Absorption Kinetics of Ethanolamine Plasmalogen and Its Hydrolysate in Mice

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Abstract: Ethanolamine plasmalogen (PlsEtn), a subclass of ethanolamine glycerophospholipid (EtnGpl), has been reported to have many biological and dietary functions. In terms of PlsEtn absorption, some studies have reported that PlsEtn is re-esterified at the sn-2 position using lymph cannulation and the everted jejunal sac model. In this study, we aimed to better understand the uptake kinetics of PlsEtn and increase its absorption. We thus compared the uptake kinetics of PlsEtn with that of the lyso-form, in which the fatty acid at the sn-2 position was hydrolyzed enzymatically. Upon administration of EtnGpl (extracted from oysters or ascidians, 75.4 mol% and 88.4 mol% of PlsEtn ratio, respectively), the plasma PlsEtn species in mice showed the highest levels at 4 or 8 hours after administration. In the contrast, administration of the EtnGpl hydrolysate, which contained lysoEtnGpl and free fatty acids, markedly increased the plasma levels of PlsEtn species at 2 h after administration. The area under the plasma concentration-time curve (AUC), especially the AUC₂₈, of PlsEtn species, was higher with hydrolysate administration than that with EtnGpl administration. These results indicate that EtnGpl hydrolysis accelerated the absorption and metabolism of PlsEtn. Consequently, using a different experimental approach from that used in previous studies, we reconfirmed that PlsEtn species were absorbed via hydrolysis at the sn-2 position, suggesting that hydrolysis in advance could increase PlsEtn uptake.

Key words: absorption, ethanolamine glycerophospholipid, lysophospholipid, lysoplasmalogen, plasmalogen

1 Introduction

Ethanolamine glycerophospholipid (EtnGpl) is a major class of phospholipids (PL) found in biological membranes¹⁻⁶. EtnGpl exists in the following three forms with alkyl, alkenyl, or acyl linkages at the sn-1 position of the glycerol moiety: 1-O-alkyl-2-acyl-sn-glycero-3-phosphethanolamine (PakEtn), 1-O-alkenyl-2-acyl-sn-glycero-3-phosphoethanolamine (PlsEtn), and 1,2-diacyl-sn-glycero-3-phosphoethanolamine (PtdEtn), respectively. The alkenylacyl form is called plasmalogen. The aliphatic moiety at the sn-1 position of PlsEtn consists of fatty alcohols 16:0l, 18:0l, or 18:1l, whereas the sn-2 position mainly consists of polyunsaturated fatty acids (PUFA) such as 22:6n-3 (docosahexaenoic acid, DHA), 20:5n-3 (eicosapentaenoic acid, EPA), and 20:4n-6 (arachidonic acid, AA). When exposed to stress triggered by inflammation, plasmalogen-specific phospholipase A₂ hydrolyzes PlsEtn into lysophospholipids (lysoPL) and PUFA. PUFA can be metabolized to eicosanoids and docosanoids, which exhibit various bioactivities. The physiological significance of lysoPlsEtn itself has been unclear, but long chain aldehydes released from lysoPlsEtn are known to act as signaling lipids⁷.

PlsEtn content in mammalian species varies considerably depending on tissues; the brain and nervous system contain 60%–90% of EtnGpl, plasma and red blood cells 50%–60%, the colon approximately 35%, while the liver contains the lowest levels (2%–5%).¹⁻⁴. PlsEtn levels in blood and organs are maintained by a combination of the regulation in its biosynthesis and degradation⁷. Recently, PlsEtn has attracted attention due to its altered levels in...
specific diseases. PlsEtn levels in the brain and blood are lower in patients with Alzheimer’s disease and in those with other neurological diseases compared to the levels in healthy subjects. PlsEtn metabolism is also profoundly dysregulated in dedifferentiated colon mucosa.

In contrast, some marine invertebrates (e.g., ascidians, oysters, and mussels) are known to be rich in PlsEtn along with DHA and EPA, and their dietary functions are rather interesting. Dietary PlsEtn from ascidians improves impaired memory in model rats of Alzheimer’s disease (9), PlsEtn, especially species bearing DHA, suppress neuronal apoptosis in vitro. Dietary PlsEtn from ascidians suppresses the formation of aberrant crypt foci and colon inflammation in colon cancer.

To effectively utilize dietary functions of PlsEtn, it is necessary to understand its digestion, absorption, and metabolism. In terms of PlsEtn absorption, Hara et al. reported the presence of a small but significant amount of PlsEtn in the lymph but not in the portal vein of rats after duodenal infusion of a phospholipid fraction containing PlsEtn, thereby indicating that PlsEtn was absorbed in vivo. Interestingly, Nishimukai et al. have reported that the composition of PlsEtn differs between the administered lipid emulsion and the collected lymph, thus implying structural changes in PlsEtn during absorption.

In light of the aforementioned studies, we compared the uptake kinetics of PlsEtn with that of the lyso-form in the plasma of mice to better understand the uptake kinetics of PlsEtn and to increase the absorption of PlsEtn. EtnGpl containing high levels of PlsEtn was prepared from oyster or ascidian muscle, and lysoEtnGpl with a high level of lysoPlsEtn was obtained from EtnGpl by enzymatic hydrolysis.

2 Experimental Procedures

2.1 Materials and reagents

Japanese oysters (Crassostrea gigas) were purchased from a local fish market in Hokkaido and were lyophilized within one day of purchasing and crushed. Freeze-dried ascidian (Halocynthia roretzi) muscle was provided by Yaizu Suisankagaku Industry Co., Ltd. (Shizuoka, Japan). Standard phospholipid species were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA), and 18:0ol/20:5-PlsEtn was purified according to previously reported methods. All other reagents used were purchased from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan) unless stated otherwise.

2.2 Preparation of EtnGpl and the lyso-form from oysters and ascidians

EtnGpl was prepared from oysters or ascidian muscle as per modification of our previously reported method. LysoEtnGpl was hydrolyzed from EtnGpl using Brockhoff’s method. EtnGpl was purified using silica column chromatography, and the purified EtnGpl was hydrolyzed with phospholipase A2 (PLA2) from Crotalus atrox venom (Sigma-Aldrich, St. Louis, USA) in 0.22 M NaCl, 20 mM CaCl2, 1 mM EDTA-2Na, and 0.05 M MOPS (pH 7.2) at 30°C. After the reaction, the lipophilic fraction containing lysoEtnGpl and free fatty acids (FFA) was obtained by solvent fractionation.

2.3 Animals and plasma preparation

Male ICR mice were obtained from CLEA Japan, Inc. (Tokyo, Japan) at 7 weeks of age and were housed under pathogen-free conditions in micro-isolator cages at 22 ± 1°C under a 12-h light/12-h dark cycle. The mice were acclimated for 1 week with CE-2 diets (CLEA Japan, Inc.) and then used for the absorption experiments. After a 12-h starvation period, the following samples were administered into the stomach by oral gavage: for Experiment 1, the control group was administered with 0.5 mL of 5% NaHCO3 and 1% sodium taurocholate as the vehicle, the EtnGpl group was administered with 0.5 mL of oyster EtnGpl (390 μmol/kg body weight) sonication-emulsified in the vehicle, and the LysoEtnGpl group was administered oyster LysoEtnGpl (390 μmol/kg body weight) plus FFA emulsified in vehicle; for Experiment 2, the control group was administered with 0.5 mL of 5% NaHCO3, 6.7% sodium taurocholate, and 1.67% fatty acid-free bovine serum albumin as the vehicle, the EtnGpl group was administered with 0.5 mL of ascidian EtnGpl (390 μmol/kg body weight) sonication-emulsified in the vehicle, and the LysoEtnGpl group was administered with ascidian LysoEtnGpl (390 μmol/kg body weight) emulsified in the vehicle. Meanwhile, the Mix group was administered with a mixture of ascidian EtnGpl and LysoEtnGpl (195 μmol/kg body weight) emulsified in the vehicle. The mice were then anesthetized with sodium pentobarbital (65 mg/kg BW, i.p.) at the scheduled time. Fresh blood samples were freshly collected from the right ventricle in tubes with EDTA-2Na, and subjected to low-speed centrifugation (15 min, 1,000 × g, 4°C) for plasma separation. The plasma was stored at −80°C until use. All animal experiments were performed with protocols approved by the Animal Care and Use Committee and according to the Obihiro University Guidelines (Permit Number: 28-191 and 18-180).

2.4 Lipid analysis

Fatty acid methylester and aldehyde composition were
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determined by gas chromatography\textsuperscript{19}. PL species were analyzed by HPLC with the 4000 QTRAP quadrupole/linear ion-trap tandem mass spectrometer (AB SCIEX, Tokyo, Japan)\textsuperscript{20}. To quantify the PL species, multiple reaction monitoring of the transition of parent ions to product ions was performed. Quantification of PL was performed for the following eighteen molecular species: PlsEtn\(18:0\text{ol}/18:1, 18:0\text{ol}/20:4, 18:0\text{ol}/20:5, \text{and } 18:0\text{ol}/22:6\), PtdEtn\(18:0/18:1, 18:0/20:4, \text{and } 18:0/22:6\), phosphatidylcholine (PtdCho; 18:0/18:1, 18:0/20:4, and 18:0/22:6), lysoPlsEtn\(18:0\text{ol} \text{ and } 16:0\text{ol})\), lysoPtdEtn\(18:0 \text{ and } 16:0\), and lysoPtdCho\(18:0 \text{ and } 16:0\).

2.5 Statistical analysis

Differences among all data groups were assessed using one-way ANOVA with the Fisher’s least significant difference test; \(P\) values <0.05 were considered as statistically significant. All data were analyzed using BellCurve for Excel (Social Survey Research Information Co., Ltd., Tokyo, Japan) and were expressed as mean ± standard error of the mean (SEM).

3 Results

3.1 Lipid composition of EtnGpl and the hydrolysate from oysters and ascidians

TLC analysis confirmed that the enzymatic reaction generated lysoEtnGpl and FFA from EtnGpl (Fig. 1). The alkenyl moiety in oyster EtnGpl mostly comprised 18:0ol, and the prominent acyl moieties were EPA and DHA, while the ratio of AA was much lower (Table 1). The composition of the alkenyl and acyl moieties in the hydrolysate was nearly the same. In oyster EtnGpl, PlsEtn-18:0ol/22:6 was the predominant PL species, PlsEtn-18:0ol/20:4 was a minor species, and ChoGpl species were not observed. In the hydrolysate, only lysoEtnGpl species were detected, and the most prominent PL species was lysoPlsEtn-18:0ol.

The alkenyl moiety in ascidian EtnGpl mostly comprised 18:0ol, and the prominent acyl moiety was EPA, whereas AA was a minor moiety (Table 2). The composition of the alkenyl and acyl moieties in the hydrolysate was almost the same. PlsEtn-18:0ol/20:5 was also analyzed in ascidian EtnGpl because 18:0ol and EPA were abundant. In ascidian EtnGpl, PlsEtn-18:0ol/20:5 was the prominent PL species and PlsEtn-18:0ol/20:4 was a minor species. In the hydrolysate, lysoEtnGpl species, especially lysoPlsEtn-18:0ol, was the most abundant and PlsEtn-18:0ol/20:5 was observed at a lower ratio. The PlsEtn ratio of oyster and ascidian EtnGpl was found to be as 75.4 mol% and 88.4 mol% as per each carbon chain composition, respectively.

Fig. 1 LysoEtnGpl preparation by hydrolysis of EtnGpl. Ascidian EtnGpl was hydrolyzed using venom and samples before and after the reactions were subjected to TLC analysis; 1, EtnGpl standard; 2, Ascidian EtnGpl before the reaction; 3, Ascidian EtnGpl after the reaction. Mobile phase, chloroform:methanol:water (65:25:4, by volume). Left panel: Ninhydrin detection; Right panel: Sulfate detection. EtnGpl, ethanolamine glycerophospholipid; FFA, free fatty acid; TLC, thin-layer chromatography.

3.2 Effects of administration of oyster EtnGpl and its hydrolysate on the carbon chain composition and PL species levels in plasma

Experiment 1: Chronological changes in the carbon chain composition and PL species levels in plasma were investigated after administration of oyster EtnGpl and its hydrolysate. Administration of oyster EtnGpl increased the 18:0ol ratio in the plasma, which was composed of plasmalogens, at 4 h and DHA ratio in the plasma at 1 and 4 h after administration compared to the control group (Fig. S1). Administration of the hydrolysate also increased the EPA ratio in the plasma at 1 and 2 h and decreased the AA ratio at 2 h. Furthermore, administration of the hydrolysate increased the 18:0ol ratio in the plasma at 2 h compared to that in the control and EtnGpl groups.

When compared to the control group, administration of oyster EtnGpl significantly increased the plasma levels of PlsEtn-18:0ol/18:1 and 18:0ol/20:4 at 2 and 4 h after administration, and PlsEtn-18:0ol/22:6 at 4 h (Fig. 2). Administration of the hydrolysate also markedly increased the plasma levels of PlsEtn-18:0ol/20:4 at 1 h and PlsEtn-18:0ol/22:6 at 2 h. Notably, the LysoEtnGpl group showed importantly higher levels of PlsEtn-18:0ol/20:4 and 18:0ol/22:6 at 2 h when compared to the EtnGpl group. The LysoEtnGpl group showed the highest levels of lysoPlsEtn-18:0ol at 1 and 2 h among the groups, and a significantly higher level at 4 h compared to the control group, but a meaningfully lower level than that of the EtnGpl group. The EtnGpl

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Table 1

| Carbon Chain Composition | Percentage |
|--------------------------|------------|
| 18:0ol/18:1              | 50.0%      |
| 18:0ol/20:4              | 20.0%      |
| 18:0ol/20:5              | 10.0%      |
| 18:0ol/22:6              | 20.0%      |

Table 2

| Carbon Chain Composition | Percentage |
|--------------------------|------------|
| 18:0ol/18:1              | 50.0%      |
| 18:0ol/20:4              | 20.0%      |
| 18:0ol/20:5              | 10.0%      |
| 18:0ol/22:6              | 20.0%      |
Table 1  Composition of carbon chains and phospholipid species in EtnGpl and the hydrolysate obtained from oyster samples.

|                | Oyster (mol%) |    | Oyster (mol% in PL) |    |
|----------------|---------------|----|---------------------|----|
|                | EtnGpl        |    | EtnGpl              |    |
| Acyl           |               |    |                     |    |
| 16:0           | 7.4           | 7.8| 18:0ol/18:1         | 0.2|
| 18:0           | 6.2           | 6.7| 18:0ol/20:4         | 1.2|
| 18:1n-9        | 0.7           | 0.3| 18:0ol/22:6         | 26.0|
| 18:2n-6        | 0.3           | 0.2|                     |    |
| 18:3n-3        | 1.0           | 0.6|                     |    |
| 20:4n-6        | 1.9           | 1.9|                     |    |
| 20:5n-3        | 13.9          | 13.6|                    |    |
| 22:6n-3        | 15.9          | 15.0|                    |    |
| Others         | 14.8          | 15.8|                    |    |
| Alkenyl        |               |    |                     |    |
| 16:0ol        | 2.6           | 3.0|                     |    |
| 18:0ol        | 25.9          | 26.2|                    |    |
| 18:1ol        | 1.8           | 1.6|                     |    |
| Others        | 7.4           | 7.4|                     |    |

n.d., not detected; PL, phospholipids.

Table 2  Composition of carbon chains and phospholipid species in EtnGpl and the hydrolysate obtained from ascidian muscle.

|                | Ascidian (mol%) |    | Ascidian (mol% in PL) |    |
|----------------|-----------------|----|-----------------------|----|
|                | EtnGpl          |    | EtnGpl                |    |
| Acyl           |                 |    |                       |    |
| 16:0           | 0.9             | 1.5| 18:0ol/18:1           | 1.3|
| 18:0           | 4.4             | 4.5| 18:0ol/20:4           | 2.7|
| 18:1n-9        | 3.0             | 3.4| 18:0ol/20:5           | 54.0|
| 18:2n-6        | 0.2             | n.d.| 18:0ol/22:6           | 8.9|
| 18:3n-3        | 0.3             | 1.0|                       |    |
| 20:4n-6        | 2.0             | 2.0|                       |    |
| 20:5n-3        | 31.0            | 30.6|                     |    |
| 22:6n-3        | 10.3            | 11.2|                     |    |
| Others         | 3.7             | 3.9|                       |    |
| Alkenyl        |                 |    |                       |    |
| 16:0ol        | 3.6             | 2.8|                       |    |
| 18:0ol        | 33.8            | 34.0|                    |    |
| 18:1ol        | 3.6             | 2.4|                       |    |
| Others        | 3.2             | 2.6|                       |    |

n.d., not detected; PL, phospholipids.

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Fig. 2  Time dependent changes of various phospholipid levels in the plasma after administration of oyster EtnGpl and its hydrolysate in mice. Data represent the mean ± SEM (n = 4-5). Control group, vehicle administration; EtnGpl group, administration of oyster EtnGpl (390 μmol/kg body wt.); LysoEtnGpl group, administration of oyster EtnGpl hydrolysate (390 μmol of LysoEtnGpl plus FFA/kg body wt.). Different letters indicate significant differences at p < 0.05. PlsCho, choline plasmalogen; PlsEtn, ethanolamine plasmalogen; PtdEtn, phosphatidylethanolamine.

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group showed higher levels of PtdEtn-18:0/22:6 and 18:0/18:1 at 2 h and the LysoEtnGpl group showed higher levels of PtdEtn-18:0/18:1 at 2 h. The plasma levels of other EtnGpl species containing lysoPlsEtn-16:0ol and PtdEtn-18:0/20:4 were not significantly different among the groups.

In terms of ChoGpl species that were not detected in the administered samples, when compared to the control group, the LysoEtnGpl group showed importantly higher levels of PlsCho-18:0ol/18:1 at 1, 2, and 4 h, PlsCho-18:0ol/20:4 at 2 and 4 h, and PlsCho-18:0ol/22:6 at 2 h, whereas the EtnGpl group showed higher levels of PlsCho-18:0ol/18:1 and 18:0ol/20:4, but these were not significant. The plasma levels of other ChoGpl species were not significantly different among the groups.

3.3 Effects of administration of ascidian EtnGpl, its hydrolysate, and their mixture on carbon chain composition and PL species levels in the plasma

Experiment 2: To further confirm and clarify the PlsEtn kinetics, we analyzed the plasma data until 8 h after administering ascidian EtnGpl, its hydrolysate, and the mixture with an equivalent amount of total PL. The EtnGpl source and auxiliary composition of the emulsion for this experiment was different from those in Experiment 1. Albumin was added and the concentration of sodium taurocholate increased. This was done to reduce the possibility of emulsion formation by lysoPL, which forms an emulsion more easily than PL.

In terms of carbon chain composition in the plasma, when compared with the control group, administration of ascidian EtnGpl significantly increased the plasma EPA ratio at 1, 2, and 4 h after administration and did not affect the other ratios (Fig. S2). Administration of the hydrolysate also markedly increased the plasma ratio of 18:0ol at 4 h. Additionally, administration of the hydrolysate significantly increased the plasma EPA ratio at 1 h compared to that in the EtnGpl group.

When compared to the control group, administration of ascidian EtnGpl significantly increased the plasma levels of PlsEtn-18:0ol/20:4 and 18:0ol/22:6 at 4 and 8 h after administration, PlsEtn-18:0ol/20:5 at 2, 4, and 8 h, and lysoPlsEtn-18:0ol at 4 h (Fig. 3). Concomitantly, administration of the hydrolysate also markedly increased the plasma levels of PlsEtn-18:0ol/20:4 and 18:0ol/22:6 at 1 and 2 h and the plasma levels of lysoPlsEtn-18:0ol at 1 and 8 h. Additionally, the LysoEtnGpl group showed importantly higher levels of PlsEtn-18:0ol/20:4 and 18:0ol/22:6 at 1 and 2 h and higher levels of PlsEtn-18:0ol/20:5 at 2 h when compared with the EtnGpl group. In terms of the absorption kinetics of PlsEtn species, the Mix group showed middle tendencies between the EtnGpl and LysoEtnGpl groups. Although, the plasma levels of lysoPlsEtn-16:0ol and PtdEtn-18:0/22:6, 18:0/20:4, and 18:0/18:1 were not significantly different among the groups, the plasma levels of lysoPtdEtn-18:0 and 16:0 at 8 h were meaningfully lower in the LysoEtnGpl and Mix groups than in the control and EtnGpl groups.

In terms of ChoGpl species, the LysoEtnGpl group showed significantly higher levels of PlsCho-18:0ol/20:4 at 1, 2, and 4 h, of PlsCho-18:0ol/22:6 at 4 h and higher levels of lysoPlsCho-18:0ol at 2 and 4 h. At 8 h after administration, the plasma levels of PlsCho-18:0ol/20:4 and 18:0ol/22:6 and lysoPlsCho-18:0ol in the LysoEtnGpl group were meaningfully decreased and were almost the same as those in the control group, whereas these levels in the EtnGpl group increased and were the highest among all groups. Plasma kinetics of PlsCho species in the Mix group tended to exhibit intermediate kinetics between those of EtnGpl and LysoEtnGpl groups. Plasma levels of other ChoGpl species were not significantly different among the groups.

3.4 Effects of administration of ascidian EtnGpl, its hydrolysate, and their mixture on the AUC of PL species

The area under the plasma concentration-time curve (AUC) of each PL species was calculated by subtracting the values in the control group based on Fig. 3 (Fig. 4). As plasma was obtained from different mice at different times, average values were used and could not be tested for significant differences.

Clarified increases in AUC were observed in PL species related to the alkenyl chain 18:0ol, which was predominant in the administered samples from ascidians. Hydrolysis of EtnGpl raised the AUC of these species, especially in the early stage compared to that of untreated EtnGpl. The mixture of untreated and treated EtnGpl showed intermediate AUC levels.

4 Discussion

PlsEtn has been reported to exert many biological functions on various parts of the body. These functionalities include improvement of cognitive impairment, suppression of aberrant crypt foci formation and inflammation in the colon, suppression of cholesterol synthesis in in vitro cells, suppression of atherosclerosis formation, and alleviation of lead-cytotoxicity in in vitro liver cells. As it is important to understand PlsEtn absorption and metabolism to clarify its function, some studies on PlsEtn absorption have been performed using lymph cannulation and everted jejunal sac model. These studies indicate that the administered PlsEtn is preferentially re-esterized to AA and partly converted to PlsCho during absorption. In the present study, we analyzed the kinetics from a different perspective and compared the uptake kinetics of PlsEtn with the lyso-form in the plasma of mice. EtnGpl containing high PlsEtn concentration was prepared from oyster
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Fig. 3  Time dependent changes of various phospholipid levels in the plasma after administration of ascidian EtnGpl, its hydrolysate, and their combination in mice. Data represent the mean ± SEM (n = 3-4). Control group, vehicle administration; EtnGpl group, administration of ascidian EtnGpl (390 μmol/kg body wt.); LysoEtnGpl group, administration of ascidian EtnGpl hydrolysate (390 μmol of LysoEtnGpl plus FFA/kg body wt.); Mix group, administration of their combination (195 μmol of EtnGpl and 195 μmol of LysoEtnGpl plus FFA/kg body wt.). Different letters indicate significant difference at \( p < 0.05 \).

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and ascidian, and the hydrolysates, which were rich in lysoPtdCho and had the same carbon chain composition as EtnGpl, were prepared from EtnGpl (Fig. 1 and Tables 1 and 2). EtnGpl administration increased the plasma levels of PlsEtn and PlsCho species, whereas administration of the hydrolysates shortened the absorption and metabolism time of plasmalogen species compared to that observed with EtnGpl (Figs. 2 and 3). Moreover, hydrolysis of EtnGpl raised the AUC$_{0-8h}$ of PlsEtn and PlsCho species (Fig. 4).

As administration of oyster lysoEtnGpl increased the plasma levels of PlsEtn species and shortened the absorption time compared to that with oyster EtnGpl (Fig. 2), we inferred that the administered PlsEtn was hydrolyzed at the sn-2 position and re-esterized as reported in previous studies$^{15,16}$. However, lysoPL has also been reported to affect the absorption of other lipophilic components as well as the formation of emulsion. For instance, lysoPtdCho increases the uptake of carotenoids and cholesterol in intestinal cells$^{24,25}$; therefore, it is also possible that lysoEtnGpl stimulates the absorption of endo- and/or exo-genous PlsEtn remaining in the digestive organs. Thus, we increased the concentration of the emulsifier in Experiment 2 relative to Experiment 1 and investigated the uptake kinetics of the mixture of EtnGpl and its hydrolysate as well.

Fig. 4 Differences in the AUC of plasmalogen species in the plasma over the period of 0-8 h after the administration of ascidian EtnGpl, its hydrolysate, and their combination in mice. The ∆AUC of each group was obtained by subtracting the AUC of the control group from the AUC of each group in Fig. 3. AUC, area under plasma concentration-time curve.
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Administration of oyster and ascidian hydrolysates rich in lysoPlsEtn-18:0ol and EPA markedly increased the plasma levels of PlsEtn-18:0ol/20:4 from non-detectable levels before administration and also increased the levels of PlsEtn-18:0ol/20:5 and PlsCho species. Nevertheless, AA was observed in small amounts and the choline base was not detected in the samples (Figs. 2 and 3). Although DHA, EPA, AA, and 18:1 had different concentrations in the administered samples (Tables 1 and 2), PlsEtn bearing DHA, EPA, and AA showed nearly the same maximum level in plasma, whereas PlsEtn bearing 18:1 showed a slightly increased level. Previous reports show that administered PlsEtn is preferentially re-esterized to AA and partly converted to PlsCho[15,16]. On the other hand, administration of EPA-rich PlsEtn increased the serum levels of PlsEtn bearing EPA by about 40-fold compared to that before administration[26]. Our results suggest that during the absorption of PlsEtn, lysoPlsEtn was preferentially esterized to PUFA independent of the fatty acid composition in the administered samples, and that the stored range in each plasma PlsEtn species bearing PUFA may be almost at the same level.

Gradual changes in PlsEtn levels in blood help to understand the absorption and metabolism of PlsEtn, and the mechanism by which PlsEtn hydrolysis shortens absorption and metabolism duration (Fig. 3). Rate of increase in plasma PlsCho species was slower than that of PlsEtn species, and elimination of PlsCho species occurred immediately, although we could not ascertain whether the decrease was due to distribution to the organs and/or hydrolysis. While it cannot be directly compared, a previous study that investigated the maximum concentration of PlsCho species in lymph output showed a delay time after administration compared to that of the PlsEtn species due to enzymatic base conversion from PlsEtn[22]. The cardiovascular system is known to possess the highest PlsCho ratio of ChoGpl among organs[23]; therefore, plasma PlsCho species may be easily distributed to the cardiovascular system. Additionally, administration of lysoPlsEtn decreased several plasma PtdEtn species later after administration when compared to the control group. The total level of EtnGpl is thus tightly regulated[27]. Therefore, PtdEtn biosynthesis may be suppressed due to an increase in PlsEtn. Further clarification can be accomplished by comparing the lysoPlsEtn kinetics with PlsEtn using in vitro intestinal cells and the ex vivo everted jejunal sac model.

Administration of ascidian hydrolysate rich in lysoPlsEtn-18:0ol increased the AUC of 18:0ol-related plasmalogens until 8 h after administration compared to that of ascidian EtnGpl (Fig. 4). Continual administration of PlsEtn is reported to increase PlsEtn levels in the blood and organs of healthy rodents, and in disease models[10,12,14]. In terms of lysoPL availability, administration of lysoPtdCho with DHA has been reported to be effective in increasing the levels of PtdCho bearing DHA in rat lymph[28]. Furthermore, lysoPtdCho-DHA intake for 30 days increases DHA levels in the mouse brain[29]. Additionally, lysoPL has been recognized as a biologically active lipid mediator[30]. LysoPtdCho-DHA shows anti-inflammatory effects in in vivo mice and in vitro macrophages[31]. Therefore, continual lysoPlsEtn intake may effectively increase PlsEtn levels in the brain and the other organs, and thus, lysoPlsEtn may show biological activity. However, hydrolysis to lysoPlsEtn may reduce dietary functions of PlsEtn. Dietary PtdEtn and PlsEtn decrease serum cholesterol levels in rats and mice[20,32], whereas lysoPtdCho as a PL hydrolysate accelerates cholesterol uptake in in vivo intestinal cells[24]. Additionally, it may be of interest whether lysoPlsEtn exerts the same intestinal protection as that demonstrated by PlsEtn[33]. It is thus necessary to study continual lysoPlsEtn intake in detail.

In conclusion, our results reconfirmed that administered PlsEtn was hydrolyzed at the sn-2 position and re-esterized when PlsEtn absorption occurred via lysoPlsEtn using a different approach compared to that reported in previous studies. Overall, the hydrolysis of PlsEtn may increase the PlsEtn uptake in the plasma and organs and enhance PlsEtn availability.

Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

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as their single administrations. The uptake kinetics of their single administrations indicated tendencies that were similar to those in Experiments 1 and 2, and the administration of both (Mix group) indicated tendencies that were intermediate between those of EtnGpl and its hydrolysate (Fig. 3). Overall, these results strongly suggest that the administered PlsEtn is absorbed via the lysoPlsEtn form.

Administration of oyster and ascidian hydrolysates rich in lysoPlsEtn-18:0ol and EPA markedly increased the plasma levels of PlsEtn-18:0ol/20:5 from non-detectable levels before administration and also increased the levels of PlsEtn-18:0ol/20:4 and PlsCho species. Nevertheless, AA was observed in small amounts and the choline base was not detected in the samples (Figs. 2 and 3). Although DHA, EPA, AA, and 18:1 had different concentrations in the administered samples (Tables 1 and 2), PlsEtn bearing DHA, EPA, and AA showed nearly the same maximum level in plasma, whereas PlsEtn bearing 18:1 showed a slightly increased level. Previous reports show that administered PlsEtn is preferentially re-esterized to AA and partly converted to PlsCho[15,16]. On the other hand, administration of EPA-rich PlsEtn increased the serum levels of PlsEtn bearing EPA by about 40-fold compared to that before administration[26]. Our results suggest that during the absorption of PlsEtn, lysoPlsEtn was preferentially esterized to PUFA independent of the fatty acid composition in the administered samples, and that the stored range in each plasma PlsEtn species bearing PUFA may be almost at the same level.

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