The Formation of Diradylglycerol Molecular Species in Murine Peritoneal Macrophages Varies Dose-Dependently With Dietary Purified Eicosapentaenoic and Docosahexaenoic Ethyl Esters\textsuperscript{1,2}

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ABSTRACT  Substantial effects of dietary fish oil–derived fatty acid ethyl esters on the metabolism of diradylglycerol (DG) have recently been described. We undertook to isolate the separate effects of (n-3) eicosapentaenoic acid (EPA) and (n-3) docosahexaenoic acid (DHA) on DG metabolism. For 3 wk, male C57BL/6 mice were fed one of six purified diets in which the lipid component was either 3 g/100 g corn oil ethyl ester (COEE) (control diet) or 1 g/100 g COEE plus 2 g/100 g of EPA ethyl ester (EPEE), DHA ethyl ester (DHEE), or an EPEE:DHEE mixture. Peritoneal macrophages were analyzed for DG content and for molecular species distributions of DG and phospholipid classes. We found that the degree of incorporation of EPA and DHA into DG in macrophages was dependent on the dietary concentration of EPEE and DHEE, under basal conditions and after stimulation with platelet-activating factor, phorbol myristate acetate and lonomycin. Incorporation of EPA and DHA into phospholipids was also significant and dose dependent in each phospholipid class. For both DG and phospholipid molecular species, the incorporation of EPA in the sn-2 position was considerably greater than that of species with DHA under conditions of equimolar dietary content. These results demonstrate that 1) incorporation of EPA and DHA into DG are independent and dependent on dietary content, 2) EPA is incorporated with greater affinity than DHA and 3) these effects on DG metabolism seem to result from corresponding effects on parent membrane phospholipids. Physiologically and therapeutically relevant differences may exist between EPA and DHA. J. Nutr. 126: 2738–2745, 1996.

INDEXING KEY WORDS:
• diradylglycerol molecular species  
• mice  
• peritoneal macrophages  
• eicosapentaenoic ethyl ester  
• docosahexaenoic ethyl ester

In the present study, experimental diets with purified eicosapentaenoic ethyl ester (EPEE),\textsuperscript{4} docosahexaenoic ethyl ester (DHEE) or mixtures thereof as their lipid component were used to determine whether there exists a difference between (n-3) eicosapentaenoic acid (EPA) and (n-3) docosahexaenoic acid (DHA) in the changes in diradylglycerol (DG) metabolism that result from their incorporation into membrane phospholipids in murine peritoneal macrophages. We also investigated whether incorporation of EPA or DHA into DG is dose dependent and whether the extent of incorporation of individual (n-3) polyunsaturated fatty acids (PUFA) into membrane phospholipids, from which DG is derived, differs between EPA and DHA. Finally, this study determined whether replacement of resident fatty acids in the sn-2 position of membrane phospholipids by EPA or DHA causes different changes in total mass of DG.

\textsuperscript{1} Supported by grants from the Arthritis Society of Canada. RJS is the recipient of a Career Award from the Pharmaceutical Manufacturers’ Association of Canada-Health Research Foundation and the Medical Research Council of Canada. PAM is the recipient of a Graduate Studentship from the Pharmaceutical Manufacturers’ Association of Canada-Health Research Foundation and the Medical Research Council of Canada.

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\textsuperscript{4} Abbreviations used: AA, arachidonic acid; ACS, aqueous counting scintillant; COEE, corn oil ethyl ester concentrate; DAG kinase, sn-1,2-diacylglycerol kinase; dPCS, heat-deactivated fetal calf serum; DG, diradylglycerol; DMPA, dimethylphosphatidic acid; DHA, (n-3) docosahexaenoic acid; DHEE, (n-3) docosahexaenoic ethyl ester; EPA, (n-3) eicosapentaenoic acid; EPEE, (n-3) eicosapentaenoic ethyl ester; HHBSS, Hanks’ balanced salt solution containing 10 mmol/L HEPES, PA, phosphatidic acid; PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PMA, phorbol myristate acetate; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid.

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Recent in vivo findings showed that when mice are fed diets that contain 10 g/100 g of either corn oil ethyl ester or mixed [n-3] PUFA ethyl ester, the latter group shows substantial enrichment of DG molecular species with five and six double bonds, reflecting EPA and DHA incorporation at the sn-2 position in peritoneal macrophage phospholipids [Marignani and Sebaldt 1995 and 1996a]. Earlier in vitro studies [Sebaldt and Marignani 1995] showed that the [n-3] PUFA EPA [20:5(n-3)] and DHA [22:6(n-3)], when added to macrophage culture media, alter the formation of DG molecular species to substantially differing extents under equimolar conditions. Those results indicated that [n-3] PUFA, in addition to altering eicosanoid biosynthesis [Croft et al. 1987, DeCaterina et al. 1995, Hubbard et al. 1994, Whelan et al. 1991], also have the potential to significantly modify a critical second messenger of intracellular signal transduction. This may account, at least in part, for some of the less well-understood actions of [n-3] PUFA [Chan et al. 1993, Huang et al. 1986, Hubbard et al. 1994, Pascale et al. 1993].

MATERIALS AND METHODS

Materials. sn-1,2-Diacylglycerol kinase [DAG kinase], ionomycin and phorbol myristate acetate [PMA] were obtained from Calbiochem [La Jolla, CA]. Diethylamine pentaacetic acid [DEPAC], imidazole, ATP, D,L-dithiothreitol [DTT] and phospholipase C [no. 7147] were from Sigma Chemical [St. Louis, MO]. Diazold was from Aldrich Chemical [Milwaukee, WI], and RPMI 1640 media without l-glutamine, heat-deactivated fetal calf serum [dFCS], penicillin, streptomycin and Hanks’ balanced salt solution (containing calcium and magnesium) were from Gibco [Grand Island, NY]. l-Glutamine was from Fisher [Unionville, ON, Canada]. Culture dishes were from Corning [Corning, NY] and Falcon [Franklin Lakes, NJ]. [γ-32P]ATP and [32P]-H3PO4 were from DuPont NEN [Markham, ON, Canada]. 1-O-Alkyl-2-acetyl-sn-glycero-3-phosphocholine [PAF] and dioleoylglycerol standards were from Avanti Polar Lipids [Alabaster, AL]. Aqueous counting scintillant [ACS] was from Amersham [Oakville, ON, Canada].

Animal care and diets. Specific pathogen-free 3- to 4-week-old male C57BL/6 mice [Charles River Laboratory, Montreal, PQ] were acclimated for 24 h in microisolator cages with access to standard nonpurified diet and water. Mice were randomly allocated to cages [4/5-cage], individually weighed and monitored for growth weekly. Mice were fed purified diets for 3 wk. Diets were prepared by reconstituting basal mix [TD89047, Harlan Teklad Diets, Madison, WI] with lipid. The composition of basal mix was such that addition of 3 g of lipid to 97 g of basal mix reconstituted a complete purified diet that contained 21.5% casein, 0.32% DL-methionine, 48.4% sucrose, 16.1% cornstarch, 5.4% cellulose, 3.8% mineral mix [AIN-76], 1.1% vitamin mix [AIN-76A], and 0.22% choline bitartrate. Corn oil ethyl ester concentrate [COEE], EPEE and DHEE were graciously supplied by the NIH/NOAA Fish Oil Test Materials Program [Charleston, SC]. For the control diet [Diet A], basal mix was reconstituted with 3 g COEE per 100 g diet. For the experimental diets [Diets B–F], basal mix was reconstituted with 2 g [n-3] PUFA ethyl ester and 1 g COEE/100 g diet, where the former was as follows—Diet B, 0 g EPEE and 2 g DHEE; Diet C, 0.5 g EPEE and 1.5 g DHEE; Diet D, 1 g EPEE and 1 g DHEE; Diet E, 1.5 g EPEE and 0.5 g DHEE; Diet F, 2 g EPEE and 0 g DHEE. Table 1 summarizes the lipid composition [3 g/100 g diet] of each diet. The COEE contained predominantly 20:5n-3 [905 mg/g] and 22:6n-3 [2.3 mg/g]. The DHEE contained principally 22:6n-3 [935 mg/g] and 20:4n-3 [7.6 mg/g]. All experimental diets contained COEE as a source of essential fatty acids and all-rac-α,-tocopheryl acetate [150 mg/kg] to prevent the increased susceptibility of the [n-3] PUFA to oxidation due to the increased degree of fatty acid unsaturation [Marignani and Sebaldt 1995]. Diets and feeding jars were changed daily. All animal procedures were approved by the McMaster University Animal Research Ethics Board.

Macrophage stimulation. After receiving purified diets for 3 wk, mice were injected intraperitoneally with 1 mL of brewer’s thioglycollate [DIFCO, Detroit, MI]. After 3 d, elicited macrophages were harvested by peritoneal lavage [80–90% macrophages], plated and enriched for adherent cells by incubation for 2 h (>98% macrophages) as described [Adams 1979]. The macrophage monolayers were washed three times with Hanks’ balanced salt solution containing 10 mmol/L HEPES, pH 7.4 [HBBSS]. The washed macrophage monolayers [15 × 10⁶ per 16 cm² well and 2 × 10⁶ per 9.62 cm² well] were cultured for an additional 16 h in RPMI 1640 containing 2 mmol/L l-glutamine, 1.25 ×
10^5 U/L penicillin and 6 mg/L streptomycin at 37°C in a 5% CO_2/100% H_2O atmosphere (Marignani and Sebaldt 1996a). To visualize the location of individual phospholipids on the two-dimensional TLC plates, 37 MBq/L of \[^{32}P\]H_3PO_4 was added to those wells destined for phospholipid analyses. After incubation, the macrophage monolayers were washed three times with HHBSS, left in 2.0 mL of HHBSS and placed in a water bath at 37°C. Macrophages were stimulated for 10 min by the addition of 400 µL of one of the following [final concentrations shown]: PAF (0.1 µmol/L), ionomycin (10 µmol/L), PMA (0.1 mg/L) or HHBSS [unstimulated control], as described (Marignani and Sebaldt 1995). All conditions were repeated in duplicate or triplicate wells for each experiment.

**Total diradylglycerol mass.** After termination of stimulation by the addition of 2 mL of methanol, wells with 2 x 10^6 macrophages were scraped into 13 x 75 mm screw-top glass tubes, and DG was extracted into chloroform using methanol and 1.0 mol/L NaCl in the aqueous phase by the Bligh and Dyer (1959) procedure as described (Sebaldt et al. 1992). Diradylglycerol was derivatized to phosphatidic acid (PA) in the presence of DAG kinase and 1.85 GBq/L \[^{32}P\]ATP, separated from ceramides by TLC and quantified as previously described (Marignani and Sebaldt 1995).

**Determination of diradylglycerol molecular species.** After termination of stimulation by the addition of 2 mL of methanol, macrophages were scraped and DG was extracted and derivatized to \[^{32}P\]PA as described above except that \[^{32}P\]ATP was used at 11.1 GBq/L. After TLC, \[^{32}P\]PA spots were scraped into 13 x 75 mm screw-top glass tubes, extracted from the silica and further derivatized to \[^{32}P\]dimethylphosphatidic acid (DMPA) and fractionated by argentation TLC, into bands corresponding to the total double bond content (0–6) in the two fatty acid residues as previously described (Marignani and Sebaldt 1995).

**Determination of phospholipid molecular species.** Macrophages were washed three times with HHBSS to remove excess \[^{32}P\]H_3PO_4. Phospholipids were extracted into chloroform, using methanol and 0.1 mol/L HCl in the aqueous phase, by the Bligh and Dyer (1959) method as described (Sebaldt et al. 1992). Phospholipid classes phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS) and phosphatidylethanolamine (PE) were separated by two-dimensional TLC on 5% Mg acetate–treated, heat-activated Silica Gel HLF plates (Analtech, Newark, DE) and extracted, converted successively to DG, \[^{32}P\]PA and DMPA, and analyzed as described (Marignani and Sebaldt 1995).

**Nitrogen environment.** The oxidation of PUFA was minimized by the extensive use of nitrogen throughout the preparation of the diets and throughout sample preparation and analyses. The ethyl esters were infused with nitrogen and sealed under a blanket of nitrogen and stored at -70°C. Peroxide value determinations were periodically performed on all lipid ethyl esters to monitor any undesirable increases in peroxides (American Oil Chemists Society 1973). Solvents were bubbled with nitrogen immediately prior to use. Sample tubes were sealed with Parafilm under nitrogen prior to centrifuging or vortexing. For overnight storage, all samples were dried under a stream of nitrogen, sealed with Parafilm and kept at -20°C.

**Statistical analyses.** Results are expressed as means ± SEM. For results shown in Figure 3, differences between diets were considered statistically significant when P values were less than 0.05 by Student's two-tailed t test calculated using Fig.P version 6.0c software (Biosoft, Cambridge, U.K.). For results shown in Figures 1 and 2, the relationship between dose (dietary EPEE or DHEE) and mol/100 mol product (proportion in total sample of corresponding molecular species fraction) was examined by both linear and quadratic regression analyses for each molecular species fraction. These were performed using Fig.P version 6.0c. To determine whether quadratic regression analyses significantly improved the goodness of fit over linear regression, F values for linear and quadratic regressions were calculated as shown below (Kelman and Whiting 1980) and were compared at P = 0.05.

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\frac{(SS_{\text{linear}} - SS_{\text{quadratic}})}{(df_{\text{linear}} - df_{\text{quadratic}})} = SS_{\text{quadratic}} / df_{\text{quadratic}}
\]

We found that quadratic regression analyses significantly improved the goodness of fit over that obtained with linear regression for the dose-response curves of molecular species fractions with five double bonds, but not for those with four or six double bonds.

**RESULTS**

**Animal growth.** No difference in weight was found between diet groups on day 0, when mice weighed approximately 12 g. Overall, mice gained approximately 10 g after 3 wk of consuming experimental diets. Weight gain did not differ significantly among diet groups.

**Diradylglycerol molecular species.** The results shown in Figure 1 are the first demonstration that a direct dose-response relationship exists between the dietary concentration of EPEE or DHEE (ranging from 0 to 2 g/100 g diet) and the proportion of DG molecular species with five or six double bonds, respectively (Fig. 1, Diets B–F). This relationship was confirmed by regression analyses. Goodness of fit by linear regression analyses for the proportions of molecular species with four and six double bonds was not significantly improved by quadratic regression, whereas quadratic regression analyses provided a significantly better fit (by F test, as described in Materials and Methods) for the proportions of molecular species with five double bonds.
bonds. Regression analyses were performed for the results under basal and all stimulation conditions. The shapes of the dose-response curves (Fig. 1, a–d) were similar among (a) basal DG and DG formed after (b) PAF, (c) PMA or (d) ionomycin stimulation. Therefore, only the results for basal DG (Fig. 1a) will be described in further detail.

The proportion of basal DG molecular species with four double bonds (Fig. 1a, left) was 38 mol/100 mol in macrophages from mice fed the control diet (Diet A) and 19–20 mol/100 mol in macrophages from mice fed the experimental diets (Diets B–F). However, among the experimental diets, there was no relationship between diet and the proportion of basal DG with four double bonds. The incorporation of EPA (five double bonds) was 3 mol/100 mol in macrophages from mice fed the control diet (A) and 12–30 mol/100 mol in macrophages from mice fed the experimental diets (B–F) (Fig. 1a, middle), whereas incorporation of DHA (six double bonds) was 4 mol/100 mol in macrophages from mice fed the control diet (A) and 15 mol/100 mol in macrophages from mice fed the experimental diets (F–B) (Fig. 1a, right). The proportion of DG with five and six double bonds clearly varied directly with EPA and DHA concentrations in the diet, respectively, as described above. In all cases, the regression coefficients were significantly different from zero (P < 0.05).

**Phospholipid molecular species.** The results shown in Figure 2 demonstrate a dose-response relationship between the dietary concentration of EPEE or DHEE (ranging from 0 to 2 g/100 g diet) and the proportion of phospholipid molecular species with five or six double bonds, respectively, in macrophages from mice fed experimental diets (Fig. 2, Diets B–F). This relationship was confirmed by both linear and quadratic regression analyses and is similar to the results for DG. Goodness of fit by linear regression analyses for the proportions of molecular species with four and six double bonds was not significantly improved by quadratic regression, whereas quadratic regression analyses provided a significantly better fit (by the F test, see Materials and Methods) for the proportion of molecular species with five double bonds. Regression analyses were performed for all phospholipid classes. Even though the proportions of phospholipid molecular species differed among classes, the shapes of the dose-dependency curves were similar (Fig. 2 a–d). Therefore, only the results for PC will be described in further detail.

The proportion of PC molecular species with four double bonds (Fig. 2a, left) was 29 mol/100 mol in macrophages from mice fed the control diet (Diet A) and

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**FIGURE 1** Diradylglycerol (DG) molecular species analyses from basal and stimulated peritoneal macrophages from mice fed purified diets. Macrophages were prepared and left unstimulated or stimulated for 10 min with 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) [0.1 μmol/L], phorbol myristate acetate (PMA, 0.1 mg/L) or ionomycin (10 μmol/L). Results for basal and stimulated DG are shown for molecular species fractions with four (left), five (middle) and six (right) double bonds and are expressed as mol/100 mol of total diradylglycerol (means ± SEM, n = 4). Control (A) and experimental (B–F) diets are defined in Table 1. Correlation coefficients are shown for quadratic regressions for fraction five and for linear regressions for fractions four and six.
**FIGURE 2** Phospholipid (PL) molecular species analyses from peritoneal macrophages from mice fed purified diets. Macrophage preparation and PL separation and analyses are described in Materials and Methods. Results are shown for molecular species fractions with four (left), five (middle) and six (right) double bonds and are expressed as mol/100 mol of total phospholipid (means ± SEM, n = 4). Control (A) and experimental (B–F) diets are defined in Table 1. Correlation coefficients are shown for quadratic regressions for fraction five and for linear regressions for fractions four and six.
FIGURE 3 Total basal diradylglycerol (DG) mass in unstimulated peritoneal macrophages from mice fed purified diets. Results are expressed as pmol/10⁶ macrophages [means ± SEM, n = 6]. A: Comparison of effects of [n-3] eicosapentaenoic ethyl ester (EPEE), [n-3] docosahexaenoic ethyl ester (DHEE) and corn oil ethyl ester concentrate (COEE) on total DG mass. Total dietary lipid concentration was held constant at 3 g/100 g, including at least 1 g/100 g COEE. The remaining 2 g/100 g lipid was COEE [A], EPEE [F or DHEE [B]]. B: Dose-effect relationship between EPEE and DHEE and total DG mass. Total dietary lipid concentration was held constant at 3 g/100 g, including 1 g/100 g COEE. The remaining 2 g/100 g lipid was [n-3] polyunsaturated fatty acid [PUFA] ethyl ester [EPEE and DHEE], and the EPEE:DHEE varied from 0.5:1.5 [C] to 1:1 [D] to 1:5:0.5 [E]. Differences were considered statistically significant when P values were less than 0.05. The DG mass in diet groups A and E is significantly different from others (*P < 0.05). The DG mass in diet group F is significantly different from others (**P < 0.05).

evidence that fatty acids with five double bonds are retained with greater affinity than those with six in macrophage membrane PC, PS, PE and PI. Presumably it is as a result of this that the proportion of DG formed with five double bonds at the sn-2 position is correspondingly greater than that with six when equimolar amounts of EPA and DHA ethyl esters are present in the diet. We have shown this to be true for both basal and stimulated DG. We have also analyzed our DG and phospholipid samples (unpublished data, not shown) by a reverse-phase HPLC method described previously (Seballdt et al. 1992), which resolves DG species in a different pattern than does argentation TLC. These analyses show that the increases in fatty acids with five and six double bonds correspond to peaks coeluting with the diacyl subclass of sn-1-palmitoyl and sn-1-stearoyl DG with sn-2-EPA and sn-2-DHA, respectively. These results support the view that the physiological effects of EPA and DHA are potentially distinct.

The magnitude of the differences between EPA and DHA warrants further investigation.

Overall, the molecular species profiles of DG with four, five and six double bonds under basal as well as PAF-, PMA- and ionomycin-stimulated conditions were similar. This may suggest that, regardless of the stimulant introduced to macrophages in culture, the accessibility of phospholipid-incorporated PUFA as a group to hydrolyzing phospholipases remains tightly regulated (Galli et al. 1993, Lokesh and Kinsella 1994, Walsh et al. 1994).

Diradylglycerol molecular species formed in the various experimental diet groups were significantly different from one another and from those in the control diet groups, indicating that even a modest change in the PUFA component in the diet has a significant impact on DG composition. Common to the experimental diets was 1 g/100 g COEE as a source of essential fatty acid. Interestingly, molecular species with four double bonds remained stable regardless of the composition [EPA vs. DHA] of the [n-3] PUFA component in the diet (Fig. 1), whereas the proportions of DG molecular species fractions five [containing EPA] and six [containing DHA] reflected the dietary lipid composition. Specifically, the mol/100 mol of fraction five changed as much as 10 mol/100 mol for each dietary increment change of 0.5 g EPEE per 100 g diet, compared with a 2 to 5 mol/100 mol change in the proportion of fraction six for each dietary increment of 0.5 g DHEE per 100 g diet. These results likely reflect corresponding changes in membrane phospholipids (see below) but may also suggest that phospholipase activity towards phospholipid-incorporated EPA is greater than that for incorporated DHA. The DHA moiety may impose steric hindrance that does not permit access by and subsequent action of phospholipases. Because these differences between EPA and DHA are also observed in vitro (Seballdt and Marignani 1995), they are unlikely to reflect differences in their intestinal absorption.
Molecular species in each phospholipid class (Fig. 2) parallel the DG molecular species findings (Fig. 1) in that the degree of EPA incorporation into membrane phospholipids was greater than that of DHA incorporation. This was true for all phospholipid classes analyzed, suggesting that the affinity for incorporation into membrane phospholipids of murine peritoneal macrophages is greater towards EPA than DHA. These results are consistent with our earlier studies (Marignani and Sebaldt 1995) in which we reported that the proportion of phospholipid molecular species with five double bonds was disproportionately greater than that with six, given the ratio of species present in the diet in those studies.

Quantification of DG revealed that the decrease in basal total DG mass paralleled the decrease in the DHEE component and the increase in the EPEE component of the diet (Fig. 3B). Diet B (DHEE without EPEE) reduced DG mass to 45% of control, whereas Diet F (EPEE without DHEE) reduced DG mass to 33% of control, and the reductions are significantly different. This suggests that the incorporation of (n-3) PUFA into membrane phospholipids attenuates basal levels of total DG mass in murine peritoneal macrophages. Furthermore, EPA more readily attenuates the formation of basal DG than does DHA.

The incorporation of EPA or DHA at the sn-2 position of DG and phospholipid molecular species is largely at the expense of AA incorporation (Marignani and Sebaldt 1995). The substitution of AA at the sn-2 position by EPA and/or DHA has been associated with numerous pharmacological and physiological changes, particularly in inflammatory mediating cells (Needleman et al. 1979, Whelan et al. 1991, Huang et al. 1986, Croft et al. 1987, Chan et al. 1993, Chilton et al. 1993, Fowler et al. 1993a) and more recently in neoplastic diseases (Hardardottir and Kinsella 1992, Hubbard et al. 1994). At equimolar dietary concentration (Diet D), EPA incorporation into DG is approximately 2.5 times greater than DHA incorporation, for basal DG as well as for PAF-, PMA-, and ionomycin-stimulated DG (Fig. 1). Moreover, the incorporation of EPA into PE and PS is approximately 4.5 times greater than that of DHA, and the incorporation of EPA into PC and PI is approximately 2.5 times greater than that of DHA (Fig. 2). The substitution of AA by EPA and DHA in PS and PE could potentially affect membrane structure and metabolic function, such as PE conversion to PC and signaling pathways where enzyme activity is dependent upon anionic PS.

Other investigators have reported related interesting effects of (n-3) PUFA ethyl esters. Both phospholipid and DG molecular species consist of diacyl, alkenylacyl and alkenylacyl subclasses. Work by Chapkin and colleagues has shown that a large proportion of PL-incorporated EPA and DHA is substituted into the diacyl subclass followed by lesser amounts into alkenylacyl and alkenylacyl subclasses (Akoh and Chapkin 1990, Chapkin and Carmichael 1990, Chapkin et al. 1992, Fowler et al. 1993b). Furthermore, total DG mass in unstimulated and concanavalin A-stimulated murine splenocytes from mice fed diets with purified EPA ethyl esters was lower than that found in mice fed diets with purified DHA ethyl esters (Fowler et al. 1993b). Our results for total DG mass and for molecular species distributions of DG and phospholipids extend these and our own earlier findings (Marignani and Sebaldt 1995) and also demonstrate a dose-response relationship. In addition, we very recently examined macrophage interleukin-6 production in tissue culture studies (unpublished data) and protein kinase C activation in lipid vesicle studies (Marignani and Sebaldt 1996b), where we found pronounced and differential effects of EPA and DHA.

The implications of dietary supplementation with EPA, DHA or mixtures of these (n-3) PUFA in terms of the resulting altered formation of DG in signal transduction are not fully understood. The incorporation of (n-3) PUFA into membrane phospholipids and the eventual formation of DG in both reduced total mass and with EPA or DHA esterified at the sn-2 position may be of significance in a number of receptor-mediated signal transduction pathways. Thus far, the only well-described physiological function for DHA is to participate in the activation of protein kinase C (Nishizuka 1986). Potentially, lesser amounts and structurally modified sn-1,2-DG may alter the activation of protein kinase C and affect the regulation of signaling pathways that control cell growth and differentiation.

LITERATURE CITED


Seballdt, R. J. & Marignani, P. A. (1995) Diacylglycerol (DG) formation is altered by n-3 highly unsaturated fatty acids with marked differences between eicosapentaenoic (EPA) and docosa

