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# Alteration in plasma docosahexaenoic acid levels following oral administration of ethyl icosapentate to rats

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ABSTRACT

*Objectives:* Ethyl icosapentate, a prodrug of eicosapentaenoic acid (EPA), has been prescribed to not only hyperlipidemia, but also psychotic patients. We have examined the impact of an orally administered polyunsaturated fatty acid (PUFA), ethyl icosapentate, on the plasma concentrations of seven other types of fatty acids and one metabolite (3-hydroxybutyrate, 3-HB) using rats.
*Design:* and Methods: A commercial omega-3 PUFA, EPA, formulation (ethyl icosapentate, Epadel®) was administered orally to Sprague-Dawley rats (15, 50, 100 mg/kg, $n = 4$–8) and changes in the plasma fatty acid concentrations were investigated by HPLC using fluorescence detection.
*Results:* The concentration of an *n*-3 PUFA, docosahexaenoic acid (DHA), was significantly increased from $11.6 \pm 1.45$ (0 h) to $25.9 \pm 6.54$ μM (6 h) in rat plasma ($n = 8$, $p = 1.88 \times 10^{-2}$) at a dose of 100 mg/kg, as was the EPA concentration from $2.58 \pm 0.16$ (0 h) to $6.03 \pm 2.20$ μM (1 h) ($n = 8$, $p = 2.09 \times 10^{-2}$), whereas concentrations of other fatty acids, such as α-linolenic acid, palmitoleic acid, arachidonic acid, linolenic acid, and oleic acid, were not significantly changed. In addition, the concentration of the ultimate fatty acid metabolite, 3-hydroxybutyrate (3-HB), was significantly increased (from $94.6 \pm 10.2$ to $217 \pm 43.4$, $p = 5.41 \times 10^{-3}$) 12 h after oral administration of ethyl icosapentate ($n = 8$, 100 mg/kg).
*Conclusions:* This result suggests that intake of the EPA formulation contributed not only to an increase in EPA concentration, but also to increases in DHA and 3-HB concentrations *in vivo*.

## 1. Introduction

There have been many reports on the role of polyunsaturated fatty acids (PUFAs) in psychiatric diseases [1–3]. These reports suggest that a considerable deficiency in PUFAs may occur in psychiatric diseases, such as schizophrenia. Indeed, decreased serum or plasma PUFA levels have been reported in patients with schizophrenia by several groups [1,4,5]. Previously, a fish oil add-on therapy (including PUFAs with anti-psychotics) has been reported to ameliorate symptoms in inpatients with schizophrenia [6].

Among the PUFAs, which can contain both *n*-3 and *n*-6 PUFAs (Fig. S1), the *n*-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported as especially good for human health and a mixed formulation has been marketed as a daily supplement promoting health benefits. PUFA formulation tablets, such as the EPA ethyl ester ethyl icosapentate (Epadel®), or the mixed formulation of both EPA and DHA ethyl esters (Lotriga®), have been prescribed for anti-hyperlipidemia, but are also currently being

*Abbreviations:* 3-HB, 3-hydroxybutyrate; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

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prescribed in some clinics to patients with psychosis. Accumulating evidence suggests both of these *n*-3 PUFAs (EPA and DHA) are important for human brain health.

There have been some reports on the influence of *n*-3 PUFA intake on other fatty acids including *n*-3 or *n*-6 PUFA concentrations in human and rodents *in vivo* [7,8]. In the previous reports, however, changes in fatty acid concentrations were examined at interval of week [7], or triglyceride of EPA or DHA was fed with a commercial diet, CE-2, to mice [8]. Therefore, there have been few reports on the time-course profiles of changes in fatty acids within 2 days after dosing of *n*-3 PUFA. In the present study, one *n*-3 PUFA, eicosapentaenoic acid (EPA), formulation (ethyl icosapentate, Epadel®) was administered orally to Sprague-Dawley rats and changes in the plasma concentrations of 7 types of fatty acids were investigated by HPLC using fluorescence detection. In addition, the ultimate metabolite of β–oxidation from fatty acids, 3-hydroxybutyrate (3-HB), which is well known as one of the ketone bodies (Fig. S2), was also determined after ethyl icosapentate administration to rats, as it has recently been reported that 3-HB suppressed oxidative stress by inhibiting histone deacetylase activity [9].

## 2. Materials and methods

### 2.1. Chemicals

*α*-Linolenic acid (ALA), palmitoleic acid (PLA), arachidonic acid (AA), linoleic acid (LA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), oleic acid (OA), docosahexaenoic acid ethyl ester (DHA ethyl ester), and heptadecanoic acid used as internal standards (IS) were purchased from Sigma-Aldrich (St. Louis, MO). Dimethylformamide (DMF) and perchloric acid (60%) were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Chloroform, *n*-heptane, and trifluoroacetic acid (TFA, HPLC-grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Triphenylphosphine (TPP) and 2,2′-dipyridyl disulfide (DPDS) were purchased from Tokyo Chemical Industries, Ltd. (Tokyo, Japan). HPLC grade acetonitrile ($CH_3CN$) and methanol (MeOH) were purchased from Kanto Kagaku Kogyo (Tokyo, Japan). All chemicals were analytical-grade reagents. The EPA formulation, Epadel S300®, was purchased from Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan). 4-[2-(*N*, *N*-dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DBD-ED) [10] and 4-nitro-7-piperazino-2,1,3-benzoadiazole (NBD-PZ) [11] were purchased form Tokyo Chemical Industries Co., Ltd. (Tokyo, Japan).

### 2.2. Purity analysis of ethyl icosapentate formulation by LC-TOF-MS

Extraction method of ethyl icosapentate from commercial capsule was performed according to our previous fatty acid analysis method [12]. Three hundred mg of ethyl icosapentate formulation (corresponding to 15 tablets) were suspended in 3.0 mL of saline. An aliquot (100 μL) was sampled into a glass tube with a screw cap, then extracted with 800 μL of chloroform, 100 μL of MeOH, and 100 μL of *n*-heptane. The extract (600 μL organic layer) was evaporated to dryness under reduced pressure and the residue was dissolved in 50 μL of DMF/$CH_3CN$ (50/50) (see Supplementary material 1). After filtration with a 0.2 μm Cosmospin filter G® (Nacalai Tesque Inc., Kyoto, Japan), an aliquot of the resultant solution (10 μL) was injected onto an LC-time of flight-mass spectrometer (LC-TOF-MS). Detailed conditions of the LC-TOF-MS are described in Supplementary material 1.

### 2.3. HPLC-fluorescence detection of fatty acids in rat plasma

Determinations of fatty acids in rat plasma were performed according to a previously described method [10,12] with minor modifications.

Ten microliters of rat plasma were mixed with 10 μL of 50 μM heptadecanoic acid ($C_{17:0}$) as an internal standard (IS) and 10 μL of $CH_3CN$. Then, 30 μL of perchloric acid (60%) was added for deproteinization. Finally, 800 μL of chloroform, 100 μL of MeOH, 100 μL of *n*-heptane, and 100 μL of $H_2O$ were added to extract the fatty acids. The mixture was vortexed for 1.0 min and centrifuged at 3,000 × *g* for 5 min. The organic layer (600 μL) was then transferred to a brown tube and evaporated to dryness (20 min) under reduced pressure using a centrifugal concentrator, VC-36N (TAITEC Co., Ltd., Saitama, Japan) without heating.

The dried residue was dissolved in 50 μL of DMF, and mixed with 50 μL of 10 mM DBD-ED in DMF, 50 μL of 140 mM TPP in $CH_3CN$, and 50 μL of 140 mM DPDS in $CH_3CN$ to derivatize the fatty acids to fluorescence derivaties (Fig. S3). The resultant mixture was vortexed for 1 min and incubated at room temperature for 120 min. The reaction solution was diluted 5-fold with mobile phase A, filtered with 0.2 μm Cosmospin filter G® (Nacalai Tesque Inc., Kyoto, Japan), and injected (50 μL) to an HPLC system as described below. HPLC conditions and calibrations are described in Supplementary materials 2. The obtained chromatograms are shown in Fig. S4. Each concentration of the fatty acids in rat plasma was determined by each calibration curve constructed by plotting peak area ratio of fatty acids to I.S. against standard concentration ($n = 4$) (Fig. S8 (a)–(g)).

### 2.4. HPLC-fluorescence detection for 3-HB

3-HB was determined by HPLC-fluorescence detection according to a previously described method with minor modification [13] (Fig. S5) using 5.0 μL of rat plasma and following the procedure described in Supplementary material 3. The obtained chromatograms are shown in Fig. S6. Each concentration of 3-HB in rat plasma was determined by each calibration curve constructed by plotting peak area ratio of 3-HB to I.S. against standard concentration ($n = 4$) (Fig. S8 (h)).

### 2.5. Animal experiments

This study was approved by the Animal Care Committee located in Toho University (No. 17-51-369, 18-52-369). Sprague-Dawley rats (male, 7-week-old) were purchased from Charles River Japan (Kanagawa, Japan) and housed in an environmentally controlled room at least 1 week before use. One package of Epadel S300® (300 mg) was added to 20 mL of sterilized saline (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) and vortexed under gentle heating to prepare an emulsion solution (15 mg/mL).

The emulsion solution was administered orally to rats [15 ($n = 4$), 50 ($n = 8$), and 100 ($n = 8$) mg/mL/kg], and the absolute values of ethyl icosapentate to rats were $5.33 \pm 0.21$ (15 mg/kg), $17.9 \pm 0.28$ (50 mg/kg), and $32.5 \pm 1.00$ mg (100 mg/kg), respectively.

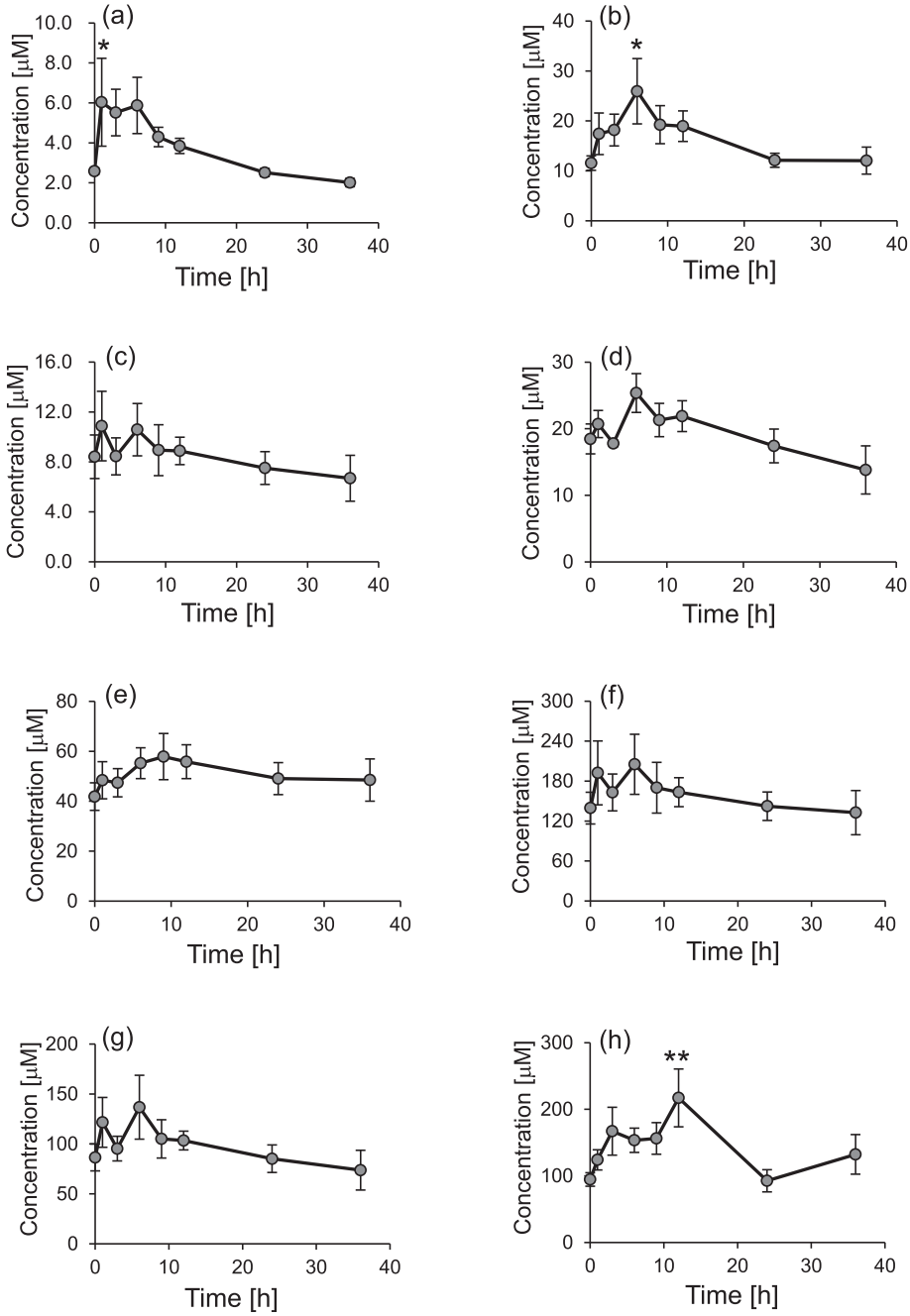


**Fig. 1.** Time-course profiles of plasma fatty acids (a–g) and 3-HB (h) concentrations after oral administration of ethyl icosapentate (100 mg/kg) ($n = 8$) (a) EPA, (b) DHA, (c) ALA, (d) PLA, (e) AA, (f) LA, (g) OA, (h) 3-HB
*: $p < 0.05$, **: $p < 0.01$.

The blood (approximately 200 μL) was drawn from the jugular vein through a heparinized syringe at 0, 1, 2, 4, 6, 9, 12, 24, and 36 h after the administration. The blood was centrifuged at 3,000 × *g* for 15 min at 4 °C and the obtained plasma was stored at −80 °C until analysis.

### 2.6. Statistical analysis

The differences between the fatty acid concentrations at each time point relative to time 0 were analyzed for statistical significance using the Dunnett test. A *p*-value below 0.05 was considered different.

The increment of fatty acid concentration at each time point (fatty acid concentration at each time – fatty acid concentration at 0 h) was used to calculate the increment of area under the curve (*Δ* AUC) values by the trapezoidal method.

## 3. Results and discussion

In the present study, a suspended PBS solution of ethyl icosapentate was administered orally to Sprague-Dawley rats to examine the resulting alterations of plasma fatty acid levels, especially those of EPA and DHA. The solution of ethyl icosapentate for dosage in $CH_3CN$/DMF (50/50) was subjected into LC-TOF-MS analysis; the data reveal that the formulation contains only ethyl icosapentate and no both EPA and DHA (Figs. S7c and d). In addition, no DHA ethyl ester was included in the formulation (Fig. S7e). This result was consistent with a previous analysis of the ethyl icosapentate formulation reported by Kodama et al. [14].

As a consequence of oral administration of ethyl icosapentate, EPA levels in rat plasma increased gradually and reached a maximum concentration at 1–6 h (Fig. 1a).

The EPA concentration was increased from 2.58 ± 0.16 (0 h) to 6.03 ± 2.20 μM (1 h) ($n = 8$, $p = 2.09 \times 10^{-2}$) after oral ethyl icosapentate administration at a dose of 100 mg/kg.

The levels decreased slowly after 6 h, returning to near initial levels at 24 h. Although a tendency for slightly increased levels (ALA, PLA, AA, LA, and OA) were observed in the other fatty acids examined by HPLC, only DHA, which is an *n*-3 PUFA like EPA, showed a significant difference (Fig. 1b). The concentration of DHA was significantly increased from 11.6 ± 1.45 (0 h) to 25.9 ± 6.54 μM (6 h) in rat plasma ($n = 8$, $p = 1.88 \times 10^{-2}$) at a dose of 100 mg/kg.

Since EPA is transformed to DHA by two elongase steps, a Δ6-desaturation and peroxisomal β-oxidation, in the metabolic pathway of *n*-3 PUFAs (Fig. S1), it is likely that the increase in plasma DHA concentration was caused by administration of the EPA formulation. The DHA levels in rat plasma increased gradually and reached the maximum concentration at 6 h. The $T_{max}$ of DHA was delayed compared to the $T_{max}$ of EPA, suggesting that DHA may be metabolically produced from EPA upon dosing with ethyl icosapentate. The DHA levels decreased slowly and were back to near the initial concentration at 24 h. As shown in Fig. S7, the LC-TOF-MS analysis of the ethyl

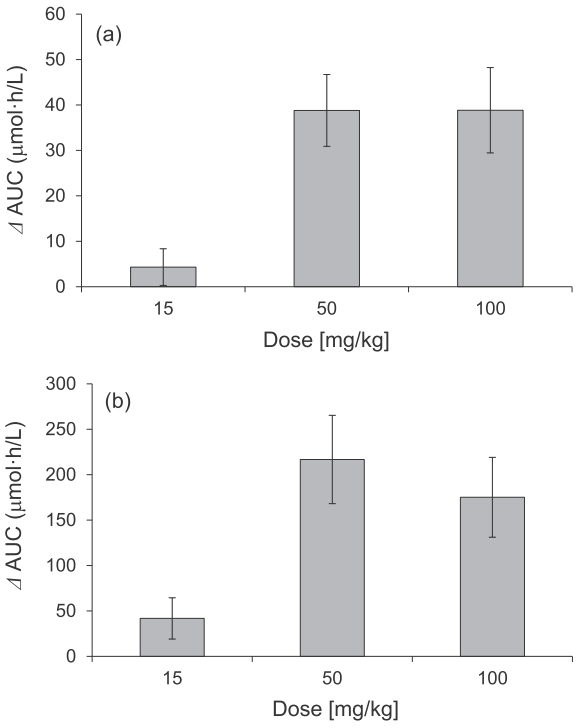


**Fig. 2.** *Δ* Area under the curve (*Δ* AUC) of plasma EPA (a) and DHA (b) concentrations of rats dosed at 15, 50, and 100 mg/kg after oral administration of Epadel® to rats ($n = 4$–8).

icosapentate formulation showed no PUFA peaks (including EPA and DHA) in the chromatogram, indicating these fatty acids were not present in the EPA formulation itself. In addition, since no DHA ethyl ester was also contaminated in the formulation, the observed increases in plasma EPA and DHA levels may be ascribed to metabolism of ethyl icosapentate *in vivo*. As expected, the $\Delta$ AUC of the plasma EPA and DHA were increased more at a dose of 50 and 100 mg/kg than at 15 mg/kg of ethyl icosapentate (Fig. 2). These results also suggest that DHA was produced from EPA, as the lack of significant increases in DHA concentrations at ethyl icosapentate doses above 50 mg/kg suggests saturation of the absorption or metabolism processes.

Regarding 3-HB, which is the ultimate fatty acid metabolite, significant increases in the plasma 3-HB levels were observed 12 h after oral dosing of ethyl icosapentate (Fig. 1h). The 3-HB metabolite is produced by β-oxidation of free fatty acids in the mammalian body; therefore, it is reasonable that the $T_{max}$ of 3-HB was delayed considerably compared to that of EPA or DHA. The 3-HB was significantly increased from $94.6 \pm 10.2$ (0 h) to $217 \pm 43.4$ at 12 h ($p = 5.41 \times 10^{-3}$) after oral administration of ethyl icosapentate ($n = 8$, 100 mg/kg). Given that 3-HB could suppress oxidative stress by inhibiting histone deacetylase activity [9], increased 3-HB plasma levels may exert some pharmacological effects on the intake of ethyl icosapentate. Therefore, *in vivo* production of 3-HB and the anti-oxidative effect of ethyl icosapentate should be considered in future studies.

The present study has a limitation on species difference between rats and human. Although dosed amount of ethyl icosapentate was considerably different, $T_{max}$ of EPA in plasma has been reported at approximately 6 h after oral dosing (2,700 mg) in human [15], but the present data revealed that $T_{max}$ of EPA was observed at 1 h after oral dosing (approx. 32.5 mg/rat), suggesting that an absorption of ethyl icosapentate was too rapid in rats than human. In addition, care should be taken on the species difference on the metabolic ratio of EPA to DHA followed by 3-HB, because it has been reported that no increase in plasma DHA concentration occurred in response to dietary supplementation of EPA in human [16]. Therefore, the present results may occur specifically in rats. In future, a clinical study exploring whether production of DHA and 3-HB occur or not after single oral administration of ethyl icosapentate in human should be necessary.

## 4. Conclusions

In addition to increasing the plasma concentrations of EPA, intake of the EPA formulation, ethyl icosapentate, also leads to increases of DHA and 3-HB through *in vivo* metabolic pathways for *n*-3 PUFAs in rats.

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## Declaration of competing interest

There are no conflicts of interest to report.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2019.e00143.

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