Dietary lipids were provided by the National Institutes of Health (Fish Oil Test Material Program, Southeast Fisheries Center, Charleston, SC, U.S.A.). The amounts of the major dietary constituents were (g/100 g): casein, 20.00; DL-methionine, 0.30; sucrose, 44.00; corn starch, 21.98; cellulose, 6.00; AIN[76] mineral mix [18], 3.50; AIN[76] vitamin mix [18], 1.00; choline chloride, 0.20; t-butylhydroquinone, 0.02; fatty acid ethyl ester mixture, 3.00. The composition of the diet with regard to the major dietary fatty acid esters, as determined by gas chromatography [18], is shown in Table 1. The animals were maintained on these diets for 10 days.

Isolation of nuclei

Nuclei from mouse livers were isolated by the method of Nicotera *et al.* [19]. All steps and centrifugations were performed on ice or at 4 °C. Livers from four mice were excised and rinsed in ice-cold 50 mM-Tris/HCl, pH 7.5. The livers were minced with scissors and homogenized with six strokes of a Potter–Elvehjem homogenizer in 6 vol. (w/v) of TKM/0.25 mM-sucrose buffer (0.25 mM-sucrose, 50 mM-Tris/HCl, pH 7.5, 25 mM-KCl, 5 mM-$MgCl_2$) containing leupeptin and pepstatin at a final concen-

Abbreviations used: EPA, eicosapentaenoic acid ($C_{20:5,n-3}$); DHA, docosahexaenoic acid ($C_{22:6,n-3}$); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; SAF, safflower oil ethyl esters.

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Table 1. Fatty acid composition (weight %) of dietary ethyl esters

Only the major ethyl esters (> 1 %) are reported. n.d., not detected (< 0.1 %).

Fatty acid ethyl ester	Dietary group...	Composition (weight %) SAF	EPA	DHA
$C_{16:0}$		14.6	10.5	11.9
$C_{18:0}$		3.6	2.5	2.7
$C_{18:1,n-9}$		16.2	11.2	12.0
$C_{18:2,n-6}$		62.1	55.1	62.0
$C_{20:5,n-3}$		n.d.	16.9	n.d.
$C_{22:6,n-3}$		n.d.	n.d.	8.3

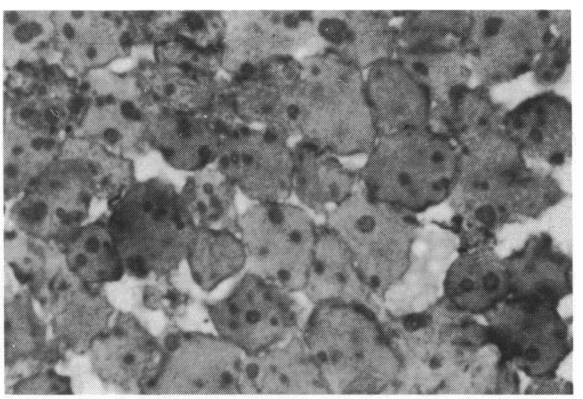

Fig. 1. Electron microscopy of mouse liver nuclear preparations

The diameter of the nucleus is 5–7 μm.

tration of 0.5 μg/ml each. This homogenate was centrifuged at 700 *g* for 10 min, the supernatant was decanted and the pellet was re-homogenized in 6 vol. of fresh TKM/0.25 mM-sucrose buffer with six strokes. The homogenate was centrifuged as above and the supernatant was again decanted. The pellet was resuspended in 2 vol. of TKM/0.25 mM-sucrose, then 1 vol. of the sample was gently mixed with 2 vol. of TKM/2.3 M-sucrose. TKM/2.3 M-sucrose (1 vol.) was placed into a centrifuge tube and 3 vol. of the above sample was layered on top. The tubes were centrifuged at 37000 *g* for 30 min. The supernatant was aspirated and the nuclei pellet was resuspended in TKM buffer and pelleted at 1000 *g* for 5 min. This final nuclei pellet was resuspended in 50 mM-Tris/HCl and divided into portions. For microscopy, fresh samples were analysed; for all other assays, samples were stored at −80 °C until analysis.

Marker enzyme assays

The nuclei samples were analysed for contamination by 5'-nucleotidase (plasma membranes), glucose-6-phosphatase (microsomes) and cytochrome *c* oxidase activities (mitochondria). 5'-Nucleotidase activity, with 5'-AMP as substrate, was assayed by the method of Michell & Hawthorne [20] followed by quantification of phosphate release by the method of Fiske & Subbarrow [21]. Glucose-6-phosphatase was assayed by the method of Swanson [22] using glucose 6-phosphate, sodium salt, as substrate. Phosphate release was subsequently determined by the method of Fiske & Subbarrow [21]. Cytochrome *c* oxidase activity was assessed by the method of Smith [23]. Protein concentration was determined by a modification of the Lowry assay [24].

Microscopy

Pelleted nuclei were fixed in 3% glutaraldehyde/2% paraformaldehyde in 0.1 M-cacodylate buffer for 2 h, post-fixed with 1% OsO_4 followed by dehydration and embedding in Epon–Araldite. For light-microscopy analysis, 1 μm sections were cut and stained with Toluidine Blue and photographed with a Zeiss Photomicroscope III (Carl Zeiss, Inc., Thornwood, NY, U.S.A.) and photographed on Kodak Technical pan film. For electron microscopy, ultrathin sections were stained with uranyl acetate and lead and examined with a Zeiss 10C transmission electron microscope.

Lipid analysis

The isolation and characterization of diacylglycerobenzoates derived from PC and PE were performed as previously described [15,16].

Statistical analysis

Data were analysed by one-way analysis of variance, followed by inspection of all differences between means by using the least-significant-difference test.

RESULTS AND DISCUSSION

To date, no attempt has been made to characterize the *sn*-1,2-diacyl molecular species of nuclei phospholipids. Therefore, to determine the *sn*-1 and *sn*-2 fatty acid moieties of the predominant nuclear phospholipid classes (PC and PE) [1], isolated intact nuclei from mouse liver were depleted of any microsomal, mitochondrial or plasma membrane contaminants. This was confirmed by measuring the activity of various marker enzymes (Table 2). In addition, the purity of nuclear preparations was corroborated using light and electron microscopic analysis (Fig. 1).

Isolated diradylglycerobenzoates revealed only the presence of diacyl species in nuclear PC and PE (results not shown). The

Table 2. Enzyme activities in liver nuclei relative to concentrations in liver homogenates

Values are means from duplicate analyses performed on four pooled livers.

	5'-Nucleotidase		Glucose-6-phosphatase		Cytochrome *c* oxidase	
	Homogenate	Nuclei	Homogenate	Nuclei	Homogenate	Nuclei
Specific activity	210.4	8.7*	467.2†	16.7†	32.1†	0.6†
Relative specific activity (%)	100	4.1	100	3.6	100	1.9

* pmol/min per mg of protein.
† nmol/min per mg of protein.

Table 3. Molecular species composition of PC

The 1,2-diacylglycerobenzoates were prepared and subsequently chromatographed by isocratic reverse-phase h.p.l.c. as described in the Materials and methods section. Peaks were detected at 230 nm. Molecular species were chromatographed using acetonitrile/propan-2-ol (7:3, v/v). Values are means ± S.E.M. from pooled livers ($n = 4$). Peak no. corresponds to the h.p.l.c. elution profile of specific phospholipid-derived diacylglycerobenzoates. Row values with the same or no superscripts are not significantly different ($P > 0.05$); tr, trace amounts (< 0.1 %)

Peak no.	Molecular species	Dietary group...	Composition (mol %) SAF	EPA	DHA
1	18:2–20:5,*n*−3		tr	tr	0.3±0.2
2	18:2–20:4,*n*−6		2.4±0.9^{a}	0.8±0.1^{b}	0.2±0.0^{c}
3	18:1–20:5,*n*−3		tr^{b}	tr^{b}	0.2±0.0^{a}
4	18:1–22:6,*n*−3		tr^{b}	tr^{b}	5.5±0.2^{a}
5	16:0–20:5,*n*−3		tr^{b}	11.2±0.2^{a}	tr^{b}
6	18:2–18:2,*n*−6		6.2±0.2^{c}		
	16:0–22:6,*n*−3; 18:2–18:2,*n*−6			16.1±0.1^{b}	25.0±0.9^{a}
7	18:1–20:4,*n*−6		4.6±0.2^{a}	2.8±0.2^{b}	1.0±0.1^{c}
8	16:0–20:4,*n*−6		11.2±0.6^{a}	5.0±0.0^{b}	3.9±1.3^{b}
9	16:0–22:5,*n*−6		0.8±0.3^{a}	tr^{b}	tr^{b}
10	18:0–20:5,*n*−3		tr^{c}	4.3±0.3^{a}	1.3±0.2^{b}
11	18:0–22:6,*n*−3; 18:1–18:2,*n*−6		4.4±0.2^{c}	7.4±0.4^{b}	9.3±0.3^{a}
12	16:0–18:2,*n*−6; 16:1–18:1,*n*−9		13.5±0.4^{b}	14.1±0.4^{b}	17.4±0.3^{a}
13	18:0–22:5,*n*−3		tr^{b}	0.8±0.0^{a}	tr^{b}
14	18:0–20:4,*n*−6		8.7±0.4^{a}	3.7±0.1^{b}	3.1±0.2^{b}
15	18:1–18:1,*n*−9		tr^{b}	0.7±0.4^{ab}	1.0±0.1^{a}
16	16:0–18:1,*n*−9		24.2±0.6^{b}	23.4±0.6^{b}	26.8±0.6^{a}
17	18:0–18:2,*n*−6; 16:0–16:0		2.4±0.4^{b}	3.3±0.3^{a}	1.5±0.2^{b}
18	18:0–18:1,*n*−9		9.2±0.7^{a}	5.0±1.0^{b}	2.9±0.1^{b}
19	16:0–18:0		0.2±0.1	0.5±0.3	0.3±0.0
20	18:0–18:0		10.2±1.1^{a}	1.0±0.1^{b}	0.4±0.2^{b}

Table 4. Molecular species composition of PE

For further details, see the legend to Table 3.

Peak no.	Molecular species	Dietary group...	Composition (mol %) SAF	EPA	DHA
1	18:2–20:5,*n*−3		tr^{b}	tr^{b}	0.2±0.0^{a}
2	18:2–20:4,*n*−6		1.3±0.2^{a}	0.1±0.0^{b}	0.2±0.0^{b}
3	18:1–20:5,*n*−3		tr^{c}	2.4±0.3^{a}	0.8±0.1^{b}
4	18:1–22:6,*n*−3		2.7±0.2^{b}	tr^{c}	8.9±0.3^{a}
5	16:0–20:5,*n*−3		tr^{b}	10.3±0.6^{a}	tr^{b}
6	18:2–18:2,*n*−6		13.8±0.7		
	16:0–22:6,*n*−3; 18:2–18:2,*n*−6			31.6±0.2^{b}	38.8±1.0^{a}
7	18:1–20:4,*n*−6		3.2±1.2	2.6±1.5	3.4±0.6
8	16:0–20:4,*n*−6		12.4±0.8^{a}	4.8±0.4^{b}	5.1±0.7^{b}
9	16:0–22:5,*n*−6		2.8±0.3^{a}	tr^{b}	tr^{b}
10	18:0–20:5,*n*−3		tr^{c}	7.5±0.5^{a}	3.0±0.6^{b}
11	18:0–22:6,*n*−3; 18:1–18:2,*n*−6		6.9±0.2^{c}	12.5±0.8^{b}	16.0±0.5^{a}
12	16:0–18:2,*n*−6; 16:1–18:1,*n*−9		4.6±0.3	4.4±1.0	3.7±0.2
13	18:0–22:5,*n*−3		tr^{b}	2.0±0.1^{a}	0.3±0.1^{b}
14	18:0–20:4,*n*−6		21.3±1.6^{a}	9.9±0.1^{b}	10.1±0.4^{b}
15	18:0–22:5,*n*−6		1.3±0.2^{a}	tr^{b}	tr^{b}
16	18:1–18:1,*n*−9		0.4±0.0	0.3±0.2	0.6±0.2
17	16:0–18:1,*n*−9		8.3±0.4^{a}	6.1±0.4^{b}	6.0±0.4^{b}
18	18:0–18:2,*n*−6; 16:0–16:0		tr	tr	0.2±0.2
19	18:0–20:3,*n*−6		tr	0.3±0.2	0.1±0.0
20	18:0–22:4,*n*−6		tr^{b}	tr^{b}	0.2±0.0^{a}
21	18:0–18:1,*n*−9		5.9±0.4^{a}	2.6±0.5^{b}	1.2±0.1^{b}
22	16:0–18:0		1.1±0.2^{a}	tr^{b}	0.3±0.0^{b}
23	18:0–18:0		6.7±0.4^{a}	tr^{b}	0.2±0.1^{b}

effect of dietary ethyl ester supplementation on the molecular species composition of the predominant phospholipid classes (PC and PE) in nuclei is shown in Tables 3 and 4. In SAF nuclei, the major species in PC was 16:0–18:1,*n*−9 (24.2 %); for PE it was 18:0–20:4,*n*−6 (21.3 %). Substantial differences in molecular species profiles between SAF, EPA and DHA ethyl ester fed animals were noted. In general, there was depletion at the *sn*-2 position of *n*−6 PUFA and substitution with *n*−3 PUFA. Specifically, 18:2–20:4,*n*−6, 18:1–20:4,*n*−6, 16:0–20:4,*n*−6 and 18:0–20:4,*n*−6 in PC, and 18:2–20:4,*n*−6, 16:0–20:4,*n*−6

and 18:0–20:4, $n-6$ in PE, were significantly decreased ($P < 0.05$) relative to SAF-fed animals. EPA feeding significantly increased ($P < 0.05$) 16:0–20:5,$n-3$, 16:0–22:6,$n-3$, 18:0–20:5,$n-3$ and 18:0–22:6,$n-3$ in PC and PE relative to SAF feeding. The enhancement of nuclear 22:6,$n-3$-containing species is consistent with the bioconversion of 20:5,$n-3$. This synthetic sequence involves four steps: 20:5,$n-3\rightarrow$ 22:5,$n-3\rightarrow$24:5,$n-3\rightarrow$24:6,$n-3\rightarrow$22:6,$n-3$ [25]. In comparison, DHA supplementation significantly increased ($P < 0.05$) 22:6,$n-3$-containing species: 18:1–22:6,$n-3$, 16:0–22:6,$n-3$ and 18:0–22:6,$n-3$ in PC, and 18:1–22:6,$n-3$, 16:0–22:6,$n-3$ *and* 18:0–22:6,$n-3$ in PE. Interestingly, low levels of 20:5,$n-3$-containing species were present in nuclei of DHA-fed rats. The increase in 20:5,$n-3$ species relative to SAF feeding is consistent with the retroconversion of DHA to EPA *in vivo*. Previous studies have shown that dietary DHA can be retroconverted to EPA in both animals and humans [26,27]. The retroconversion process requires a single cycle of peroxisomal β-oxidation with 4-enoyl-CoA reductase and 3,2-enoyl-CoA isomerase as auxiliary enzymes [26,27].

The presence of phospholipid inside the nucleus is important in view of its ability to regulate gene function [2], nucleosome structure [7], RNA synthesis [7] and activation of DNA polymerase alpha [8]. Nuclear lipids may produce an intranuclear signalling system affecting protein kinase C activity which could phosphorylate specific nuclear proteins [8]. Interestingly, diet-induced differences in the fatty acid composition of the nuclear envelope and intranuclear phospholipids have been linked to alterations in nuclear membrane lipid fluidity and nuclear RNA release [3–5]. In addition, it has been recently reported that the composition of PUFA in nuclear phospholipids may modulate cell profileration in mammalian cells [6].

In conclusion, the present data demonstrate that the molecular species composition of nuclear PC and PE can incorporate and retain $n-3$ PUFA at the expense of *sn*-2 18:2,$n-6$ and 20:4,$n-6$. The extensive modification of nuclear phospholipid molecular species may have implications for lipid-mediated signal transduction in the cell nucleus and, therefore, the regulation of gene functions. Further studies are necessary to elucidate the effect of dietary $n-3$ PUFA on nuclear enzyme activity and intranuclear signal transduction.

We thank Dr. Robert Burghardt of the College of Veterinary Medicine Image Analysis Laboratory for performing the microscopy analysis, and Ms. Kim Coble for excellent technical assistance. This project was supported by NIH grant DK 41693 and the USDA Cooperative State Research Service, Project No. H-6983.

Received 8 January 1992/30 March 1992; accepted 13 April 1992

REFERENCES

1. Mato, J. M. (1990) in Phospholipid Metabolism in Cellular Signaling, pp. 9–18, CRC Press, Boca Raton, FL
2. Manzoli, F. A., Martelli, A. M., Capitani, S., Maraldi, N. M., Rizzoli, R., Barnabei, O. & Cocco, L. (1989) Adv. Enzyme Regul. **28**, 25–34
3. Herlan, G., Giese, G. & Wunderlick, F. (1979) Exp. Cell Res. **118**, 305–309
4. Venkatraman, J. T. & Clandinin, M. T. (1988) Biochim. Biophys. Acta **940**, 33–42
5. Venkatraman, J. T., Lefebvre, Y. A. & Clandinin, M. T. (1986) Biochem. Biophys. Res. Commun. **135**, 655–661
6. Ishihara, H., Tamiya-Koizumi, K., Kuriki, H., Yoshida, S. & Kojima, K. (1991) Biochim. Biophys. Acta **1084**, 53–59
7. Cocco, L., Gilmour, R. S., Ognibene, A., Letcher, A. J., Manzoli, F. A. & Irvine, R. F. (1987) Biochem. J. **248**, 765–770
8. Sylvia, V. L., Joe, C. O., Norman, J. O., Curtin, G. M. & Busbee, D. L. (1986) Biochem. Biophys. Res. Commun. **135**, 880–885
9. Iritani, N., Fukuda, H. & Matsumura, Y. (1988) Biochim. Biophys. Acta **963**, 224–230
10. Upreti, G., deAntueno, R. J. & Wood, R. (1983) J. Natl. Cancer Inst. **70**, 559–566
11. Upretti, G. C. & deAntueno, R. J. & Wood, R. (1983) J. Natl. Cancer Inst. **70**, 567–573
12. Cane, T. C. (1991) FASEB J. **5**, 2160–2166
13. Voss, A., Reinhart, M., Sankarappa, S. & Sprecher, H. (1991) J. Biol. Chem. **266**, 19995–20000
14. Fernandes, G., Venkatraman, J., Khare, A., Horbeck, G. & Friedrichs, W. (1990) Proc. Soc. Exp. Biol. Med. **193**, 16–22
15. Chapkin, R. S., Akoh, C. C. & Miller, C. C. (1991) J. Lipid Res. **32**, 1205–1213
16. Akoh, C. C. & Chapkin, R. S. (1990) Lipids **25**, 613–617
17. Holub, B. J. & Kuksis, A. (1978) Adv. Lipid Res. **16**, 1–125
18. Chapkin, R. S. & Carmichael, S. L. (1990) J. Nutr. **120**, 825–830
19. Nicotera, P., McConkey, D. J., Jones, D. P. & Orennius, S. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 453–457
20. Michell, R. H. & Hawthorne, J. N. (1965) Biochem. Biophys. Res. Commun. **21**, 333–338
21. Fiske, C. H. & Subbarrow, Y. (1925) J. Biol. Chem. **66**, 375–400
22. Swanson, M. A. (1955) Methods Enzymol. **2**, 541–543
23. Smith, L. (1953) Methods Biochem. Anal. **2**, 427–434
24. Peterson, G. L. (1977) Anal. Biochem. **83**, 346–356
25. Kaizer, L., Boyd, N. F., Kriukov, V. & Tritchler, D. (1989) Nutr. Cancer **12**, 61–68
26. Fischer, S., Vischer, A., Preac-Mursic, V. & Weber, P. C. (1987) Prostaglandins **34**, 367–375
27. Gronn, M., Christensen, E., Hagve, T. & Christophersen, B. (1991) Biochim. Biophys. Acta **1081**, 85–91