Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [^{13}C]DHA in phosphatidylcholine

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Abstract The amount and distribution of [^{13}C]docosahexaenoic acid (DHA) in plasma, platelet, and erythrocyte lipid classes were followed as a function of time (1 to 72 h) in young adults after ingestion of a single dose of [^{13}C]DHA esterified in a phosphatidylcholine (PC), in using gas chromatography combustion–isotope ratio mass spectrometry. [^{13}C]DHA first appeared in plasma non-esterified fatty acids (NEFA) and triglycerides (TG), with a maximal appearance at 6 h and a further decline, then being delayed 3-fold compared to [^{13}C]DHA ingested in triglycerides. Lysophosphatidylcholine (LPC) was also enriched in [^{13}C]DHA, due mainly to earlier hepatic secretion, and plateaued at 6 h, whereas phosphatidylethanolamine (PE) and phosphatidylcholine (PC) containing [^{13}C]DHA plateaued at 9 h. The labeling of erythrocyte and platelet phospholipids exhibited different kinetics, probably involving different metabolic pathways for [^{13}C]DHA incorporation in cell membranes. Computation of the relative contribution of LPC and NEFA for delivery of [^{13}C]DHA to blood cells showed that the supply to platelets occurred through NEFA. In contrast, [^{13}C]DHA was carried by both LPC and NEFA to erythrocytes, which differs from what was previously been observed after intake of triglycerides labeled with [^{13}C]DHA where LPC was the only source of [^{13}C]DHA for erythrocytes. We conclude that the lipid form of ingested DHA affects markedly its kinetics and partly its metabolic fate.—Lemaitre-Delaunay, D., C. Pachiaudi, M. Laville, J. Pousin, M. Armstrong, and M. Lagarde. **Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [^{13}C]DHA in phosphatidylcholine.** ***J. Lipid Res.*** **1999.** 40: **1867–1874.**

Supplementary key words docosahexaenoic acid • stable isotope • plasma • platelets • erythrocytes

Marine oils, which contain high levels of polyunsaturated fatty acids (PUFA) of the n–3 family such as docosahexaenoic (DHA, 22:6n–3) and eicosapentaenoic (EPA, 20:5n–3) acids (1), may have beneficial roles in the prevention of cardiovascular diseases (2, 3). N–3 PUFA exert antiatherothrombotic effects through modulation of blood lipids (4) and lipoproteins (5). Furthermore, it has been found that long chain polyunsaturated n–3 fatty acids could decrease plasma triglyceride levels (6) and cause a prolongation of bleeding time. The incorporation of n–3 PUFA in cell membranes also modifies eicosanoid production resulting in altered platelet and leukocyte reactivities (7, 8). DHA, a minor component of human plasma lipids, is a major fatty acid of phospholipids in the brain and retina (9). This fatty acid is required for the development of visual acuity and learning in humans (10, 11). Although the biological properties of DHA have been documented, the mechanisms by which this fatty acid is taken up by the brain and blood cells, the pathway of its absorption, and its transport and dynamic exchanges within blood lipid pools for the assimilation by human tissues remain largely unknown. Lysophosphatidylcholine (LPC), a second form of blood phospholipid (12), could be a transport system for PUFA (13) when secreted by the liver and could enhance the PUFA absorption and distribution (14). The transport of DHA to target cells has been reported in humans after a single ingestion of this ^{13}C-labeled fatty acid esterified in triglycerides (15). It has been shown that plasma albumin carries DHA in two forms, as non-esterified fatty acids (NEFA) which supply this fatty acid to platelets, and as LPC which delivers it to erythrocytes. The mechanism of cellular uptake of fatty acids presumably involves a carrier-mediated and a passive transmembrane translocation (16). On the other hand, the uptake of PUFA by red

Abbreviations: BHT, butylated hydroxytoluene; CE, cholesterol ester; DHA, docosahexaenoic acid; GCC-IRMS, gas-chromatography combustion–isotope ratio mass spectrometry; GLC, gas–liquid chromatography; HPLC, high performance liquid chromatography; LCAT, lecithin:cholesterol acyltransferase; LPC, lysophosphatidylcholine; NEFA, non-esterified fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; TG, triglycerides.

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blood cells may be considered as a marker for their accretion into brain (17) and retina (18) lipids. It is now accepted that the fatty acid–albumin complex is a major route by which fatty acids reach the brain (19–21). More recently, it has been found that 2-acyl-LPC bound to albumin could be an efficient delivery form of unsaturated fatty acids to the developing rat brain (22). The brain preferentially takes up DHA from lysoPC-DHA compared with non-esterified DHA (23).

According to in vivo studies in humans and animals (24), oral administration of labeled PC leads to the rapid appearance of labeled PC in plasma, with rather weak formation of labeled TG. The use of double-labeled PC, [^{3}H]choline and a ^{14}C-labeled fatty acid, showed that the ^{3}H/^{14}C ratio of plasma PC was only half of the absorbed PC (22). After oral administration, PC is more than 90% absorbed by the intestinal mucosa via conversion to LPC and reesterification (25, 26). The absorbed PC is then incorporated into chylomicrons (26) and, after degradation to the TG-rich particles, taken up by the high density lipoprotein (HDL) fraction (25). A small proportion of these PC is taken up without prior hydrolysis.

Little information is available on PC in relation to the possible incorporation into human tissues and circulating cells. The aim of the present study was to assess whether circulating [^{13}C]DHA-PC and subsequent [^{13}C]DHA-LPC in plasma are preferential forms of DHA transport compared to [^{13}C]DHA-TG and non-esterified [^{13}C]DHA. This has been done by comparing the kinetics of [^{13}C]DHA appearance in plasma and red cells after a single dose of DHA ingested either in PC (the present study) or in TG (our previous study).

MATERIALS AND METHODS

Chemical

Docosahexaenoic acid and eicosapentaenoic acid methyl esters as well as internal standards for gas chromatography (heptadecaenoic acid, cholesteryl heptadecanoate, phosphatidylcholine diheptadecanoyl, phosphatidylethanolamine diheptadecanoyl, triheptadecanoyl glycerol, lysophosphatidylcholine heptadecanoyl) were purchased from Sigma-Chimie (L'Isle d'Abeau, France). All solvents were analytical or HPLC grade from SDS (Peypin, France) or Merck (Darmstadt, Germany). Silica gel 60 plates and Superspher HPLC column were purchased from Merck (Darmstadt, Germany).

Synthesis of phosphatidylcholine containing [^{13}C]DHA

Phosphatidylcholine (PC) containing [^{13}C]DHA was produced by growing a microalgae (*Crypthecodinium cohnii*) strain in a defined medium containing d-[1-^{13}C]glucose. After harvesting the cells by centrifugation, the biomass was freeze-dried and lipids were extracted with hexane–isopropanol–water 5:5:1 (v/v/v). Phosphatidylcholine purification was carried out on a silica column (55 × 5 cm). The lipid extract (60 g) was first eluted with butanol and acetone. Then phosphatidylcholine was eluted by ethanol–water 85:15 (v/v) and ethanol–butanol 1:2 (v/v) mixtures and purified later by preparative liquid chromatography. The fatty acid composition of the PC is reported in **Table 1**. DHA represented 54.70% of the total fatty acids with a ^{13}C abundance of 8 atom%. It was esterified mainly at the *sn*-2 position.

TABLE 1. Fatty acid composition of phosphatidylcholine ingested

Fatty Acids	mol%
12:0	0.39
14:0	15.56
16:0	19.57
18:0	1.39
16:1n–7	0.18
18:1n–9	8.30
22:6n–3 (DHA)	54.70

Data are expressed as mol% of total fatty acids.

Human studies

Three healthy male volunteers (age: 35.6 ± 1.1; body mass index: 20.8 ± 0.5 kg/m^2; glucose: 5.0 ± 0.5 mm; blood cholesterol: 5.2 ± 0.6 mm; triglycerides: 1.36 ± 0.44 g/L) signed a written consent form after being informed about the purpose and modalities of the study. The scientific protocol was approved by the institutional human ethical committee. Subjects were instructed to maintain their usual diet, but to exclude alcohol 24 h before the protocol as well as marine food a week before and during the experiment. Subjects, fasted overnight, consumed 250 mg of tracer PC mixed in yogurt (3.5% lipids, 3.7% proteins, 5% carbohydrates) and had a breakfast (120 g of bread and 30 g of jam) immediately after. At various periods of time (1 to 72 h) after ingestion, blood was taken by venipuncture on ACD as anticoagulant (0.8% citric acid, 2% citrate, 2.45% dextrose, pH 4.5). A blood sample was also collected just before the tracer ingestion to measure the basal ^{13}C abundance in various lipid pools.

Preparation of plasma and blood cells

Blood samples transferred to plastic tubes kept in ice were centrifuged at 100 *g* for 15 min at 4°C to obtain platelet-rich plasma. This was acidified to pH 6.4 with citric acid and centrifuged at 900 *g* for 12 min at 4°C to obtain the platelet pellet and platelet-poor plasma. This plasma was collected, centrifuged at 1850 *g* for 10 min at 4°C to eliminate the remaining platelets, and frozen at −20°C with 5 × 10^{-5} m butyl hydroxytoluene (BHT). The platelet pellet was resuspended into a Tyrode-N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) buffer (in mm: 135 NaCl, 2.68 KCl, 0.46 $NaHPO_4$, 1 $MgCl_2$, 5 HEPES, 5.5 glucose, 1 EDTA; pH 6.4) (27). This cell suspension was immediately centrifuged at 1000 *g* for 10 min at 4°C. Then the new platelet pellet was resuspended in 2 ml of Tyrode-HEPES buffer (pH 7.35) without EDTA and frozen at −20°C with BHT (5 × 10^{-5} m). For preparation of erythrocytes, the pellet resulting from centrifugation of whole blood at 100 *g* was diluted in Tyrode-HEPES buffer and centrifuged at 100 *g* for 10 min at 4°C. After removing the supernatant, red blood cells were diluted with 9% NaCl and centrifuged at 2000 *g* for 10 min at 4°C. This procedure was repeated twice and Tyrode-HEPES was added to red cells prior to freezing, in the presence of 5 × 10^{-5} m BHT.

Extraction and separation of lipids

Total lipids were extracted from blood cells and plasma according to Bligh and Dyer (28). Lysophosphatidylcholine (LPC) from plasma and erythrocytes was prepared by spotting the lipid extracts on silica gel 60 plates which were developed, with chloroform–methanol–water 65:25:4 (v/v/v) as the mobile phase. Other lipids from plasma were first separated into neutral lipids and NEFA with the solvent mixture chloroform–methanol 80:8 (v/v). A second chromatography step was performed with chloroform–methanol–40% aqueous methylamine 60:20:5 (v/v/v) to separate phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Neutral lipids were extracted from the silica gel with chloroform–methanol

90:10 (v/v) and further fractionated into cholesteryl esters (CE) and triglycerides (TG) on thin-layer chromatography (TLC) plates developed with hexane–diethyl ether–acetic acid 80:20:1 (v/v/v). PE and PC from platelets and erythrocytes were purified by TLC using chloroform–methanol–40% aqueous methylamine as the mobile phase after predeveloping the plates with chloroform–methanol 80:8 (v/v). Lipid classes were scraped off the plate and treated for 90 min with 5% H_2SO_4 in methanol to obtain FA methyl esters (29).

Quantification of lipids by gas–liquid chromatography (GLC)

Heptadecanoic acid, cholesteryl heptadecanoate, diheptadecanoyl phosphatidylcholine, diheptadecanoyl phosphatidylethanolamine, triheptadecanoyl glycerol, and heptadecanoyl lysophosphatidylcholine were added before lipid extraction, when appropriate, and the absolute amounts of fatty acid methyl esters were determined by GLC analysis, relative to the known amount of added 17:0. A Delsi chromatograph model DI200 equipped with a Ross injector and a SP2380 capillary column (30 m × 0.32 mm) (Supelco, Bellefonte, PA) was used for these analyses. The oven temperature was held at 145°C for 5 min and raised to 215°C at 2°C/min (30).

Purification of fatty acyl methyl esters

HPLC with a 5 μm Superspher 100 C18 column (4.6 mm internal diameter × 250 mm) and isocratic elution with acetonitrile–water 80:20 (v/v), at a flow rate of 2 ml/min was used to separate fatty acyl methyl esters (31). The fatty acyl methyl esters were detected by UV absorbance at 210 nm. The DHA methyl ester was collected, taken to dryness under nitrogen, and redissolved into isooctane for GCC–IRMS analysis.

Gas chromatography combustion–isotope ratio mass spectrometry (GCC–IRMS) analysis

Analyses were performed using an isotope ratio mass spectrometer (SIRA 12; VG Isogas, Middlewitch, UK) interfaced with a gas–liquid chromatograph (5890A; Hewlett-Packard, Evry, France) equipped with a Ross injector (240°C) and a capillary column (30 m × 0.32 mm, Supelco, Bellefonte, PA). The oven temperature was maintained at 180°C for 0.5 min and then raised at 25°C/min to 245°C where it was maintained for 5 min. Helium was used as a carrier gas (injector inlet pressure, 26 psi). The gas–liquid chromatograph effluent was diverted to a flame ionization detector until elution of DHA methyl ester. The effluent was then switched to a catalytic furnace filled with CuO and maintained at 800°C. The effluent from the furnace containing CO_2 and H_2O generated from DHA methyl ester, flowing in the continuous helium flux, was driven into a water trap at −100°C before its ionization by electron impact in the source of the GCC–IRMS. The different isotopomers were collected onto three different collectors at mass-to-charge ratio (m/z) 44 (main ion: $^{12}C^{16}O^{16}O$), 45 ($^{13}C^{16}O^{16}O$, $^{12}C^{16}O^{17}O$), and 46 ($^{12}C^{17}O^{17}O$, $^{12}C^{16}O^{18}O$, $^{13}C^{16}O^{17}O$). Ions at m/z 44, 45, and 46 were continuously recorded until the return of the 44 signal to the baseline value. Isotopomers at m/z 44 and 45 were measured, leading to the $^{13}C/^{12}C$ ratio. Before and after the CO_2 peaks generated from DHA, a CO_2 sample reference of known enrichment, calibrated against the international standard (Pee Dee Belemnite: PDB), was automatically injected into the mass spectrometer (32).

Expression of results

The $^{13}C/^{12}C$ ratios of the sample and of the reference were used to calculate the δ per 1000 value ($\delta^{13}C‰$) of the sample using the following formula:

$$\delta^{13}C‰ = \frac{(^{13}C/^{12}C)\ \text{sample} - (^{13}C/^{12}C)\ \text{reference}}{(^{13}C/^{12}C)\ \text{reference}} \times 10^3 \quad \textit{Eq. 1)}$$

where the reference is the international PDB standard, which is CO_2 obtained from the carbonate shell of the cretaceous mollusc, *Belemnitella americana*, from the Pee Dee formation in South Carolina.

The [^{13}C]DHA appearance in biological samples is also expressed as the absolute amount of [^{13}C]DHA in lipid pools by multiplying the [^{13}C]DHA dilution by the endogenous DHA concentration quantitated by GLC.

Calculations

To determine the contribution of NEFA and LPC to [^{13}C]DHA delivery to platelets and erythrocytes, a mathematical model, briefly presented here, was used. It should be noted that NEFA and LPC are the only providers for platelets and erythrocytes for the mathematical model considered here. As all possible transport phenomenon are not accounted for, the mathematical model is based mainly on the dynamic aspect of the total amount of [^{13}C]DHA present in the providers. Two time-dependent functions, t_1 and t_2, express the total amount of [^{13}C]DHA carried by NEFA and LPC. C_1 and C_2, also time-dependent functions, represent the total amount of [^{13}C]DHA in platelets and erythrocytes, verifying the relationships:

$$\frac{d}{dt}C_1(t) = \alpha\gamma_1[t_1(t) - t_1^{as}] + \beta\gamma_2[t_2(t) - t_2^{as}] \quad \textit{Eq. 2)}$$

$$\frac{d}{dt}C_2(t) = (1-\alpha)\gamma_1[t_1(t) - t_1^{as}] + (1-\beta)\gamma_2[t_2(t) - t_2^{as}] \quad \textit{Eq. 3)}$$

The constants α, β, γ_1, γ_2 represent the relative contribution of NEFA to platelets, the relative contribution of LPC to erythrocytes, the fractions of NEFA and LPC involved in our process, respectively. These constants were identified by means of experimental data. The constants t_1^{as} and t_2^{as} stand for the asymptotic values of functions t_1 and t_2 reached when the process does not evolve anymore. These constants were obtained from experimental data.

By integrating equations (2) and (3) from time t_i to time t_{i+1} the following expressions are obtained for functions C_1 and C_2:

$$C_1(t_{i+1}) - C_1(t_i) = \alpha\gamma_1\int_{t_i}^{t_i+1}[t_1(s) - t_1^{as}]ds + \beta\gamma_2\int_{t_i}^{t_i+1}[t_2(s) - t_2^{as}]ds \quad \textit{Eq. 4)}$$

$$C_2(t_{i+1}) - C_2(t_i) = (1-\alpha)\gamma_1\int_{t_i}^{t_i+1}[t_1(s) - t_1^{as}]ds + (1-\beta)\gamma_2\int_{t_i}^{t_i+1}[t_2(s) - t_2^{as}]ds \quad \textit{Eq. 5)}$$

By choosing the collection of times $\{t_i\}_{i=1}^{i=9}$ as the times where measurements took place ($t_1 = 1$, $t_2 = 2$, $t_3 = 4$, $t_4 = 6$, $t_5 = 9$, $t_6 = 12$, $t_7 = 24$, $t_8 = 48$, $t_9 = 72$) and by assuming that function t_1 and t_2 vary linearly in $[t_i, t_{i+1}]$, numerical values for functions C_1 and C_2 depending on α, β, γ_1, γ_2 at times t_j are obtained. If $\hat{C}_1(t_i)$, $\hat{C}_2(t_i)$ denote the measured values of total amounts of [^{13}C]DHA in platelets and in erythrocytes, respectively, then the constants α, β, γ_1, γ_2 are calculated according to a least square minimizing procedure between these values and the ones provided by numerical simulations equations 4 and 5:

$$\underset{\alpha,\beta,\gamma1,\gamma2}{\text{Inf}} \sum_{i=1}^{i=9}[\hat{C}_1(t_i) - C_1(t_i)]^2 + [\hat{C}_2(t_i) - C_2(t_i)]^2 \quad \textit{Eq. 6)}$$

RESULTS

Natural ^{13}C abundance was measured on pure, commercially available DHA methyl ester. Analyses were performed with 300 ng of the methyl ester, which gave a similar intensity to the standard CO_2. $\delta^{13}C‰$ values were -26.6 ± 0.2 (mean of 10 determinations), with less than 1% variation. This reproducibility ensured precise determinations of low ^{13}C abundance after intake.

Lipid forms of [^{13}C]DHA in plasma

[^{13}C]DHA appeared rapidly in TG and NEFA. Six hours after ingestion of the tracer, the labeling was maximal in both fractions (**Fig. 1A**), with $\delta^{13}C‰$ values of $+1329 \pm 313$ and $+538 \pm 110$, respectively. The abundance at 6 h was 2.47-fold higher in the TG than in NEFA. Between 6 and 24 h after ingestion, the [^{13}C]DHA abundance decreased in both fractions to reach a plateau, with minimal values of $+76.1 \pm 39.4$ in TG and $+26.2 \pm 19.4$ in NEFA observed at 72 h post-ingestion. In contrast (Fig. 1B), the labeling of PE and PC was more progressive until 9 h and plateaued from 9 to 72 h where the [^{13}C]DHA abundance was higher in PE than in PC. For example, the $\delta^{13}C‰$ values were $+197.9 \pm 121.5$ and $+97.2 \pm 43.6$, respectively, at 24 h and $+180.7 \pm 89.3$ and 100.2 ± 43.9, respectively, at 48 h. In PE, the abundance tended to decrease at the later points of the kinetics with $\delta^{13}C‰$ values of $+197.9 \pm 121$ at 24 h and $+123.6 \pm 63.1$ at 72 h. For LPC, a plateau was reached from 6h with $\delta^{13}C‰$ values slightly lower than those of PC between 9 and 72 h. The labeling of CE was lower and increased in function of time.

[^{13}C]DHA appearance in these lipid species was also expressed as the absolute amount of [^{13}C]DHA at various time points (**Fig. 2**). [^{13}C]DHA accumulation in NEFA and LPC (Fig. 2A) was lower than in other lipid classes (Fig. 2B). Six hours after ingestion, the [^{13}C]DHA concentration in NEFA was 33.7-fold lower than in TG but 7.3-fold higher than in LPC. In contrast, the [^{13}C]DHA concentration was slightly higher in LPC than in NEFA after 24 h, reaching 1.48-fold at 72 h. Finally, the [^{13}C]DHA accumulation in PC was more than 3.4-fold higher when compared to PE after 9 h.

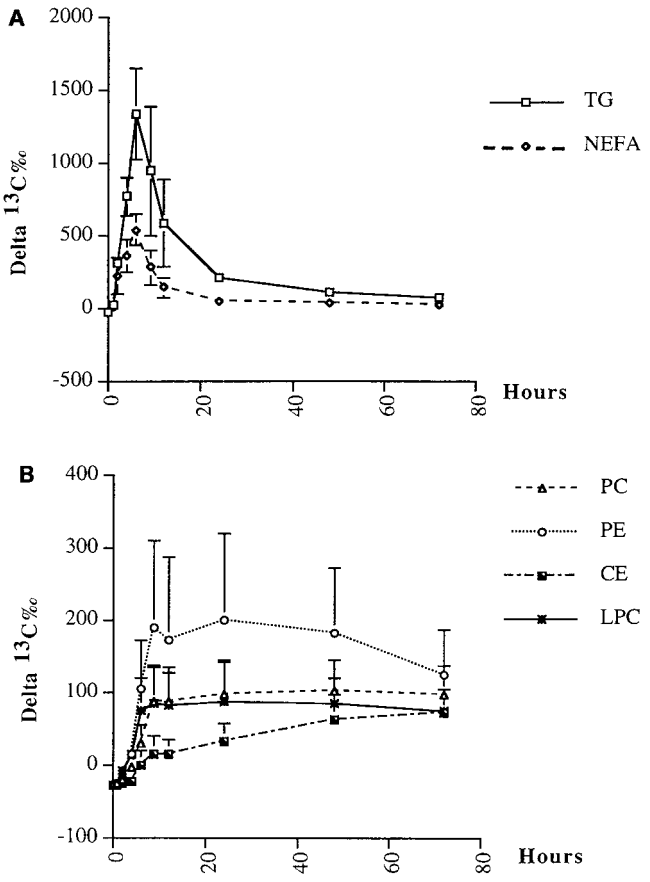

Fig. 1. Time course of [^{13}C]DHA, expressed in $\delta^{13}C‰$, in human plasma triglycerides (TG), non-esterified fatty acids (NEFA) (A), phosphatidylcholine (PC), phosphatidylethanolamine (PE), lysophosphatidylcholine (LPC), and cholesteryl esters (CE) (B), as a function of time after the ingestion of a tracer dose of [^{13}C]DHA-PC. Each point represents the mean ± SD of determinations from three human subjects.

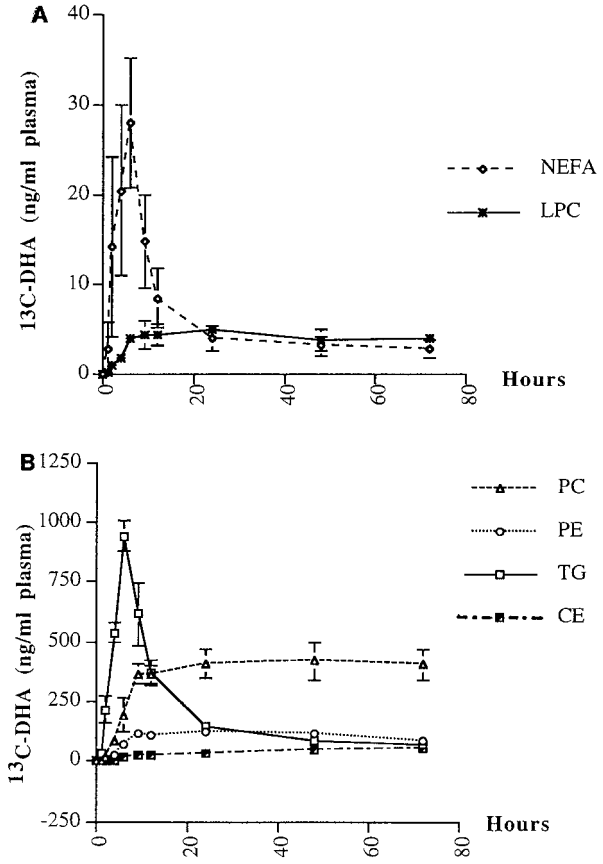

Fig. 2. Concentration of [^{13}C]DHA, expressed in ng/ml plasma, in human plasma non-esterified fatty acids (NEFA), lysophosphatidylcholine (LPC) (A), phosphatidylcholine (PC), phosphatidylethanolamine (PE), triglycerides (TG), and cholesteryl esters (CE) (B), as a function of time after the ingestion of a single dose of [^{13}C]DHA-PC. The [^{13}C]DHA amount in each lipid class was calculated from the $\delta^{13}C‰$ and from the DHA endogenous concentrations evaluated by GC. Each point represents the mean ± SD of determinations from three human subjects.

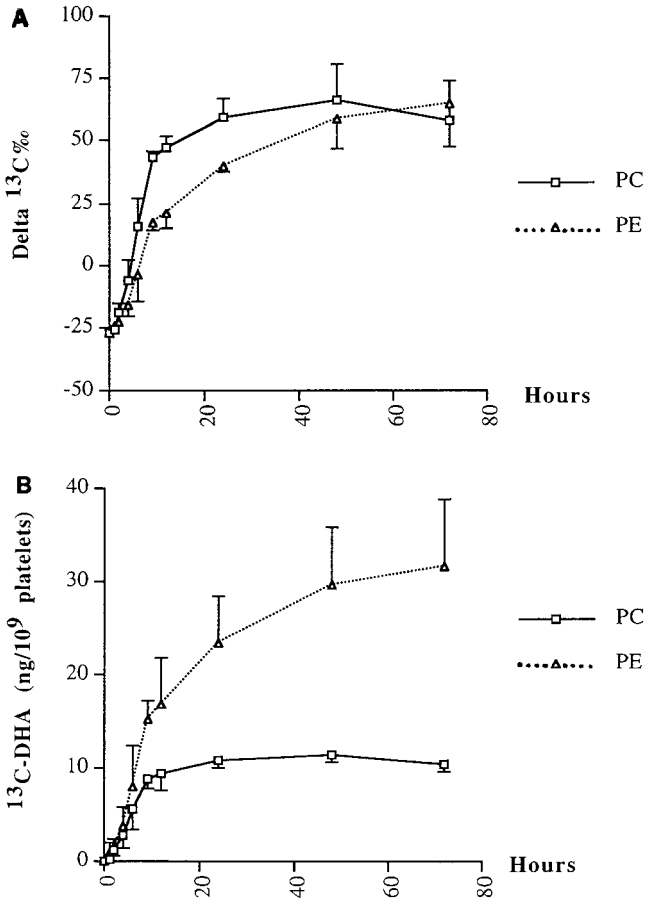

Fig. 3. Kinetics for the [^{13}C]DHA accumulation in platelet phosphatidylcholine (PC) and phosphatidylethanolamine (PE), expressed in δ^{13}C‰ (A) and in ng/10^9 platelets (B). [^{13}C]DHA concentration in PC and PE was calculated from the δ^{13}C‰ abundance and the endogenous DHA concentrations quantitated by GC. Each point represents the mean ± SD of determinations from three human subjects.

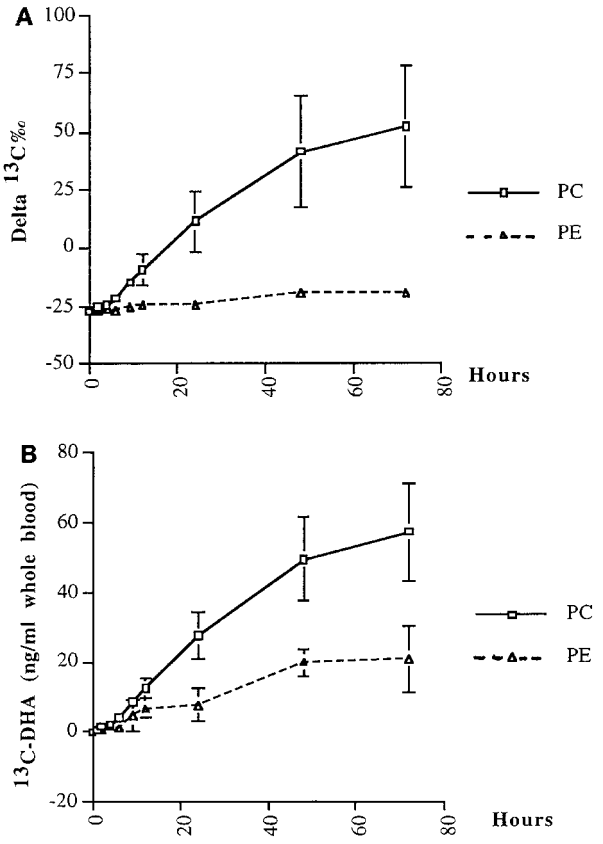

Fig. 4. Kinetics for the [^{13}C]DHA accumulation in erythrocyte phosphatidylcholine (PC) and phosphatidylethanolamine (PE), expressed in δ^{13}C‰ (A) and in ng/ml whole blood (B). [^{13}C]DHA concentration in PC and PE was calculated from the δ^{13}C‰ abundance and the endogenous DHA concentrations, quantitated by GC. Each point represents the mean ± SD of determinations from three human subjects.

Uptake of [^{13}C]DHA by platelets and erythrocytes

An increase in the labeling in platelet PC (**Fig. 3A**) occurred till 24 h post-ingestion, followed by a plateau between 24 and 72 h. The [^{13}C]DHA abundance increased also in platelet PE but the labeling in PC was higher than in PE from 6 to 24 h, and very close after 48 and 72 h. The amount of [^{13}C]DHA stored in platelet phospholipids was also calculated (Fig. 3B). [^{13}C]DHA was preferentially incorporated in PE, being 3-fold higher than in PC, after 72 h.

The uptake of [^{13}C]DHA by erythrocytes was different. A slow and progressive increase of PC labeling was observed, as a function of time (**Fig. 4A**). Values ranged from −27.3 ± 0.8 (baseline) to −9.7 ± 6.8 (12 h) and +51.9 ± 26.5 (72 h). The labeling of erythrocyte PE was lower, attaining −19.5 ± 2.2 at 72 h. When the results are expressed in the amount of [^{13}C]DHA found in these two compartments (Fig. 4B), a preferential accumulation occurred in erythrocyte PC. On the other hand, the incorporation of [^{13}C]DHA into erythrocyte PE was weak. A small amount of [^{13}C]DHA appeared in erythrocyte LPC (**Fig. 5**), with an increase until 9 h and a maximal amount between 9 h and 72 h. This plateau was attained when the incorporation of [^{13}C]DHA started to rise into PC. At the latter time point, the LPC content of [^{13}C]DHA was +0.06 ± 0.02 ng/ml of whole blood.

The contribution of NEFA and LPC to supply [^{13}C]DHA to blood cells was investigated. We calculated that the relative contribution factor of NEFA involved in the [^{13}C]DHA supply to erythrocytes and platelets was $\gamma 1 = 0.32$ and that the relative contribution factor of LPC to this supply was $\gamma 2 = 1.0$. The computed relative contribution factors of NEFA and LPC to platelets were $\alpha = 0.069$ and $\beta = 0$, respectively. The computed relative contribution factors of NEFA and LPC to erythrocytes were $1 - \alpha = 0.931$ and $1 - \beta = 1.0$, respectively. It appears that the DHA supply to platelets occurred through NEFA, $\alpha(\gamma 1) = 0.022$, and not through LPC, $\beta(\gamma 2) = 0$, due to the isotope enrichment pattern in LPC which does not fit with that in platelets. In contrast, these two lipid compartments are involved in DHA supply to erythrocytes. The value of coefficient $(1 - \beta)(\gamma 2) = 1$ relates to the amount of DHA available through LPC, with NEFA being another provider according to the following equation:

$$(1 - \alpha)\gamma 1 = 0.299 \qquad \textit{Eq. 7})$$

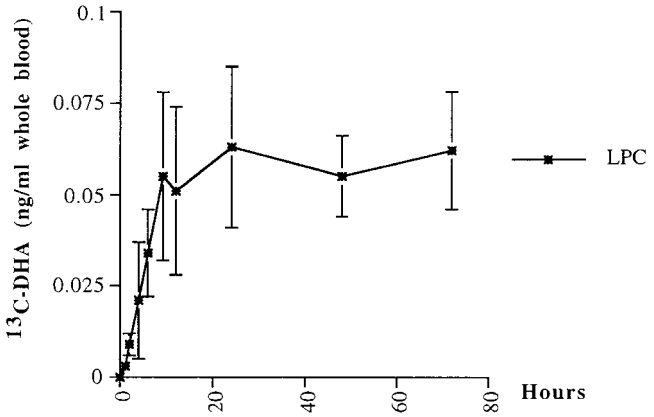

Fig. 5. Concentration of [^{13}C]DHA, expressed in ng/ml whole blood, in human erythrocyte lysophosphatidylcholine (LPC) as a function of time after the ingestion of a single dose of [^{13}C]DHA-PC. The [^{13}C]DHA amount in each lipid class was calculated from the δ^{13}C‰ abundance and the DHA endogenous concentrations evaluated by GC. Each point represents the mean ± SD of determinations from three human subjects.

Ingestion of [^{13}C]DHA-TG and [^{13}C]DHA-PC: comparison

Previous studies (15) under the same conditions, except for the form of DHA ingested, have shown that ingestion of a single dose of [^{13}C]DHA-TG leads to the appearance of a [^{13}C]DHA peak in plasma TG and NEFA after 2 h. In plasma LPC and PC, the incorporation of [^{13}C]DHA was more progressive until 6 and 12 h, respectively, and a plateau was observed in both compartments thereafter. LPC was found to be the main vehicle for DHA to erythrocytes and NEFA the major one to leukocytes and platelets.

In the present study, after ingestion of a single dose of [^{13}C]DHA-PC corresponding to the same dose of [^{13}C]DHA, it appears that [^{13}C]DHA peaked in plasma TG and NEFA only after 6 h, being delayed 3-fold compared to [^{13}C]DHA-TG intake. The plateau in plasma LPC was also obtained after 6 h. But, in the present study, the incorporation of [^{13}C]DHA into PC was slightly faster with a plateau after 9 h instead of 12. Also, the uptake of [^{13}C]DHA by the blood cells was quite different. The esterification of [^{13}C]DHA in platelet PC and PE was delayed and was about 2-fold lower, while that in the erythrocyte PE and PC was slightly higher compared to [^{13}C]DHA-TG intake.

DISCUSSION

The high precision of the measurements made with GCC–IRMS makes significant the low variations obtained in ^{13}C/^{12}C ratios from one compound, even if its ^{13}C abundance over the basal value is low (33). In addition, the direct measurement of the ^{13}C/^{12}C ratio from CO_2 is independent of the compound recovery from biological samples and avoids the use of internal standards from the beginning of the laboratory treatment (31). This allowed us to follow [^{13}C]DHA in various blood lipid pools in humans after a single small dose intake of [^{13}C]PC-DHA (250 mg) with a ^{13}C abundance of only 8-fold the natural abundance, and to compare the [^{13}C]DHA distribution with that observed after the same intake of [^{13}C]TG-DHA (15).

Some studies have reported that the fatty acid transport in different chyle lipids depends on the vehicle (34). The predominant form of fatty acids in plasma is known to be triglycerides, which are the major dietary lipid form (about 90%) in humans. However, a substantial amount of fatty acids is also ingested in phospholipids, and it has been shown that this might influence their metabolic fate (24). As we previously found that LPC could represent a preferential vehicle of DHA to target tissues (15, 23), we addressed the question of whether the ingestion of DHA in phosphatidylcholine (PC-DHA) would affect the metabolic fate of DHA.

From the present experiment, it appears that the maximal accumulation of [^{13}C]DHA in plasma TG occurred 6 h after ingestion of [^{13}C]PC-DHA instead of 2 h when absorbed from [^{13}C]TG-DHA. In contrast to triglycerides, phospholipids are not hydrolyzed by lingual nor gastric lipases but only in the small intestine (35). PC are acted upon by pancreatic phospholipase A_2 which releases the fatty acid from the *sn*-2 position, [^{13}C]DHA in the present experiment, to form the corresponding LPC. After absorption by the enterocytes, this LPC could be reacylated into PC while the previously released fatty acid can be used for TG synthesis (36). This might explain the delay for [^{13}C]DHA to peak in TG after the ingestion of [^{13}C]PC-DHA. Then, the newly formed TG and PC can be carried to blood by the lymphatic duct (37). [^{13}C]DHA also peaked after 6 h in the plasma NEFA and was consistently and significantly lower than in plasma TG, whatever the time course point, which agrees with its predominant release from circulating [^{13}C]TG-DHA by lipoprotein lipase (38). This also explains the 3-fold delay for the peak of [^{13}C]DHA in NEFA compared to that observed after the ingestion of [^{13}C]TG-DHA (15).

[^{13}C]DHA accumulated in plasma phospholipids with a plateau from 9 h after ingestion. PE was more labeled than PC in agreement with a previous study showing that the liver ethanolamine phosphotransferase exhibits a marked specificity for 1-saturated,2-docosahexaenoyl-glycerol in contrast to the choline phosphotransferase (39). This made PE labeled twice as much as PC, although containing 4 times less [^{13}C]DHA, despite the possibility of PE being partially methylated into PC in the liver (40). Also, compared to the ingestion of [^{13}C]TG-DHA, where the plateau for the accumulation of [^{13}C]DHA into plasma phospholipids was reached after 12 h, the ingestion of [^{13}C]PC-DHA slightly shortened this time to 9 h. If significant, the origin of this difference is not clear. It may, however, suggest a facilitated recirculation of DHA in phospholipids when ingested as PC-DHA.

As in the previous study on the intake of [^{13}C]TG-DHA, some [^{13}C]DHA was accumulated in plasma with a plateau reached as soon as 6 h post-ingestion. It is unlikely that it was produced from plasma [^{13}C]PC-DHA by the lecithin: cholesterol acyltransferase (LCAT) activity that usually

transfers the acyl group from the *sn*-2 position of PC to cholesterol (41), although it has been reported that LCAT may specifically transfer some DHA from 1-palmitoyl,2-docosahexaenoyl-GPC (42, 43), because the accumulation of [^{13}C]DHA in LPC slightly precedes that in PC. In addition, it has been shown that a substantial source of plasma LPC derives directly from the liver (13, 44). The very slow accumulation of [^{13}C]DHA into cholesteryl esters also favors marginal LCAT activity acting upon [^{13}C]PC-DHA.

The incorporation of [^{13}C]DHA into platelet and red cell phospholipids was also investigated. This was restricted to PE and PC, the esterification into phosphatidylserine and phosphatidylinositol being negligible. In platelets, [^{13}C]DHA accumulated in both PC and PE although a little faster in PC. This presumably occurred via the deacylation/reacylation or the Lands pathway (45), as the availability of LPC and LPE has been well documented in platelets (46–48), as well as the presence of active acyl-CoA synthetases toward high polyunsaturated fatty acids (49, 50). Also, a preferential acylation of LPC and LPE over LPI and LPS has been reported with DHA (48). Although [^{13}C]DHA was only slightly more rapidly esterified into PC, the labeling of PE still increased after 48 h while that of PC began to decrease. This could easily be explained by the transacylation process from PC to PE as has been described in platelets from rats (51) and from humans (30). Also, three times higher [^{13}C]DHA was found in PE compared to PC after 72 h although the labeling was similar in the two phospholipids, in agreement with the preferential accumulation of DHA into PE but at a lower rate. When compared to the ingestion of [^{13}C]TG-DHA, that of [^{13}C]PC-DHA induced a small lag phase in the accumulation of [^{13}C]DHA in platelet phospholipids. This fits entirely with the 3-fold delay of the non-esterified [^{13}C]DHA peak in plasma, and agrees with the mathematic modeling which led to the conclusion that NEFA was involved only in providing [^{13}C]DHA to platelets.

In erythrocytes, the esterification of [^{13}C]DHA into PE was very low as previously reported (52) and esterification into PC did not appear saturable within the period studied (72 h). This agrees with a recent study showing an important turnover of PC in erythrocyte membranes of rabbit fed fish oil (53). In contrast to platelets, [^{13}C]LPC could be detected in erythrocytes and it appeared to plateau from 9 h after ingestion (6 h in plasma), when [^{13}C]DHA started to rise in PC. This is consistent with the hypothesis that the preferential lipid form of DHA to erythrocytes is LPC (15) and fits with previous data showing that plasma LPC are taken up by erythrocytes where they are subsequently reacylated into PC (54). If we assume, as previously reported (17), that the accretion of DHA into erythrocytes may be, at least in part, an index of that into the brain, we could consider that LPC might be a preferential vehicle of DHA to the brain, as suggested in young rats (23). Another support for this hypothesis may be taken from our previous work in the rat fed a single dose of [^{13}C]TG-DHA where the kinetics for the accumulation of [^{13}C]DHA into the brain and erythrocytes were similar (55). However, in the present study with humans fed a single dose of [^{13}C]PC-DHA, mathematic modeling led to the conclusion that, in contrast to the ingestion of [^{13}C]TG-DHA, NEFA could also provide part of [^{13}C]DHA to erythrocytes. Although we do not have any explanation for this difference, it could explain why the total uptake of [^{13}C]DHA in erythrocytes phospholipids was slightly higher compared to the ingestion of [^{13}C]-TG-DHA, while it was substantially lower in platelets. Overall, these findings reinforce the potential interest of PC-DHA over TG-DHA, which might favor the DHA uptake by erythrocytes and putatively by the brain, providing that phospholipid sources of DHA are available.

We conclude that the metabolic fate of DHA differs substantially when ingested in TG or PC both in terms of bioavailability in plasma and accumulation in target tissues.

This work was supported by INSERM and a grant from MENRT no. 94G 0164. We thank Dr. Y. Bayon for having prepared the ^{13}C-labeled PC-DHA.

Manuscript received 1 February 1999 and in revised form 16 June 1999.

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