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Enhanced Production of EPA-Derived Anti-Inflammatory Metabolites after Oral Administration of a Novel Self-Emulsifying Highly Purified EPA Ethyl Ester Formulation (MND-2119)

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Aims: MND-2119 is a novel once-daily dose self-emulsifying formulation of highly purified eicosapentaenoic acid ethyl ester (EPA-E) and is approved as an antihyperlipidemia agent in Japan. It has improved absorption and achieves higher plasma EPA concentrations at C_{max} than conventional EPA-E. In the JELIS trial, concomitant use of EPA-E with statin therapy significantly reduced atherosclerotic cardiovascular disease (ASCVD) risks. As a potential mechanism of action of EPA, endogenous formation of EPA-derived anti-inflammatory metabolites is receiving greater attention. This study aims to investigate the endogenous formation of EPA-derived anti-inflammatory metabolites following single and multiple administrations of MND-2119.

Methods: Healthy adult male subjects were randomly assigned to a nonintervention (control) group, MND-2119 2-g/day group, MND-2119 4-g/day group, or EPA-E 1.8-g/day group for 7 days (N=8 per group). Plasma fatty acids and EPA-derived metabolites were evaluated. Peripheral blood neutrophils were isolated, and the production of EPA-derived metabolites from *in vitro* stimulated neutrophils was evaluated.

Results: After single and multiple administrations of MND-2119 2 g/day, there were significant increases in plasma EPA concentration, 18-hydroxyeicosapentaenoic acid (18-HEPE), and 17,18-epoxyeicosatetraenoic acid compared with those of EPA-E 1.8 g/day. They were further increased with MND-2119 4 g/day administration. In neutrophils, the EPA concentration in the MND-2119 2-g/day group was significantly higher compared with that in the EPA-E 1.8-g/day group after multiple administration, and 18-HEPE production was positively correlated with EPA concentration. No safety issues were noted.

Conclusions: These results demonstrate that MND-2119 increases the plasma and cellular concentrations of EPA and EPA-derived metabolites to a greater extent than conventional EPA-E formulations.

Key words: Eicosapentaenoic acid, MND-2119, Metablolite, Inflammation

Introduction

Eicosapentaenoic acid (EPA), an n-3 polyunsaturated fatty acid (n-3 PUFA), is widely known to be beneficial for human health[1)]. In a Japanese community, the serum EPA/arachidonic acid (AA) ratio decreased over 10 years[2)]. Previous studies showed that a lower serum EPA/AA ratio is predictive of the incidence of cardiovascular events[3-6)]. The JELIS and REDUCE-IT trials verified that highly purified EPA ethyl ester (EPA-E) reduced the risk of cardiovascular events[7, 8)]. In Japan, highly purified EPA-E has been used clinically for treating arteriosclerosis obliterans and hyperlipidemia.

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Recently, a pharmaceutically well-designed once-daily self-emulsifying formulation of EPA-E (MND-2119) was developed to improve absorption of EPA in the gastrointestinal tract. For the effect on the serum triglyceride (TG) levels in patients with hypertriglyceridemia, 12-week treatment with once-daily dosing of MND-2119 (2 g, semel in die [SID]) was shown to be noninferior to that with twice-daily dosing of the conventional formulation of EPA-E (1.8 g, bis in die [BID], clinically utilized dosage)[9]. Long-term administration of MND-2119 2 and 4 g/day was not associated with any safety-related problems[10].

MND-2119 contains ingredients that enable EPA-E to be emulsified without bile acids. Orally ingested long-chain fatty acids including EPA-E are generally emulsified by bile acids to form micelles to be absorbed efficiently[11]. Previous studies on several n-3 PUFA formulations have reported that n-3 PUFA ethyl esters have low bioavailability when administered before meals. However, the emulsification formula of n-3 PUFA ethyl esters improved bioavailability when taken without meals[12, 13]. In the pharmacokinetic study of MND-2119 in healthy male adult subjects[14], plasma EPA concentrations following multiple adminitirations of 2 g SID after a meal were higher around C_{max} (approximately 3–4 h postdose) and remained higher throughout the 11-day dosing period than those achieved with the conventional formulation doses of 1.8 g BID after a meal.

The anti-inflammatory properties of EPA are considered associated with a reduced risk of cardiovascular disease[1, 15, 16]. In the Hisayama study, a greater risk of cardiovascular disease was associated with a lower serum EPA/AA ratio, especially in the subjects with high hsCRP levels (≥ 1.0 mg/L)[6]. Another study also showed that a lower serum EPA/AA ratio could predict the long-term prognosis in patients with acute ischemic stroke[3]. As a mechanism for this anti-inflammatory effect, specialized proresolving mediators (SPMs) and EPA-derived metabolites with proresolving actions are attracting attention[15, 17, 18]. Both 18-hydroxyeicosapentaenoic acid (18-HEPE) and 17,18-epoxyeicosatetraenoic acid (17,18-EpETE) are n-3 oxygenated products from EPA, which can be measured in plasma under a static condition. They are not just precursors of SPMs but anti-inflammatory mediators themselves, so measuring them may serve as markers of EPA-derived SPM production. A study on the inhibitory effects of resolvin E1 (RvE1) on atherogenesis in ApoE*3-Leiden mice showed the relationships between EPA-derived SPMs and cardiovascular diseases[19], and other studies showed the inhibitory effects of 18-HEPE and 17,18-EpETE on developing myocardial fibrosis with tissue remodeling[20] and pulmonary hypertension with vascular remodeling[21], respectively.

Endogenous formations of hydroxy- and epoxy-fatty acid metabolites generally correlate with their metabolic precursor PUFA levels[22]. Given the higher concentrations of plasma EPA after MND-2119 administration than those after conventional formulation administration, it was expected that MND-2119 administration would increase the plasma and cellular EPA and EPA-derived metabolites concentrations to a greater extent than that of conventional EPA-E formulations.

Aim

In this study, we determined the endogenous levels of EPA and EPA-derived hydroxy- and epoxy-fatty acid metabolites following single and multiple administrations of MND-2119 at 2 or 4 g, using EPA-E 1.8 g and nonintervention (control) as controls.

Methods

Study Organization and Ethics

This study was conducted at Kitasato University Kitasato Institute Hospital in Japan from October 2021 to December 2021. All subjects underwent the informed consent process before enrollment, as evidenced by their written informed consent. The study protocol and informed consent document were reviewed and approved by the IRB of Kitasato University Shirokane Campus before the study was initiated. The studies were conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines issued by the Ministry of Health, Labour, and Welfare of Japan and the International Conference on Harmonization. This study is registered in the Japan Registry of Clinical Trials (jRCT2031210362).

Study Subjects

Healthy male subjects aged 20–40 years with a body weight of 55.0–79.0 kg and a body mass index

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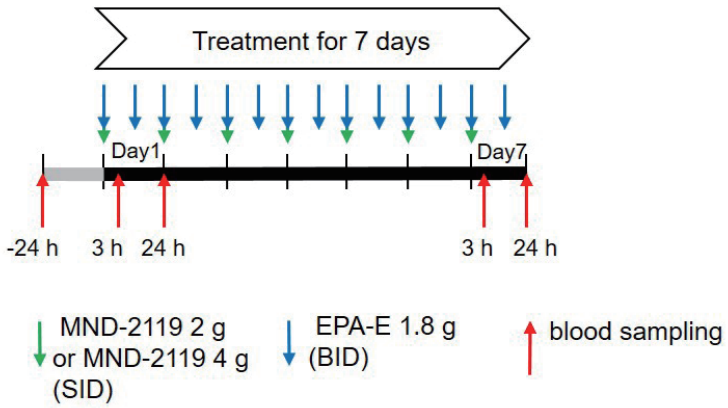

Fig. 1. Study design

Abbreviations: SID, semel in die; BID, bis in die.

between ≥ 18.5 and < 25.0 kg/m^2 were considered eligible for this study. All subjects were determined to be healthy using their medical history, physical examination, screening laboratory tests, and standard 12-lead electrocardiography. Subjects were excluded if their plasma EPA concentration was ≤ 10 μg/mL or ≥ 60 μg/mL at screening tests, or they took large amounts of food or beverages containing seafood, seaweed, EPA, or docosahexaenoic acid (DHA) within 3 days before the first dose of the study drug. During hospitalization, all subjects were provided with the same meals, which did not include seafood or seaweed.

Study Design and Treatment

This study was a single-center, open-label, randomized, parallel-group study investigating the plasma and blood cell fatty acid composition, neutrophil-derived EPA metabolites, and cytokine production after multiple oral administrations of MND-2119 in healthy male adult volunteers using both highly purified EPA-E and nonintervention as controls. A sufficient number of eligible subjects were admitted to the study site 2 days before the first dose of the study drugs, and 32 of these subjects were randomized equally to the four treatment groups: the MND-2119 2-g (MND-2119, SID, self-emulsifying EPA-E 2 g/day), MND-2119 4-g (MND-2119, SID, self-emulsifying EPA-E 4 g/day), EPA-E 1.8-g (EPADEL S900®, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan, BID, EPA-E 1.8 g/day), and control (nonintervention) groups. During the 7-day treatment period, blood samples were collected at baseline (24 h before the first dose) and 3 (Day 1 3 h), 24 (Day 1 24 h), 147 (Day 7 3 h), and 168 (Day 7 24 h) h after the first dose **(Fig. 1)**.

Preparation of Plasma and Red Blood Cells (RBCs)

EDTA-2Na containing whole blood samples were centrifuged (1200 ×g, for 10 min) to separate plasma from other blood components. Aliquots of this plasma were stored at −80℃ until assay. After plasma and buffy coat removal, RBCs were suspended in phosphate-buffered saline containing 4 mM EDTA, gently mixed, and pelleted via centrifugation (300 ×g, for 10 min). The washing procedure was repeated, and the resulting washed RBCs were transferred to polypropylene tubes and stored at −80℃ until assay.

Preparation of Neutrophils and Calcium Ionophore Stimulation

Neutrophils were isolated by the immunomagnetic cell separation method using the MACSxpress Whole Blood Neutrophil Isolation Kit, human (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Briefly, 20 mL of EDTA-2Na containing whole blood was incubated with MACSxpress Neutrophil Isolation Cocktail (Miltenyi Biotec) for 5 min at room temperature. The tube was then placed in the magnetic field of a MACSxpress Separator (Miltenyi Biotec) for 15 min. The supernatant was transferred into a new tube and centrifuged (300×g, for 10 min) followed by washing with Hanks' Balanced Salt Solution (HBSS; Thermo Fisher, Waltham, MA, USA). Slightly contaminated RBCs were removed by RBC Lysis Buffer (BioLegend, San Diego, CA, USA). The isolated neutrophils were counted and suspended in HBSS with 1.3 mM Ca^{2+} and 0.9 mM Mg^{2+} (Thermo Fisher) containing 0.1% fatty acid-free BSA (Sigma-Aldrich, St. Louis, MO, USA). Two polypropylene tubes of neutrophil suspension (5×10^6 cells/mL) were preincubated for

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5–10 min at 37 ℃, then calcium ionophore A23187 (final concentration 2.5 μg/mL, Sigma-Aldrich) or vehicle was added to these suspensions, and they were incubated for an additional 10 min. The samples were centrifuged (2400 × g, for 5 min) immediately, and the supernatants were aliquoted, frozen immediately, and stored at −80℃ until assay.

Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated via density gradient centrifugation using Ficoll-Paque PREMIUM (Cytiva, Marlborough, MA, USA) according to the manufacturer's instructions. Approximately 15 mL of EDTA-2Na containing whole blood was mixed with an equal volume of HBSS and gently layered over the Ficoll-Paque PREMIUM solution and centrifuged (400 ×g, for 40 min) without braking. The intermediate phase of PBMCs was collected and mixed with a threefold volume of Dulbecco's phosphate-buffered saline (D-PBS; Nacalai Tesque, Kyoto, Japan) followed by centrifugation (500 ×g, for 10 min). The pellet was subjected to hemolysis with an RBC Lysis Buffer (BioLegend, San Diego, CA, USA) and washed 3 times with D-PBS via centrifugation (100 ×g, for 10 min). The isolated PBMCs were counted and aliquoted, frozen immediately, and stored at −80℃ until assay.

Whole Blood Culture and Lipopolysaccharide (LPS) Stimulation

Heparinized whole blood samples were diluted with culture medium RPMI-1640 (FUJIFILM Wako Pure Chemical, Osaka, Japan) at a ratio of 1:2. Aliquots of diluted samples were incubated with LPS (Escherichia coli O111:B4; Sigma-Aldrich, St. Louis, MO, USA; in a final concentration of 0.1, 1, 10, or 100 ng/mL) for 24 h at 37℃. The samples were centrifuged (1200 ×g, for 10 min), and the supernatants were aliquoted, frozen immediately, and stored at −80℃ until assay.

Fatty Acid Analysis

Fatty acid analysis was performed by LSI Medience Corporation (Tokyo, Japan) using their commercial clinical laboratory procedures with minor modifications, with the exception of the fractionation of lipid classes (phosphatidylcholine [PC], phosphatidylethanolamine [PE], TG, nonesterified fatty acids [NEFA], and cholesterol esters [CE]) using an aminopropyl column[23]. Lipids in plasma, neutrophils, PBMCs, and RBCs were extracted using Folch extraction methods[24] and dried with nitrogen gas. The residues were reconstituted with chloroform, and portions of residue were applied to a Bond Elut NH2 cartridge (100 mg, 1 mL, 40 μm; Agilent, Santa Clara, CA, USA). The pass-through and washing solutions with 2 mL of chloroform were collected as a mixture of TG and CE. PC, PE, and NEFA were eluted with 1 mL of chloroform/methanol (3:2, v/v), 1 mL of methanol, and 1 mL of chloroform/methanol/acetic acid (100:2:2, v/v/v), respectively. The mixture of TG and CE was dried with nitrogen gas, reconstituted with 1 mL of hexane, and applied to another Bond Elut NH2 cartridge preconditioned with 4 mL of hexane. The pass-through and washing solutions with 2 mL of hexane were collected as CE. TG was eluted with 2 mL of hexane/chloroform/ethyl acetate (100:5:5, v/v/v). Prior to fatty acid composition analysis, samples were mixed with internal standards, including myristic acid-^{13}C3 (Cambridge Isotope Laboratories, Tewksbury, MA, USA), DHA-d5 methyl ester (Cayman Chemical, Ann Arbor, MI, USA), behenic acid-d4 (Cayman Chemical), and one of non-naturally occurring (or very rare) fatty acid. Samples were saponified, and fatty acid composition was measured using LC-MS/MS. Twenty-four fatty acids were quantified using the commercial analysis method, and if the concentration of four specific fatty acids (EPA, AA, DHA, and dihomo- γ -linolenic acid) was below the limit of quantification (BLQ), these values were substituted by those obtained from an additionally performed high-sensitivity analysis method.

EPA-Derived Metabolite Analysis

Solid-phase extraction was performed using OASIS HLB 3 cc Vac cartridges (Waters, Milford, MA, USA) under ice-cold conditions. Briefly, 300 μL from the plasma sample was mixed with 600 μL of ethanol and 10 μL of deuterium-labeled internal standard (20-hydroxyeicosatetraenoic acid-d6 and RvE1-d4) solution and then centrifuged (1800 ×g, for 5 min). Supernatants were collected, mixed with 4 mL of water/acetate (100:0.1, v/v), and applied to the cartridges preconditioned with 2 mL of methanol and 3 mL of water/acetate (100:0.1, v/v). Cartridges were then washed with 3 mL of water/acetate (100:0.1, v/v) and 2 mL of hexane followed by elution with 2 mL of methanol. The eluent was dried with nitrogen gas and then reconstituted in 40 μL of methanol. Subsequent LC-MS/MS analyses were performed using an HPLC system (Nexera UHPLC, Shimadzu, Kyoto, Japan) with a quadrupole mass spectrometer (Triple Quad 5500+, AB Sciex, Framingham, MA, USA) equipped with an Acquity UPLC HSS T3 column (1.8 μm, 2.1 mm I.D. × 150 mm, Waters). Samples were eluted with mobile phase A (water/acetic acid [100:0.1, v/v])

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Table 1. Demographic of subjects

	Control (N=8)	MND-2119 2 g (N=8)	MND-2119 4 g (N=8)	EPA-E 1.8 g (N=8)
Age	29.0 (7.2)	25.0 (5.9)	26.0 (4.1)	30.5 (5.6)
Height (cm)	176.55 (3.08)	172.13 (2.67)	173.44 (6.43)	175.88 (5.12)
Body Weight (kg)	67.53 (5.33)	63.05 (5.97)	64.51 (4.91)	67.09 (5.73)
BMI (kg/m^2)	21.66 (1.62)	21.30 (2.16)	21.48 (1.66)	21.69 (1.63)
Race (Asian) (%)	100	100	100	100

Abbreviations: BMI, body mass index.
Values are shown as means (SD) or frequencies.

and mobile phase B (acetonitrile/2-propanol [50:50, v/v]) in a gradient program (0–3 min: 80% A; 3–26 min: 80% A → 25% A; 26–28 min: 25% A → 0% A; 28–30 min: 0% A) with a flow rate of 0.2 mL/min. The injection volume was 5 µL, and the column temperature was 60℃. MS/MS analyses were conducted in negative ion mode, and EPA metabolites were identified and quantified by multiple reaction monitoring (MRM). The instrument parameters were as follows: curtain gas, 20 psi; ion spray voltage, −4000 V; temperature, 650℃; ion source gas 1, 40 psi; and ion source gas 2, 40 psi. MRM transitions (precursor and product ions), collision energy, and dwell time for all the analytes and internal standards are described in **Supplementary Table 1**. Compounds were quantified using calibration curves. The MRM chromatograms of the reference standard and representative specimen are shown in **Supplementary Figs. 1–3**. The lower limits of quantitation in plasma were 50 pg/mL for 18-HEPE and 17,18-EpETE and 6 pg/mL for RvE1. Culture supernatant samples were analyzed similarly with scaled-down and minor modifications. The lower limits of quantitation in culture supernatant samples were 1 pg/10^6 cells for 18-HEPE and 17,18-EpETE and 1.2 pg/10^6 cells for RvE1. These assays were performed at CMIC Pharma Science Co., Ltd. (Tokyo, Japan). The amount of EPA metabolite production was calculated as the difference between concentrations in calcium ionophore- and vehicle-added samples.

Cytokine Analysis

Concentrations of TNF-alpha, IL-1 beta, IL-6, and IL-10 in the culture supernatants were determined using a commercially available automatic enzyme-linked immunosorbent assay system and cartridge (Ella and Simple Plex Cartridge; ProteinSimple, San Jose, CA, USA). These assays were performed at Sumika Chemical Analysis Service, Ltd. (Osaka, Japan).

Safety Assessment

Laboratory safety outcome measures comprised hematology, blood biochemistry, and urinalysis. Additionally, to assess safety, vital signs (blood pressure and pulse rate), and body weight were measured and 12-lead electrocardiography was performed. Adverse events (AEs) were assessed from study drug initiation to the end of the follow-up period.

Statistical Analysis

Statistical analyses were performed using SAS Release 9.4 (SAS Institute Inc., Cary, NC, USA). Missing data were not substituted. The BLQ values were substituted by 0. The values above the limit of quantification were regarded as missing data. Values are shown as medians (IQR) unless otherwise indicated. The differences between groups were examined using the Wilcoxon rank sum test, and changes from baseline were examined using the Wilcoxon signed-rank test for statistical significance. The significance level for statistical tests was set at 5% (two sided). Multiplicity adjustment was not required because all statistical tests were exploratory. The relationships between neutrophil 18-HEPE productions and EPA concentrations in neutrophils and between changes in plasma EPA metabolite concentrations and changes in plasma EPA concentrations or EPA/AA ratios were investigated using simple linear regression analysis and Spearman's rank correlation coefficient. All statistical analyses were predefined in the protocol.

Results

Subjects

In this study, there were 32 subjects (N=8 for each group), and no subjects discontinued the study. All subjects were included in the pharmacological and safety analysis populations. The demographic characteristics of subjects are shown in **Table 1**.

Pharmacology

Plasma Total EPA and EPA/AA Ratio

Plasma total EPA concentrations and changes from baseline at each time point are shown in **Fig. 2.**

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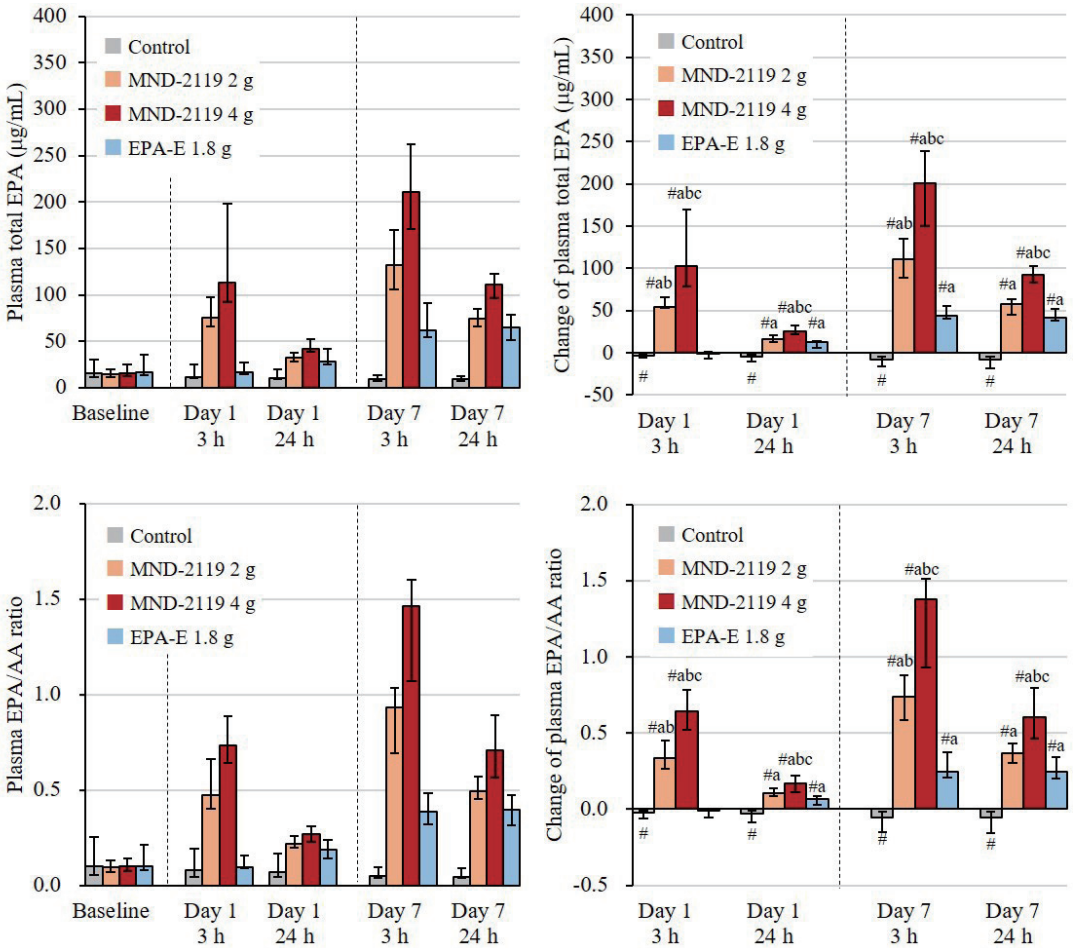

Fig. 2. Plasma total EPA concentration and EPA/AA ratio

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid.
The upper figures show the plasma total EPA concentrations and changes from baseline. The lower figures show the plasma EPA/AA ratio and changes from baseline. Results are medians and interquartile ranges of eight healthy subjects.
[#]$P<0.05$ vs. the corresponding baseline levels. [a]$P<0.05$ vs. the control group. [b]$P<0.05$ vs. the EPA-E 1.8-g group. [c]$P<0.05$ vs. the MND-2119 2-g group.

At 3 h (hour) postdose on Days 1 and 7, changes in plasma total EPA concentrations were significantly higher in the MND-2119 2-g group than in the EPA-E 1.8-g group. These results were in agreement with those from a pharmacokinetic study in healthy male adult subjects[8]. At all the time points, changes in plasma total EPA concentrations were significantly higher in the MND-2119 4-g group than in the MND-2119 2-g group. Changes in the plasma EPA/AA ratio showed a similar trend to plasma total EPA concentrations (Fig. 2). The results for other fatty acids are shown in Supplementary Table 2.

Table 2 shows EPA concentrations in plasma lipids. In the MND-2119 2-g group, an increase in the EPA concentration in the plasma TG and NEFA fractions were observed at 3 h on Days 1 and 7. EPA concentrations in the plasma PC increased with time postdose. The results for other fatty acids are shown in Supplementary Tables 3–5.

EPA-Derived Metabolites in Plasma

Plasma 18-HEPE and 17,18-EpETE concentratins at each time point are shown in Fig. 3. In the MND-2119 2-g group, changes over time in plasma EPA-derived metabolite concentrations showed a similar trend to those in plasma EPA concentrations. Plasma 18-HEPE concentrations were significantly higher in the MND-2119 2-g group than in the EPA-E 1.8-g group at 3 h on Days 1 and 7 and were significantly higher in the MND-2119 4-g group than in the MND-2119 2-g group at 3 h on Day 1 and 24 h on Day 7. Regarding the absolute concentration, the plasma 18-HEPE (median concentrations [interquartile ranges]) at 3 h on Day 1

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Table 2. EPA concentrations in plasma (mg/mL).

	Baseline	Day 1 3 h	Day 1 24 h	Day 7 3 h	Day 7 24 h
PC					
Control	8.31 (5.53, 15.21)	6.15 (5.13, 11.65)	5.04 (4.05, 9.58)	4.67 (3.38, 6.09)	4.36 (3.19, 5.70)
MND-2119 2g	7.36 (5.48, 8.79)	10.77 (9.04, 13.73)	17.82 (17.06, 22.19)	37.05 (34.15, 42.10)	35.50 (30.05, 38.41)
MND-2119 4g	7.26 (5.89, 10.5)	15.50 (10.88, 16.88)	24.46 (23.07, 28.81)	53.17 (50.01, 63.82)	50.34 (44.08, 55.72)
EPA-E 1.8g	7.37 (6.01, 16.67)	6.35 (5.72, 12.74)	16.03 (13.56, 20.06)	31.79 (24.19, 36.42)	30.47 (23.17, 37.71)
PE					
Control	0.60 (0.30, 0.71)	0.44 (0.22, 0.62)	0.30 (0.17, 0.43)	0.23 (0.19, 0.31)	0.19 (0.00, 0.28)
MND-2119 2g	0.39 (0.27, 0.57)	0.86 (0.63, 0.99)	1.10 (0.82, 1.26)	2.03 (1.64, 2.74)	1.76 (1.33, 2.30)
MND-2119 4g	0.36 (0.30, 0.63)	0.88 (0.72, 1.05)	0.93 (0.89, 1.62)	2.14 (1.96, 3.29)	1.73 (1.38, 2.87)
EPA-E 1.8g	0.41 (0.27, 0.67)	0.35 (0.25, 0.56)	0.58 (0.44, 1.03)	1.65 (1.26, 1.95)	1.30 (1.07, 1.64)
CE					
Control	4.56 (3.43, 8.62)	3.96 (3.20, 8.01)	3.59 (2.69, 7.16)	2.94 (1.66, 4.27)	3.01 (1.70, 3.92)
MND-2119 2g	4.50 (3.48, 6.75)	4.45 (3.11, 5.51)	8.01 (7.11, 9.72)	25.24 (22.20, 26.62)	24.65 (21.99, 28.31)
MND-2119 4g	4.93 (3.76, 7.14)	5.34 (3.86, 7.18)	9.57 (8.13, 11.28)	33.53 (25.72, 41.44)	31.03 (26.58, 43.00)
EPA-E 1.8g	5.04 (3.50, 12.03)	4.16 (3.41, 10.81)	6.04 (4.51, 11.04)	14.79 (14.25, 24.67)	20.98 (14.57, 25.80)
TG					
Control	2.05 (1.70, 3.57)	1.53 (1.26, 3.02)	1.44 (1.16, 2.29)	1.31 (1.04, 1.80)	1.10 (0.91, 1.59)
MND-2119 2g	1.80 (1.50, 2.89)	60.17 (53.16, 65.02)	4.51 (3.77, 5.48)	65.59 (45.81, 105.92)	9.47 (7.35, 10.60)
MND-2119 4g	1.92 (1.27, 2.80)	94.15 (75.35, 164.93)	5.99 (4.85, 8.37)	118.94 (78.64, 172.46)	13.22 (9.99, 16.32)
EPA-E 1.8g	1.91 (1.37, 4.33)	4.73 (2.31, 5.50)	5.02 (4.12, 9.01)	11.76 (9.67, 23.48)	8.96 (5.93, 12.03)
NEFA					
Control	0.00 (0.00, 0.20)	0.00 (0.00, 0.00)	0.00 (0.00, 0.17)	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
MND-2119 2g	0.00 (0.00, 0.09)	0.48 (0.38, 0.54)	0.09 (0.00, 0.24)	0.66 (0.51, 0.79)	0.35 (0.29, 0.44)
MND-2119 4g	0.00 (0.00, 0.19)	0.83 (0.54, 0.91)	0.22 (0.21, 0.26)	1.08 (0.97, 1.46)	0.66 (0.57, 0.77)
EPA-E 1.8g	0.00 (0.00, 0.11)	0.00 (0.00, 0.00)	0.17 (0.08, 0.25)	0.22 (0.19, 0.27)	0.30 (0.25, 0.35)

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; CE, cholesterol ester; TG, triglyceride; NEFA, non-esterified fatty acid. Values are shown as medians (IQR) of 8 healthy subjects.

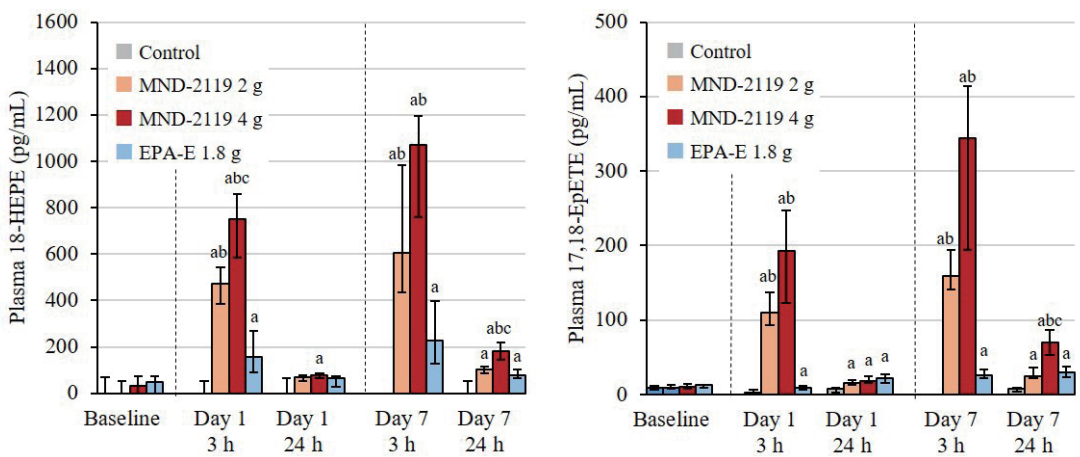

Fig. 3. Plasma 18-HEPE and 17,18-EpETE concentrations

Abbreviations: 18-HEPE, 18-hydroxyeicosapentaenoic acid; 17,18-EpETE, 17,18-epoxyeicosatetraenoic acid; EPA, eicosapentaenoic acid. Results are medians and interquartile ranges of eight healthy subjects.
[a]$P<0.05$ vs. the control group. [b]$P<0.05$ vs. the EPA-E 1.8-g group. [c]$P<0.05$ vs. the MND-2119 2-g group.

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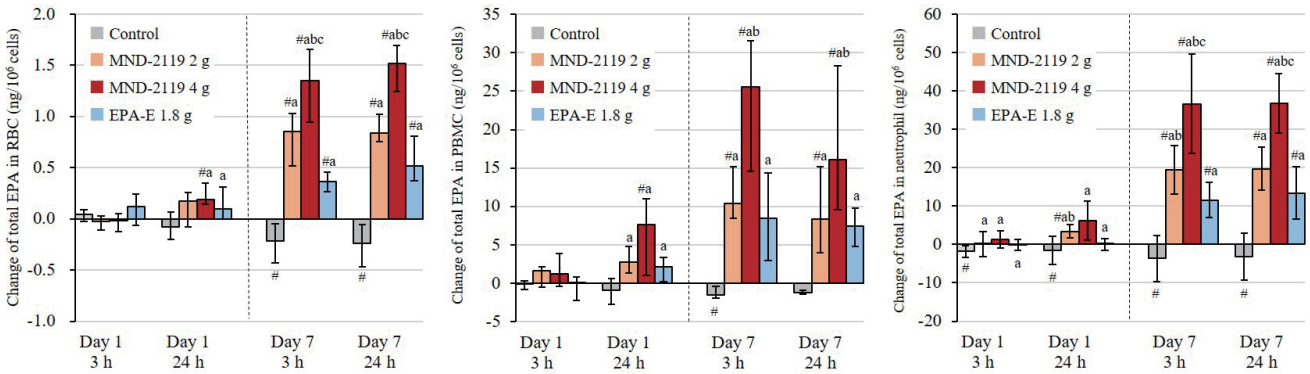

Fig. 4. Total EPA concentration in blood cells

Abbreviations: EPA, eicosapentaenoic acid; RBC, red blood cells; PBMC, peripheral blood mononuclear cells.
Figures show the changes in blood cell total EPA from baseline. Results are medians and interquartile ranges of eight healthy subjects.
[#]$P<0.05$ vs. the corresponding baseline levels. [a]$P<0.05$ vs. the control group. [b]$P<0.05$ vs. the EPA-E 1.8-g group. [c]$P<0.05$ vs. the MND-2119 2-g group.

increased up to 471.50 pg/mL (386.69, 541.30) and 751.67 pg/mL (585.33, 858.82) in the MND-2119 2- and 4-g groups, respectively, compared with 0.00 pg/mL (0.00, 54.83) and 156.12 pg/mL (92.27, 271.10) in the control and EPA-E 1.8-g groups, respectively. Plasma 17,18-EpETE concentration showed a similar trend to plasma 18-HEPE concentration. At 3 h on Day 1, plasma 17,18-EpETE concentrations were 109.99 pg/mL (92.65, 137.39) and 192.36 pg/mL (122.80, 247.17) in the MND-2119 2- and 4-g groups, compared with 2.60 pg/mL (0.00, 6.55) and 8.62 pg/mL (6.57, 11.11) in the control and EPA-E 1.8-g groups. The plasma concentrations of these EPA-derived metabolites were positively correlated with plasma EPA concentrations and EPA/AA ratios **(Supplementary Figs. 4 and 5)**. RvE1 levels under static condition were BLQ in most plasma samples (data not shown).

Total EPA in Blood Cells

The changes in total EPA concentration in RBCs, PBMCs, and neutrophils from baseline at each time point are shown in **Fig. 4**. The results for other fatty acids are shown in **Supplementary Tables 6–8**. In blood cells, there were only slight changes in total EPA concentration on Day 1, but it increased significantly after 7-day multiple administrations in the MND-2119 2-g, MND-2119 4-g, and EPA-E 1.8-g groups. The increase in EPA concentrations in neutrophils was significantly greater in the MND-2119 2-g group than in the EPA-E 1.8-g group at 24 h on Day 1 and 3 h on Day 7. Changes in total EPA concentrations in neutrophils and RBCs were significantly higher in the MND-2119 4-g group than in the MND-2119 2-g group at 3 and 24 h on Day 7.

EPA-Derived Metabolite Production by Neutrophils

EPA-derived metabolites including RvE1, 18-HEPE, and 17,18-EpETE were measured from peripheral blood neutrophils stimulated with calcium ionophore. RvE1 and 17,18-EpETE levels were BLQ in most samples. The amounts of 18-HEPE produced in some samples were also BLQ, making group comparisons difficult. However, in a correlation analysis of 18-HEPE production and the total EPA concentration in neutrophils on Day 7 (3 and 24 h), Spearman's rank correlation coefficient was 0.655 (test of noncorrelation, $P<0.001$), indicating a positive correlation between them **(Fig. 5)**. Taking into account that the increase in total EPA concentrations in neutrophils at 3 h on Day 7 was significantly higher in the MND-2119 2-g group than in the EPA-E 1.8-g group, MND-2119 2 g may be superior to EPA-E 1.8 g in terms of 18-HEPE production. Besides that, the cytokine production from LPS-stimulated ex vivo whole blood culture changed little over time among treatment groups.

Safety

The incidence of AEs reported in the study in the control, MND-2119 2-g, MND-2119 4-g, and EPA-E 1.8-g groups was 12.5% (1/8 subjects), 62.5% (5/8 subjects), 37.5% (3/8 subjects), and 25.0% (2/8 subjects), respectively. The incidence of adverse drug reactions in these groups in the same order was 0% (0/8 subjects), 62.5% (5/8 subjects), 37.5% (3/8 subjects), and 25.0% (2/8 subjects), respectively **(Supplementary Table 9)**.

The only AE reported in two or more subjects in any group was diarrhea (one in the control group, two each in the MND-2119 2- and 4-g groups, and one in the EPA-E 1.8-g group). All the AEs reported in the

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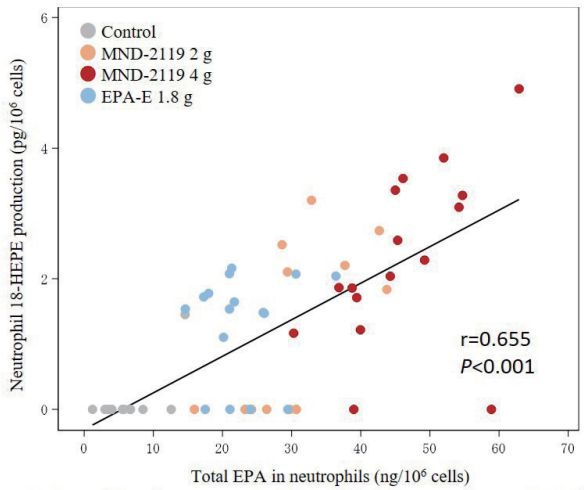

Fig. 5. Association between neutrophil total EPA concentrations and 18-HEPE production

Abbreviations: EPA, eicosapentaenoic acid; 18-HEPE, 18-hydroxyeicosapentaenoic acid.
Plots represent data of all groups at Day 7 3 and 24 h (N = 64).

study were mild and nonserious, did not result in discontinuation of study treatment, and were resolved. There were no notable issues in laboratory values, vital signs, body weights, or electrocardiograms.

Discussion

This study has clarified the elevation of plasma and cellular EPA and EPA-derived metabolite concentrations following single and multiple administrations of MND-2119. Increases in plasma 18-HEPE and 17,18-EpETE concentrations after a single administration of MND-2119 2 g exceeded that of EPA-E 1.8 g. They were further increased with MND-2119 4 g administration. Neutrophil EPA concentrations significantly increased after multiple administrations of MND-2119 2 g compared with those of EPA-E 1.8 g. Additionally, the level of 18-HEPE released from activated neutrophils was positively correlated with neutrophil EPA concentrations.

At 3 h postdose, plasma EPA concentration significantly increased in the MND-2119 2-g group than in the EPA-E 1.8-g group, reproducing the result of a previous study[14]. This result appeared to be attributable to not only a higher dose at the time of MND-2119 administration (SID formulation) but also improved absorption efficiency with self-emulsification. EPA concentrations in the plasma TG fraction increased at 3 h after administration of MND-2119. Intestinally absorbed free fatty acids are actively incorporated into chylomicrons as ester forms[25]. Most of the absorbed EPA are included in chylomicrons at 3 h postdose. Simultaneously, concentrations of both plasma-free EPA and EPA-derived metabolites also increased. Although a number of clinical research studies have analyzed EPA-derived metabolite concentrationsin plasma after multiple administrations of n-3 PUFA formulations[22], few studies have analyzed EPA-derived metabolite concentrations in plasma after a single dose[26, 27]. This study showed that the plasma concentrations of 18-HEPE and 17,18-EpETE increased in just 3 h after administration of a self-emulsifying EPA-E formulation. Because the endogenous levels of hydroxyl- and epoxy-fatty acid metabolites depend on the dosage, formulation, and duration of PUFA administration, it is difficult to compare the absolute metabolites concentrations with those of other reported studies. However, the plasma-free 18-HEPE and 17,18-EpETE concentrations increased immediately to unprecedented high levels after MND-2119 administration. This unique feature of MND-2119 would imply the potential new application in the acute phase treatment, such as suppressing inflammatory reactions and arrhythmias after acute myocardial infarction[28] in addition to the continuing treatment of hypertriglycemia to reduce the risk of ASCVD.

The bioactivities of 18-HEPE, 17,18-EpETE as potential mechanisms of anti-inflammatory action of high-dose EPA formulations, have been attracting attention. A relationship between 18-HEPE and cardiovascular diseases is reported in a nonclinical study about the inhibitory effect of 18-HEPE on the progression of cardiac remodeling and heart failure[20] and clinical studies about a negative correlation between aggravation of atherosclerosis and blood

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18-HEPE levels[29]. Additionally, nonclinical studies demonstrated that 18-HEPE prevented retinal dysfunction via BDNF upregulation in diabetic retinopathy[30] and inhibited metastases/growth of melanoma[31]. Also, it has been reported that 17,18-EpETE has inhibitory effects on allergic reactions in the skin and intestinal tract[32-34] and on vascular remodeling in pulmonary hypertension[21] in nonclinical studies. 18-HEPE is a precursor of E-series resolvins. 17,18-EpETE is also metabolized to secondary product 12-hydroxy-17,18-epoxyeicosaptetraenoic acid, which has anti-inflammatory activity[35, 36]. This study revealed that the increase in EPA-derived primary products, 18-HEPE and 17,18-EpETE, was higher after administering MND-2119 than that after administering the conventional EPA-E formulation. Therefore, MND-2119 is a potential new therapeutic agent for inflammatory disease including ASCVD. Additionally, it may be expected to have a prophylactic effect, in terms of readiness to generate secondary products such as RvEs immediately in response to inflammatory stimuli. In daily clinical practice, it is not easy to test the status of these EPA-derived anti-inflammatory metabolites. However, as shown in **Supplementary Figs.4 and 5**, plasma EPA concentration and EPA/AA ratio positively correlated with the concentration of EPA-derived metabolites, and these clinical tests may be useful in estimating the status of EPA-derived metabolites in patients.

Unlike plasma EPA concentrations, EPA concentrations in RBCs, PBMCs, and neutrophils increased after multiple administrations of MND-2119. It is considered that a significant amount of EPA was taken up by bone marrow, emerged in blood a few days later, and was observed as an increase in EPA concentration in blood cell components in this study. In neutrophils, however, a transient modest increase in EPA concentration was observed at 3 h on Day 7 after administration of MND-2119 2 g compared with that of EPA-E 1.8 g. This result suggests that EPA in circulating blood was partially taken up by neutrophils. Neutrophil EPA concentrations showed a positive correlation with 18-HEPE production after calcium ionophore stimulation. On the basis of these results, MND-2119 is expected to increase the amount of EPA distributed in neutrophils and thereby increase 18-HEPE production.

The results of the analyses of EPA-derived metabolite concentrations in plasma and neutrophils have interesting implications on the production pathways of EPA metabolites. Given that plasma concentrations of 18-HEPE and 17,18-EpETE increased prior to the distribution of EPA into neutrophils and PBMCs after MND-2119 administration, CYP enzymes expressed in vascular endothelial cells[37], they may contribute to the production of 18-HEPE and 17,18-EpETE. Moreover, 18-HEPE and 17,18-EpETE may have protective effects on vascular endothelial cells. The effects of MND-2119 on vascular endothelial cell function through 18-HEPE and 17,18-EpETE production should be further investigated.

MND-2119 has several potential applications in acute diseases associated with inflammation. Our previous study demonstrated that early EPA treatment after percutaneous coronary intervention reduces acute inflammatory responses in patients with acute myocardial infarction[28]. Furthermore, previous studies showed a possible beneficial effect of omega-3 PUFAs on acute respiratory distress syndrome and sepsis[38]. In critically ill patients with acute respiratory distress syndrome, omega-3 PUFAs in enteral immunomodulatory diets may be associated with an improvement in early oxygenation and length in stay of intensive care[39]. In these cases, an early increase in EPA-derived anti-inflammatory metabolites of MND-2119 might contribute to an improvement in outcomes. Further studies are required to clarify the therapeutic effects of MND-2119 on acute diseases associated with inflammation.

Conclusion

This study has demonstrated that the administration of MND-2119 rapidly increases plasma 18-HEPE and 17,18-EpETE to unprecedented high levels beyond those of conventional EPA-E formulations, following the increase in plasma EPA concentrations. Additionally, this study showed that multiple administrations of MND-2119 increased cellular EPA concentrations in neutrophils and PBMCs. These results highlight the possibility that the pharmacological action of MND-2119 is superior to that of conventional formulations in terms of anti-inflammatory effects. Further investigation of the effect of MND-2119 will be needed in a targeted patient population.

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Conflicts of Interest

Toru Miyoshi and Hiroshi Ito have received research/grant support from Mochida Pharmaceutical Co., Ltd. Toru Miyoshi, Makoto Arita and Hiroshi Ito have honoraria from Mochida Pharmaceutical Co., Ltd. Satoko Naoe, Hiroyuki Wakabayashi, Takashi Yano, Takuya Mori and Shingo Kanda are employed by Mochida Pharmaceutical Co., Ltd.

Author Contributions

TM from Okayama University, SN, HW, TY, MA and HI substantially contributed to the study conceptualization and interpretation of the results. TM from Mochida Pharmaceutical Co., Ltd. supervised the conduct of this study. SK developed the statistical analysis plan and conducted statistical analyses. TM from Okayama University, SN and HW wrote the manuscript with input from all authors. All authors critically revised the report, commented on drafts of the manuscript, and approved the final report.

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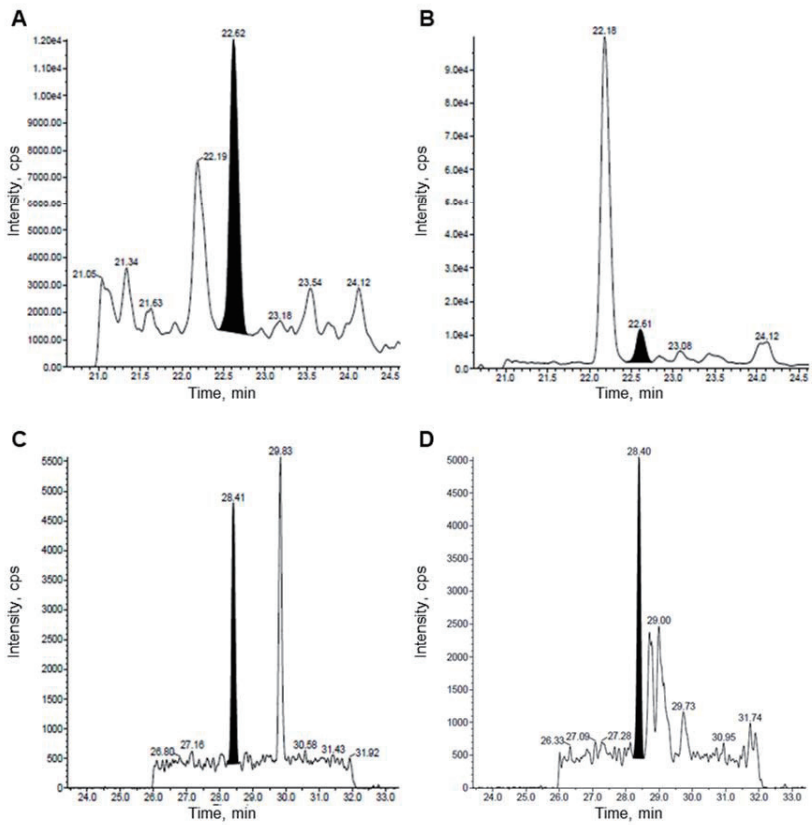
Supplementary Table 1. MRM transitions (precursor ion and product ion), collision energy and dwell time for all the analytes and internal standards used in this study

Compound	Precursor ion: m/z	Product ion: m/z	Collision energy (V)	Dwell time (msec)
18-HEPE	317	255 (215)	-20 (-18)	150
17,18-EpETE	317	215	-18	150
Resolvin E1	349	107	-28 (-26)	150
20-HETE-d6	325	281	-24	150
Resolvin E1-d4	353	109	-28	150

Parameters used for analysis of plasma samples are indicated. Values in parentheses indicate parameters modified for analysis of culture supernatants.



SupplementaryFig. 1. 18-HEPE chromatograms

18-HEPE MRM (317/255) chromatograms of the reference standard in plasma (A) and a representative plasma specimen (B). 18-HEPE MRM (317/215) chromatograms of the reference standard in culture supernatant (C) and a representative culture supernatant specimen (D).

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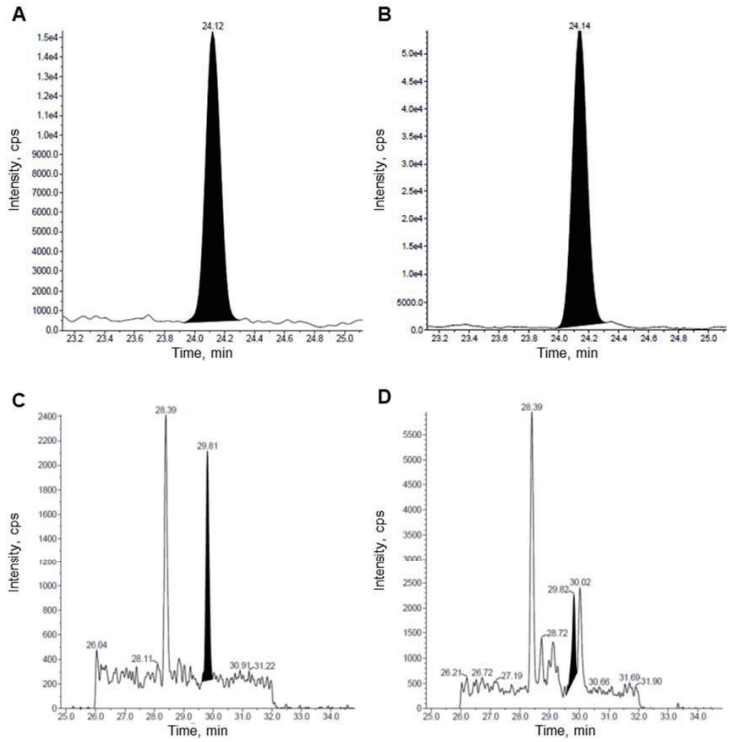
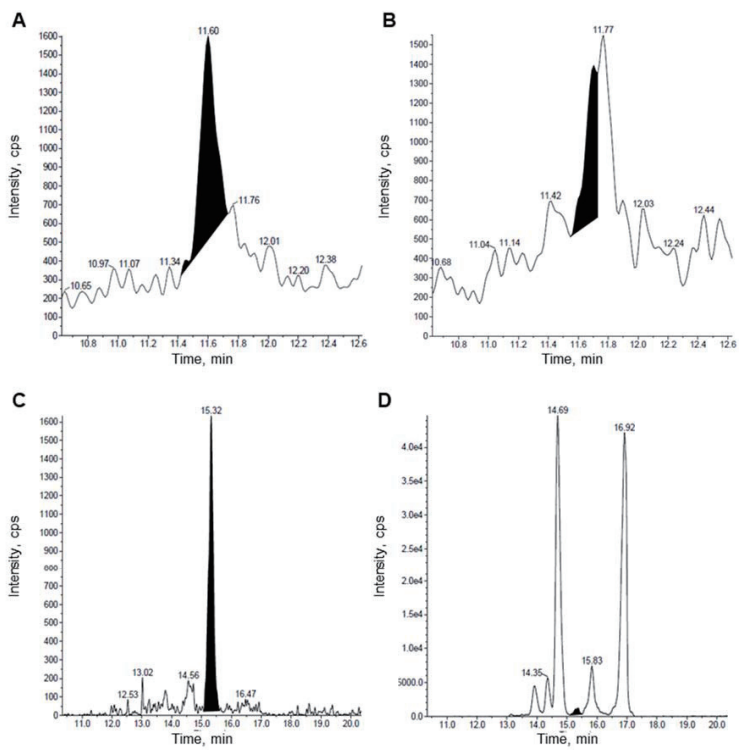


SupplementaryFig. 2. 17,18-EpETE chromatograms

17,18-EpETE MRM (317/215) chromatograms of the reference standard in plasma (A) and a representative plasma specimen (B). 17,18-EpETE MRM (317/215) chromatograms of the reference standard in culture supernatant (C) and a representative culture supernatant specimen (D).



SupplementaryFig. 3. RvE1 chromatograms

RvE1 MRM (349/107) chromatograms of the reference standard in plasma (A) and a representative plasma specimen (B). RvE1 MRM (349/107) chromatograms of the reference standard in culture supernatant (C) and a representative culture supernatant specimen (D).

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Supplemetary Table 2. Total fatty acids in plasma

	Baseline (median, μg/mL)				Day1 3h change from baseline (median, μg/mL)				Day1 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.79	1.98	0.00	0.00	0.00	0.27	1.67	0.00	-0.79	-1.24	0.00	0.00
myristic acid	14.72	18.41	11.42	12.50	-2.63	-0.71	-0.49	-0.02	-2.26	-4.44	-0.98	-0.68
myristoleic acid	0.88	0.95	0.82	0.60	-0.34	-0.26	-0.19	-0.09	-0.10	-0.11	0.01	-0.01
palmitic acid	501.57	532.15	542.56	492.81	-13.30	-3.74	-2.49	45.42	-43.40	-42.72	-25.19	20.33
palmitoleic acid	48.86	48.68	53.75	41.94	-7.51	-3.81	-3.60	0.41	-1.83	-2.49	-2.33	4.25
stearic acid	162.43	169.35	159.23	152.41	1.16	2.79	4.43	10.18	-18.34	-10.97	-5.66	-0.45
oleic acid	580.34	610.57	620.70	536.08	15.60	58.99	27.02	111.36	25.97	-82.85	-39.47	19.99
linoleic acid	796.59	820.79	840.84	812.12	-14.80	49.27	33.63	72.89	-43.27	-89.64	-84.50	-6.14
linolenic acid	15.49	21.74	15.67	17.05	5.02	9.62	12.50	7.94	-2.21	-5.19	-1.28	-1.79
γ-linolenic acid	11.55	11.91	8.61	12.35	-0.65	-1.27	0.97	-0.39	-2.50	-3.35	-2.27	-1.83
arachidic acid	1.47	1.31	1.23	1.28	0.60	1.05	1.20	1.12	-0.12	-0.01	0.00	0.03
eicosenoic acid	4.32	4.76	4.65	3.67	0.38	1.02	1.20	1.15	-0.26	-0.68	-0.18	0.23
eicosadienoic acid	6.95	6.85	6.74	6.29	-0.63	-0.20	-0.50	0.10	-0.53	-1.03	-0.74	0.10
dihomo-γ-linolenic acid	34.40	34.57	35.27	32.07	0.12	-1.86	-0.30	1.80	-1.31	-5.36	-4.68	-0.67
5,8,11-eicosatrienoic acid	1.95	1.80	1.87	1.45	-0.14	-0.08	-0.11	-0.01	-0.32	-0.35	-0.29	-0.06
arachidonic acid	169.82	158.06	169.02	174.66	1.80	8.71	1.05	13.42	-5.07	1.10	-3.92	3.63
eicosapentaenoic acid	15.36	15.08	15.47	16.36	-3.64	54.82	102.20	-0.38	-5.14	15.85	25.94	13.19
behenic acid	1.19	1.06	0.98	1.29	0.26	0.49	0.64	0.44	-0.10	-0.01	0.05	0.03
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	4.78	4.65	4.67	4.34	-0.17	-0.03	0.07	0.44	-0.21	-0.49	-0.18	0.42
docosapentaenoic acid	17.31	18.94	15.20	16.87	-0.81	1.18	2.25	0.74	-1.28	0.92	2.99	2.99
docosahexaenoic acid	63.47	66.14	75.26	68.38	-2.25	1.37	-1.61	0.76	-5.89	-5.12	-7.63	0.71
lignoceric acid	1.31	1.27	1.11	1.49	0.23	0.31	0.32	0.25	-0.08	-0.04	-0.01	0.00
nervonic acid	1.95	1.71	1.75	2.13	0.13	0.26	0.37	0.06	0.00	0.07	0.15	0.03
EPA/AA ratio	0.10	0.10	0.10	0.10	-0.02	0.33	0.64	-0.01	-0.03	0.10	0.17	0.07

	Day7 3h change from baseline (median, μg/mL)				Day7 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.82	0.65	0.79	-0.31	-0.66	0.00	0.00
myristic acid	-3.64	-3.73	-1.10	-0.72	-3.78	-3.68	0.12	-0.38
myristoleic acid	-0.32	-0.31	-0.24	-0.08	-0.10	0.00	0.12	0.08
palmitic acid	-46.60	-29.38	-39.04	4.26	-48.24	-37.98	-52.59	-10.80
palmitoleic acid	-9.21	-7.53	-8.95	-0.15	-2.98	-0.46	-2.79	2.41
stearic acid	-6.32	0.89	-3.25	-11.65	-18.51	-9.87	1.17	-17.34
oleic acid	-31.66	-1.70	-9.38	6.11	-23.87	-41.70	-46.41	-16.89
linoleic acid	-3.89	-51.36	-110.04	1.79	-42.03	-141.81	-160.62	-112.31
linolenic acid	5.16	9.67	10.30	6.48	-0.19	-1.62	0.21	-0.34
γ-linolenic acid	-2.11	-2.50	-2.10	-2.72	-3.57	-4.61	-3.32	-3.06
arachidic acid	0.63	0.87	0.75	0.72	-0.08	-0.13	0.09	-0.15
eicosenoic acid	0.39	0.35	0.37	0.50	-0.06	-0.88	-0.68	-0.17
eicosadienoic acid	-0.57	-0.92	-1.16	-0.61	0.00	-0.81	-1.34	-0.32
dihomo-γ-linolenic acid	-6.99	-12.25	-13.89	-6.90	-8.00	-12.74	-14.89	-6.89
5,8,11-eicosatrienoic acid	-0.56	-0.68	-0.78	-0.39	-0.68	-0.75	-0.76	-0.36
arachidonic acid	-3.67	2.94	-9.29	-1.42	-5.05	-10.25	-17.87	-4.20
eicosapentaenoic acid	-8.26	110.32	200.42	44.10	-8.53	57.90	92.37	41.74
behenic acid	0.19	0.45	0.45	0.31	-0.09	-0.03	0.02	-0.12
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	0.04	-0.66	-1.33	-0.55	-0.14	-0.73	-1.06	-0.47
docosapentaenoic acid	-1.57	13.88	18.45	9.71	-1.90	12.74	15.87	11.23
docosahexaenoic acid	-5.49	-6.87	-15.32	-5.47	-7.01	-8.84	-18.07	-9.38
lignoceric acid	0.10	0.25	0.15	0.18	-0.11	-0.10	0.01	-0.20
nervonic acid	0.21	0.31	0.31	0.12	0.14	0.19	0.20	0.08
EPA/AA ratio	-0.06	0.74	1.37	0.24	-0.06	0.37	0.61	0.24

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid.
Values are shown as medians of 8 healthy subjects.

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Supplementary Table 3. Plasma fatty acids in PC

	Baseline (median, μg/mL)				Day1 3h change from baseline (median, μg/mL)				Day1 24h change from baseline (median, g/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	2.20	2.22	1.73	1.86	-0.38	-0.32	-0.09	-0.17	-0.46	-0.41	-0.28	-0.20
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	-	-	-	-	-	-	-	-	-	-	-	-
palmitoleic acid	5.21	4.34	5.06	3.73	-0.03	-0.24	-0.14	0.19	-0.30	-0.06	-0.14	0.35
stearic acid	-	-	-	-	-	-	-	-	-	-	-	-
oleic acid	106.26	86.48	88.21	80.31	-7.10	-1.70	0.42	4.87	-12.05	-7.26	-6.34	2.63
linoleic acid	193.21[a)]	210.17[a)]	208.37[a)]	203.05[a)]	-	2.81[a)]	-5.77[a)]	0.47[a)]	-1.33[a)]	-17.48[a)]	-39.02[a)]	-14.29[a)]
linolenic acid	1.57	1.61	1.48	1.64	-0.19	-0.26	-0.22	-0.16	-0.38	-0.33	-0.41	-0.46
γ-linolenic acid	1.40	1.08	0.81	1.13	-0.28	-0.19	-0.05	-0.20	-0.54	-0.32	-0.23	-0.34
arachidic acid	0.93	0.79	0.76	0.87	-0.01	0.03	0.07	0.05	-0.04	-0.03	-0.02	0.02
eicosenoic acid	1.67	1.77	1.70	1.65	-0.01	-0.01	0.02	0.09	-0.10	-0.08	-0.08	-0.08
eicosadienoic acid	4.29	3.72	4.08	3.97	-0.18	-0.10	-0.39	0.03	-0.33	-0.38	-0.56	-0.28
dihomo-γ-linolenic acid	24.48	23.56	24.39	23.21	-0.57	-0.46	-0.83	1.46	0.01	-2.79	-3.81	0.14
5,8,11-eicosatrienoic acid	0.82	0.61	0.74	0.64	-0.05	-0.05	-0.04	-0.02	-0.17	-0.10	-0.17	-0.04
arachidonic acid	89.59	77.08	90.62	91.82	3.52	3.19	-0.16	5.82	0.26	1.85	-4.53	3.38
eicosapentaenoic acid	8.31	7.36	7.26	7.37	-2.16	3.90	5.88	-1.05	-3.26	10.08	17.51	6.27
behenic acid	0.73	0.65	0.62	0.78	-0.02	0.00	-0.03	0.00	-0.03	-0.03	-0.02	-0.04
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	2.77	2.37	2.71	2.69	0.00	-0.02	-0.16	0.11	-0.14	-0.08	-0.21	0.04
docosapentaenoic acid	9.86	10.47	9.77	10.84	-0.01	0.09	-0.56	0.04	-0.13	0.99	0.61	0.43
docosahexaenoic acid	46.20	46.30	53.26	50.15	-1.04	-0.77	-2.87	1.21	-0.73	-1.86	-5.01	-1.08
lignoceric acid	1.01	0.87	0.96	1.08	-0.03	-0.01	-0.07	-0.06	-0.10	-0.05	-0.12	-0.05
nervonic acid	1.29	1.02	1.22	1.26	-0.07	-0.02	0.00	0.02	-0.13	-0.02	-0.07	0.01
EPA/AA ratio	0.09	0.09	0.09	0.09	-0.02	0.04	0.07	-0.02	-0.04	0.13	0.18	0.07

	Day7 3h change from baseline (median, μg/mL)				Day7 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	-0.57	-0.67	-0.10	-0.27	-0.58	-0.53	0.00	-0.17
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	-	-	-	-	-	-	-	-
palmitoleic acid	0.00	-0.16	-0.48	0.14	0.12	0.11	-0.21	0.01
stearic acid	-	-	-	-	-	-	-	-
oleic acid	-11.37	-6.78	-11.14	-4.35	-17.16	-10.47	-14.47	-4.22
linoleic acid	-3.22[a)]	-31.59[a)]	-47.94[a)]	-32.58[a)]	2.21[a)]	-39.19[a)]	-50.04[a)]	-40.82[a)]
linolenic acid	-0.10	-0.32	-0.22	-0.28	-0.28	-0.43	-0.46	-0.48
γ-linolenic acid	-0.40	-0.40	-0.27	-0.33	-0.56	-0.46	-0.33	-0.38
arachidic acid	-0.11	0.02	-0.01	-0.08	-0.13	0.03	0.05	-0.11
eicosenoic acid	0.03	-0.01	-0.07	0.03	-0.02	-0.13	-0.21	-0.07
eicosadienoic acid	0.08	-0.63	-1.35	-0.49	-0.07	-0.74	-1.37	-0.46
dihomo-γ-linolenic acid	-4.59	-8.48	-10.56	-4.94	-5.52	-8.71	-11.66	-5.14
5,8,11-eicosatrienoic acid	-0.32	-0.26	-0.40	-0.25	-0.36	-0.26	-0.42	-0.26
arachidonic acid	1.13	1.84	-7.63	-3.44	-2.16	-3.58	-13.76	-6.05
eicosapentaenoic acid	-4.35	28.08	45.28	18.90	-4.69	24.80	41.11	18.23
behenic acid	-0.14	-0.05	-0.08	-0.15	-0.10	-0.02	-0.06	-0.08
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	-0.28	-0.59	-1.05	-0.56	-0.14	-0.64	-1.05	-0.65
docosapentaenoic acid	-0.56	6.94	7.59	5.70	-0.31	8.03	7.48	6.00
docosahexaenoic acid	-4.68	-4.92	-13.64	-6.37	-4.33	-3.78	-14.58	-6.83
lignoceric acid	-0.20	-0.09	-0.18	-0.17	-0.14	-0.10	-0.17	-0.12
nervonic acid	-0.08	-0.01	-0.07	-0.05	-0.08	0.05	-0.05	-0.06
EPA/AA ratio	-0.05	0.35	0.59	0.22	-0.05	0.34	0.54	0.22

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; PC, phosphatidylcholine; ALQ, above the limit of quantification. Values are shown as medians of 8 healthy subjects unless otherwise indicated. -; The value is not shown, because there are 3 or more ALQ cases. [a)]; There are 1-2 ALQ cases.

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Supplementary Table 4. Plasma fatty acids in TG

	Baseline (median, μg/mL)				Day1 3h change from baseline (median, μg/mL)				Day1 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.86	1.07	0.56	0.68	0.54	0.87	0.84	0.89	-0.22	-0.32	0.00	-0.01
myristic acid	10.13	11.95	6.58	8.26	-2.12	0.77	1.20	1.41	-1.15	-2.62	0.89	0.38
myristoleic acid	0.64	0.63	0.45	0.43	-0.20	-0.03	0.00	0.05	-0.06	-0.07	0.05	0.04
palmitic acid	162.76	158.85[a)]	170.93[a)]	145.61[a)]	-5.78[a)]	-	-	21.68[a)]	-15.66	-6.07[a)]	-11.05[a)]	9.68[a)]
palmitoleic acid	27.73[a)]	23.23[a)]	22.81[a)]	18.07[a)]	-5.31[a)]	0.11[a)]	-0.94[a)]	3.43[a)]	0.03 [a)]	0.91 [a)]	0.75 [a)]	4.77[a)]
stearic acid	30.79	29.23	26.21	24.14	4.36	7.12	9.96	9.20[a)]	-1.62	-3.56	-0.73	1.85
oleic acid	-	-	-	-	-	-	-	-	-	-	-	-
linoleic acid	143.28	163.10[a)]	150.01[a)]	154.27[a)]	16.82[a)]	-	-	-	-4.96	-22.11[a)]	-6.15[a)]	3.21[a)]
linolenic acid	9.85	13.28	9.20	9.60[a)]	5.33	9.09	10.71[a)]	7.79[a)]	-0.91	-2.43	-0.11	-0.15[a)]
γ-linolenic acid	4.20	3.90	2.51	3.80	-0.44	0.92	1.42	1.48	-1.34	-0.49	-0.14	-0.27
arachidic acid	0.39	0.34	0.33	0.29	0.58	0.99	1.02	1.06	0.01	-0.05	0.01	0.03
eicosenoic acid	2.34	2.45	2.14	1.65	0.57	1.51	1.44	1.45	-0.10	-0.04	0.00	0.13
eicosadienoic acid	2.33	2.55	2.03	1.89	-0.26	-0.02	0.14	0.25	-0.13	-0.18	0.08	0.13
dihomo-γ-linolenic acid	2.70	2.49	2.02	2.22	-0.56	0.22	0.64	0.43	-0.53	-0.50	-0.17	0.05
5,8 11-eicosatrienoic acid	0.74	0.65	0.56	0.52	-0.12	0.01	0.01	0.00	-0.18	-0.04	-0.07	-0.05
arachidonic acid	8.80	9.57	8.71	9.13	0.44	3.32	4.48	2.59	-0.76	-0.34	0.20	0.08
eicosapentaenoic acid	2.05	1.80	1.92	1.91	-0.55	54.12	92.76	1.23	-0.84	2.27	4.43	3.37
behenic acid	0.09	0.09	0.04	0.04	0.27	0.45	0.51	0.48	0.00	-0.02	0.00	0.01
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	1.18	1.16	0.92	1.12	-0.10	0.05	0.06	0.11	-0.03	-0.10	0.00	0.01
docosapentaenoic acid	4.60	5.18	3.48	4.27	-0.57	1.47	2.70	0.35	-0.98	1.24	2.41	2.05
docosahexaenoic acid	7.79	7.84	7.76	6.41	-0.13	1.92	3.37	1.75	-1.55	-0.39	-0.12	0.07
lignoceric acid	0.00	0.08	0.04	0.00	0.22	0.24	0.31	0.26	0.00	-0.01	0.00	0.00
nervonic acid	0.04	0.08	0.00	0.00	0.10	0.10	0.16	0.15	-0.04	-0.03	0.00	0.00
EPA/AA ratio	0.24	0.22	0.23	0.24	-0.09	4.12	7.69	0.04	-0.07	0.30	0.55	0.43

	Day7 3h change from baseline (median, μg/mL)				Day7 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.61	0.77	1.23	1.00	-0.23	-0.19	0.03	-0.11
myristic acid	-1.45	-0.04	2.82	4.73	-1.91	-1.22	1.35	1.16
myristoleic acid	-0.16	-0.10	0.05	0.09	-0.07	0.02	0.18	0.12
palmitic acid	-24.73	-	17.15[a)]	18.70[a)]	-32.79	-4.63[a)]	-4.63[a)]	-7.29[a)]
palmitoleic acid	-5.87[a)]	-1.31[a)]	0.68[a)]	3.84[a)]	-1.27[a)]	2.88[a)]	4.23[a)]	3.82[a)]
stearic acid	2.78	5.27	8.70	14.28	-7.50	-1.01	-0.49	-2.82
oleic acid	-	-	-	-	-	-	-	-
linoleic acid	19.51	-	-	-	1.51	-0.98[a)]	8.01[a)]	-7.69[a)]
linolenic acid	6.01	10.84	10.67[a)]	7.03[a)]	-0.01	-1.25	0.80	-0.81[a)]
γ-linolenic acid	0.06	1.15	0.71	1.09	-1.24	-0.50	0.12	-0.06
arachidic acid	0.61	0.97	0.76	0.85	-0.10	-0.05	-0.05	-0.04
eicosenoic acid	0.28	1.28	0.92	1.05	-0.45	-0.28	-0.26	-0.08
eicosadienoic acid	-0.41	-0.01	-0.10	0.23	-0.18	-0.32	-0.18	-0.01
dihomo-γ-linolenic acid	-0.79	-0.38	-0.13	0.13	-0.71	-0.92	-0.59	-0.20
5,8,11-eicosatrienoic acid	-0.21	-0.12	-0.17	-0.05	-0.23	-0.12	-0.11	-0.09
arachidonic acid	1.22	3.30	3.77	3.52	-0.80	0.41	-0.50	-0.09
eicosapentaenoic acid	-1.12	63.75	116.71	10.70	-1.19	6.20	11.66	6.82
behenic acid	0.28	0.46	0.36	0.36	-0.09	-0.01	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	0.04	0.07	0.00	0.18	0.05	-0.08	-0.05	0.03
docosapentaenoic acid	-0.90	7.17	9.96	4.93	-1.10	4.43	5.68	3.79
docosahexaenoic acid	-0.27	1.57	2.63	1.92	-1.69	-0.29	-0.61	-0.11
lignoceric acid	0.22	0.26	0.27	0.21	0.00	-0.01	0.00	0.00
nervonic acid	0.09	0.12	0.12	0.12	-0.04	-0.02	0.00	0.00
EPA/AA ratio	-0.13	5.91	9.41	0.81	-0.11	0.85	1.45	0.76

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; TG, triglyceride; ALQ, above the limit of quantification. Values are shown as medians of 8 healthy subjects unless otherwise indicated. -; The value is not shown, because there are 3 or more ALQ cases. [a)]; There are 1-2 ALQ cases.

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Supplementary Table 5. Plasma fatty acids in NEFA

	Baseline (median, μg/mL)				Day1 3h change from baseline (median, μg/mL)				Day1 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.50	0.44	0.49	0.47	-0.46	-0.44	-0.48	-0.47	-0.05	0.00	-0.04	-0.01
myristic acid	1.55	1.86	1.99	1.88	-0.63	-0.77	-1.11	-0.87	0.03	-0.10	-0.23	-0.20
myristoleic acid	0.12	0.10	0.19	0.12	-0.12	-0.10	-0.19	-0.12	0.00	-0.03	-0.05	-0.02
palmitic acid	41.59	49.11	52.12	51.71	-12.93	-7.98	-21.63	-17.00	0.12	-0.06	-7.48	-10.12
palmitoleic acid	2.21	2.63	3.56	2.32	-2.11	-2.37	-3.38	-2.07	-0.11	-0.01	-0.98	-0.27
stearic acid	22.44	26.59	26.44	28.15	-5.56	-2.16	-6.68	-7.27	-0.18	0.61	-3.01	-3.71
oleic acid	26.38	32.76	41.81	34.87	-20.95	-27.10	-36.56	-26.86	-3.83	-3.52	-9.02	-7.24
linoleic acid	10.49	12.56	13.76	13.80	-7.85	-9.62	-10.70	-9.60	-1.26	-0.51	-2.97	-2.66
linolenic acid	0.89	0.98	1.07	1.19	-0.58	-0.67	-0.82	-0.74	-0.06	-0.10	-0.19	-0.14
γ-linolenic acid	0.18	0.20	0.23	0.24	-0.18	-0.15	-0.21	-0.22	-0.04	-0.01	-0.07	-0.04
arachidic acid	0.16	0.16	0.17	0.19	-0.04	-0.02	-0.05	-0.04	0.00	-0.01	-0.02	-0.03
eicosenoic acid	0.33	0.45	0.60	0.44	-0.30	-0.38	-0.54	-0.38	-0.02	-0.03	-0.15	-0.13
eicosadienoic acid	0.24	0.31	0.34	0.31	-0.20	-0.27	-0.30	-0.26	-0.03	-0.02	-0.07	-0.08
dihomo-γ-linolenic acid	0.09	0.18	0.16	0.16	-0.09	-0.12	-0.16	-0.16	-0.02	0.00	0.00	-0.02
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	0.81	0.71	0.87	0.75	-0.27	-0.08	-0.10	-0.10	-0.11	0.04	-0.03	-0.02
eicosapentaenoic acid	0.00	0.00	0.00	0.00	0.00	0.40	0.65	0.00	0.00	0.01	0.20	0.12
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	0.12	0.11	0.11	0.13	-0.05	-0.11	-0.10	-0.10	-0.02	-0.02	0.00	-0.02
docosapentaenoic acid	0.26	0.30	0.31	0.26	-0.24	-0.19	-0.20	-0.26	-0.05	0.00	0.02	-0.02
docosahexaenoic acid	0.96	0.92	1.14	0.88	-0.46	-0.43	-0.56	-0.47	-0.18	-0.04	-0.11	-0.14
lignoceric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nervonic acid	0.08	0.09	0.09	0.10	-0.08	-0.08	-0.09	-0.10	-0.01	-0.01	0.00	-0.08
EPA/AA ratio	0.00	0.00	0.00	0.00	0.00	0.72	0.89	0.00	0.00	0.00	0.25	0.11

	Day7 3h change from baseline (median, μg/mL)				Day7 24h change from baseline (median, μg/mL)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	-0.50	-0.24	-0.49	-0.47	0.02	0.24	0.12	-0.25
myristic acid	-0.56	-0.73	-1.16	-1.01	0.08	0.00	0.17	-0.26
myristoleic acid	-0.12	-0.10	-0.19	-0.12	0.03	0.00	0.06	-0.02
palmitic acid	-11.23	-12.77	-23.33	-19.28	2.43	0.14	-0.31	-8.41
palmitoleic acid	-2.02	-2.44	-3.42	-2.20	0.75	-0.09	1.02	-0.38
stearic acid	-5.57	-4.26	-7.85	-8.67	-1.04	0.78	-0.62	-5.11
oleic acid	-19.20	-25.72	-37.94	-29.84	5.22	-1.58	4.98	-4.62
linoleic acid	-6.66	-9.31	-11.33	-10.54	2.40	0.30	3.34	-2.15
linolenic acid	-0.52	-0.61	-0.76	-0.80	0.19	0.03	0.36	-0.19
γ-linolenic acid	-0.18	-0.19	-0.21	-0.20	0.03	-0.01	0.08	-0.02
arachidic acid	-0.04	-0.02	-0.04	-0.05	-0.03	0.00	-0.02	-0.03
eicosenoic acid	-0.30	-0.33	-0.54	-0.39	0.02	-0.07	-0.04	-0.09
eicosadienoic acid	-0.20	-0.28	-0.30	-0.29	0.04	-0.01	0.05	-0.03
dihomo-γ-linolenic acid	-0.09	-0.18	-0.16	-0.16	0.00	0.01	0.07	-0.11
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	-0.27	-0.14	-0.22	-0.29	-0.01	0.06	0.22	-0.01
eicosapentaenoic acid	0.00	0.56	1.00	0.19	0.00	0.29	0.60	0.25
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	-0.12	-0.11	-0.11	-0.13	0.01	-0.02	0.02	-0.05
docosapentaenoic acid	-0.26	-0.08	-0.10	-0.24	0.00	0.13	0.27	0.07
docosahexaenoic acid	-0.46	-0.44	-0.68	-0.49	-0.05	-0.04	0.12	-0.09
lignoceric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nervonic acid	-0.08	-0.09	-0.08	-0.10	-0.05	-0.01	0.00	-0.05
EPA/AA ratio	0.00	1.05	1.83	0.39	0.00	0.43	0.59	0.33

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; NEFA, non-esterified fatty acid.
Values are shown as medians of 8 healthy subjects.

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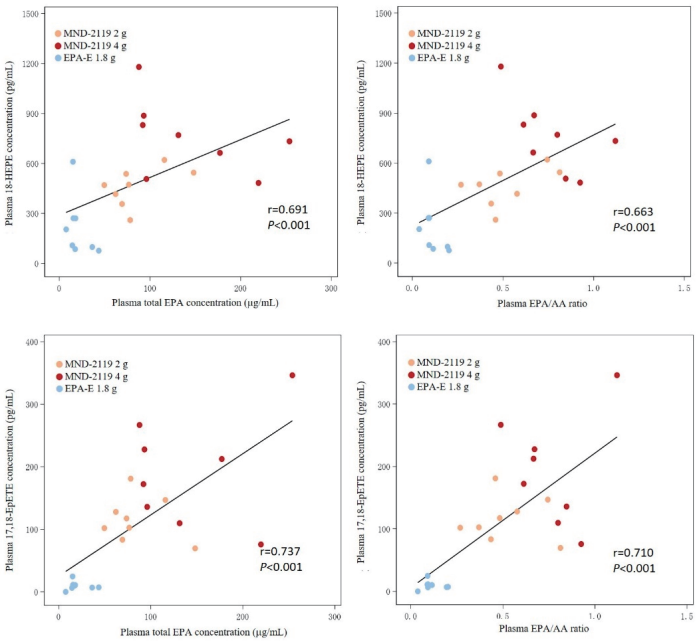
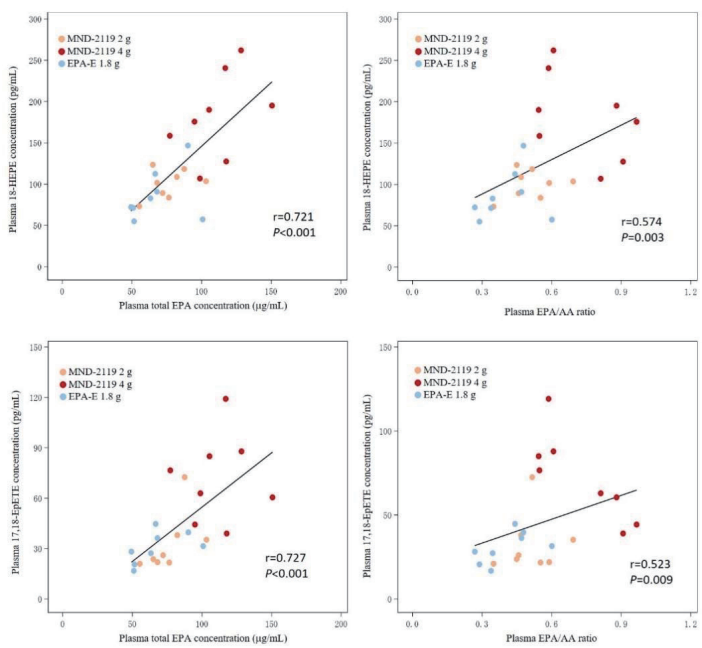


SupplementaryFig. 4. Association between plasma total EPA concentrations, EPA/AA ratio and 18-HEPE, 17,18-EpETE at Day 1 3 h

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid.
Plots show data of MND-2119 2 g group, MND-2119 4 g group and EPA-E 1.8 g group at Day 1 3 h (N = 24).



SupplementaryFig. 5. Association between plasma total EPA concentrations, EPA/AA ratio and 18-HEPE, 17,18-EpETE at Day 7 24 h

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid.
Plots show data of MND-2119 2 g, MND-2119 4 g and EPA-E 1.8 g group at Day 7 24 h (N = 24).

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Supplementary Table 6. Total fatty acids in RBC

	Baseline (median, ng/10⁶ cells)				Day1 3h change from baseline (median, ng/10⁶ cells)				Day1 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	0.09	0.11	0.20	0.00	-0.03	-0.02	-0.05	0.00	-0.05	-0.02	-0.06	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	23.69	24.59	23.53	23.23	1.73	-1.29	-2.83	3.93	-0.50	-1.04	-1.46	2.48
palmitoleic acid	0.29	0.24	0.35	0.23	0.01	-0.01	-0.01	0.02	-0.03	-0.04	-0.02	0.02
stearic acid	17.39	18.11	18.70	17.99	2.27	-0.17	-2.22	2.63	0.26	0.62	-1.11	1.60
oleic acid	16.04	16.89	17.16	15.22	0.79	-0.80	-1.75	2.41	-0.23	-0.09	-0.90	0.93
linoleic acid	13.04	13.37	13.50	12.35	0.21	-0.72	-1.90	1.92	-0.57	-1.28	-1.51	0.67
linolenic acid	0.14	0.15	0.12	0.13	0.00	-0.01	-0.01	0.02	-0.02	-0.04	-0.02	0.00
γ-linolenic acid	0.07	0.08	0.06	0.06	0.00	-0.02	-0.01	0.01	-0.01	-0.03	-0.01	0.00
arachidic acid	0.12	0.12	0.12	0.10	0.01	-0.01	-0.01	0.02	0.01	-0.01	-0.01	0.01
eicosenoic acid	0.41	0.40	0.40	0.34	0.04	-0.01	-0.06	0.07	0.01	-0.01	-0.04	0.02
eicosadienoic acid	0.41	0.37	0.41	0.41	0.03	-0.01	-0.05	0.08	0.00	0.00	-0.02	0.03
dihomo-γ-linolenic acid	1.58	1.81	1.65	1.55	0.12	-0.04	-0.07	0.24	-0.07	-0.01	-0.07	0.10
5,8,11-eicosatrienoic acid	0.05	0.05	0.06	0.04	0.01	0.00	-0.01	0.01	0.00	0.00	0.00	0.00
arachidonic acid	16.28	16.02	16.20	15.20	1.45	-0.64	-1.86	2.44	0.63	-0.63	-1.18	1.38
eicosapentaenoic acid	0.77	0.96	0.93	0.84	0.04	-0.03	-0.02	0.12	-0.08	0.17	0.19	0.10
behenic acid	0.16	0.16	0.17	0.15	0.03	-0.01	-0.01	0.04	-0.01	0.00	-0.01	0.02
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	3.83	2.99	3.15	3.35	0.35	-0.02	-0.41	0.44	0.12	-0.04	-0.21	0.28
docosapentaenoic acid	3.51	3.43	3.56	3.28	0.29	-0.02	-0.42	0.61	-0.04	-0.12	-0.18	0.35
docosahexaenoic acid	7.80	8.20	9.13	7.32	0.61	-0.17	-1.02	1.23	0.37	-0.35	-0.51	0.65
lignoceric acid	0.53	0.49	0.53	0.47	0.05	-0.04	-0.04	0.12	0.00	0.02	-0.02	0.09
nervonic acid	0.52	0.51	0.46	0.43	0.03	-0.05	-0.02	0.11	-0.01	-0.02	-0.01	0.07
EPA/AA ratio	0.05	0.06	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00

	Day7 3h change from baseline (median, ng/10⁶ cells)				Day7 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	-0.09	-0.11	-0.20	0.00	-0.09	-0.11	-0.14	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	-2.03	0.27	-1.56	-2.36	-1.98	-0.12	0.42	0.84
palmitoleic acid	-0.04	-0.06	-0.04	-0.05	-0.04	-0.05	-0.04	-0.02
stearic acid	-1.17	0.45	-1.68	-2.50	-0.88	1.22	0.33	-0.49
oleic acid	-1.02	0.33	-0.93	-2.03	-1.11	0.71	0.45	-0.11
linoleic acid	-2.20	-1.33	-2.28	-3.22	-1.62	-1.36	-2.46	-1.36
linolenic acid	-0.02	-0.03	-0.01	-0.03	-0.03	-0.04	-0.01	-0.03
γ-linolenic acid	-0.02	-0.02	-0.02	-0.02	-0.04	-0.02	-0.01	-0.01
arachidic acid	-0.02	0.00	-0.01	-0.01	-0.01	0.00	-0.01	0.01
eicosenoic acid	-0.03	-0.02	-0.05	-0.05	-0.03	0.00	-0.02	0.00
eicosadienoic acid	-0.01	-0.02	-0.03	-0.05	-0.01	-0.01	-0.01	0.01
dihomo-γ-linolenic acid	-0.09	-0.05	-0.19	-0.27	-0.18	-0.06	-0.15	-0.02
5,8,11-eicosatrienoic acid	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	0.00
arachidonic acid	-0.41	-0.05	-0.65	-1.44	-0.70	0.05	0.60	0.99
eicosapentaenoic acid	-0.22	0.86	1.35	0.37	-0.24	0.84	1.52	0.52
behenic acid	-0.02	0.01	0.00	0.00	-0.01	0.00	0.02	0.03
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	-0.15	0.10	-0.19	-0.44	-0.14	0.06	0.05	0.16
docosapentaenoic acid	-0.18	0.29	0.30	-0.10	-0.17	0.43	0.61	0.20
docosahexaenoic acid	-0.40	0.08	-0.31	-0.66	-0.45	0.09	-0.04	0.45
lignoceric acid	-0.05	0.02	0.02	-0.01	-0.02	-0.03	0.07	0.08
nervonic acid	-0.04	-0.02	0.02	0.01	-0.02	-0.03	0.03	0.10
EPA/AA ratio	-0.01	0.05	0.08	0.03	-0.01	0.05	0.08	0.03

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; RBC, red blood cells.
Values are shown as medians of 8 healthy subjects.

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Supplementary Table 7. Total fatty acids in PBMC

	Baseline (median, ng/10⁶ cells)				Day1 3h change from baseline (median, ng/10⁶ cells)				Day1 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
stearic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
oleic acid	149.71	120.07	166.01	168.41	0.00	-17.57	-130.81	-16.00	0.00	89.59	16.12	-80.95
linoleic acid	53.51	0.00	117.10	97.66	2.10	0.00	-38.97	0.00	0.00	0.00	29.60	-1.64
linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
γ-linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosenoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosadienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dihomo-γ-linolenic acid	24.99	22.64	27.66	24.42	-1.19	4.35	-5.33	1.24	-2.39	1.57	-1.08	0.92
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	211.01	176.56	218.71	219.56	9.83	35.87	-54.48	21.61	-19.68	35.02	51.95	-0.62
eicosapentaenoic acid	3.89	2.78	3.75	4.36	-0.08	1.65	1.22	0.07	-0.89	2.80	7.61	2.17
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	14.58	19.41	27.62	25.64	-1.54	-8.79	-10.27	0.29	0.00	-6.18	-0.43	-2.13
docosapentaenoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	19.06	0.00
docosahexaenoic acid	38.70	30.44	41.91	38.06	-2.79	3.64	-9.07	1.67	-6.33	1.29	6.15	1.06
lignoceric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nervonic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EPA/AA ratio	0.02	0.02	0.02	0.02	0.00	0.01	0.02	0.00	0.00	0.01	0.03	0.01

	Day7 3h change from baseline (median, ng/10⁶ cells)				Day7 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
stearic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
oleic acid	-1.27	21.77	29.64	-149.19	-53.30	8.52	18.37	-41.37
linoleic acid	0.00	0.00	0.00	-97.66	-53.51	0.00	0.00	-25.30
linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
γ-linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosenoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosadienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dihomo-γ-linolenic acid	0.10	1.11	-2.18	0.49	-3.74	-4.33	-5.14	-4.42
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	17.55	14.21	-6.41	-16.25	-14.50	-13.16	-55.52	-42.71
eicosapentaenoic acid	-1.51	10.36	25.58	8.50	-1.22	8.33	16.08	7.42
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	0.00	-17.69	-23.76	-25.64	-8.45	-19.41	-24.46	-14.95
docosapentaenoic acid	0.00	34.46	60.00	0.00	0.00	11.54	27.20	0.00
docosahexaenoic acid	2.15	-6.42	-9.75	-8.81	-5.03	-9.81	-16.99	-16.78
lignoceric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nervonic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EPA/AA ratio	-0.01	0.06	0.13	0.04	-0.01	0.06	0.12	0.05

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid; PBMC, peripheral blood mononuclear cells.
Values are shown as medians of 8 healthy subjects.

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Supplementary Table 8. Total fatty acids in neutrophil

	Baseline (median, ng/10⁶ cells)				Day1 3h change from baseline (median, ng/10⁶ cells)				Day1 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	327.61	276.29	289.60	278.40	-13.84	-35.79	-21.53	-4.64	-51.29	-39.48	-15.36	-2.74
palmitoleic acid	8.76	0.00	9.53	6.30	-1.09	0.00	-0.81	-0.26	-1.86	0.00	0.00	0.06
stearic acid	457.45	421.11	427.93	420.64	-3.43	-96.54	3.03	21.25	-35.54	-10.37	-31.40	2.62
oleic acid	785.20	645.03	677.38	659.91	-7.35	-80.16	-47.78	-5.92	-131.88	-53.82	-38.65	-32.86
linoleic acid	249.55	239.34	269.40	282.30	-7.80	-24.37	-17.40	4.44	-26.13	-15.26	-20.46	-13.50
linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
γ-linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosenoic acid	30.23	24.38	30.50	29.01	-0.16	-3.22	-1.26	1.99	-3.02	-2.52	-8.19	-0.08
eicosadienoic acid	31.86	26.99	31.45	33.58	0.48	-1.26	-3.31	1.68	-0.56	-0.08	-2.95	-0.68
dihomo-γ-linolenic acid	43.14	43.05	43.90	43.18	-3.05	-5.12	-3.70	1.60	-4.36	-2.26	-0.38	1.46
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	309.42	247.09	277.85	252.98	-9.51	-21.80	-15.12	3.86	-41.67	-2.00	-10.17	-12.31
eicosapentaenoic acid	8.11	6.10	9.25	9.90	-1.80	0.00	1.30	-0.09	-1.54	3.38	6.24	0.01
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	45.32	40.36	40.77	41.08	0.94	-3.66	-2.67	-1.02	0.80	-1.07	-3.23	0.46
docosapentaenoic acid	23.24	19.83	24.28	21.52	-0.65	-2.37	0.00	-1.07	-4.15	2.46	4.39	-0.11
docosahexaenoic acid	22.95	20.93	25.52	22.24	-1.43	-3.03	-2.39	-0.68	-4.08	-0.67	-3.33	-3.01
lignoceric acid	6.53	0.00	0.00	2.90	0.00	0.00	0.00	0.00	-0.59	0.00	0.00	0.00
nervonic acid	16.22	14.75	15.85	14.04	-1.03	-1.36	-0.21	1.00	-2.11	-0.78	-2.65	-0.75
EPA/AA ratio	0.03	0.03	0.03	0.03	0.00	0.00	0.01	0.00	0.00	0.01	0.02	0.00

	Day7 3h change from baseline (median, ng/10⁶ cells)				Day7 24h change from baseline (median, ng/10⁶ cells)			
	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g	Control	MND-2119 2g	MND-2119 4g	EPA-E 1.8g
lauric acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
myristoleic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
palmitic acid	9.90	3.46	-4.37	-13.31	-1.65	-7.25	-18.62	-15.41
palmitoleic acid	-1.60	0.00	-0.95	-6.15	-0.30	0.00	0.07	0.00
stearic acid	9.38	-61.66	43.85	19.03	39.50	-48.14	17.42	15.02
oleic acid	-37.35	3.16	-9.24	-119.36	-36.50	-79.42	-13.77	-47.58
linoleic acid	7.48	20.90	-2.03	-35.71	11.09	-12.92	-5.64	-19.91
linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
γ-linolenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
eicosenoic acid	2.13	-0.94	-4.97	-1.86	-0.18	-3.65	-7.72	-3.92
eicosadienoic acid	3.97	2.04	-6.23	-3.22	6.53	-2.87	-3.53	-3.08
dihomo-γ-linolenic acid	-3.07	-5.85	-4.29	-3.39	1.14	-7.43	-4.98	-4.14
5,8,11-eicosatrienoic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
arachidonic acid	-21.72	-18.06	-39.72	-24.09	-18.61	-23.81	-37.54	-19.80
eicosapentaenoic acid	-3.66	19.41	36.64	11.60	-3.15	19.76	36.77	13.41
behenic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
erucic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
docosatetraenoic acid	-1.27	-8.55	-10.25	-7.60	5.96	-9.84	-12.42	-8.23
docosapentaenoic acid	-2.50	14.25	25.19	8.32	-1.98	17.68	23.24	13.45
docosahexaenoic acid	-2.29	-4.25	-8.27	-5.81	-3.72	-6.68	-10.17	-3.78
lignoceric acid	-0.41	0.00	0.00	0.00	-0.82	0.00	0.00	0.00
nervonic acid	0.49	0.91	-1.86	1.39	-0.38	-0.91	0.48	1.87
EPA/AA ratio	0.00	0.10	0.16	0.05	0.00	0.10	0.17	0.06

Abbreviations: EPA, eicosapentaenoic acid; AA, arachidonic acid.
Values are shown as medians of 8 healthy subjects.

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Supplementary Table 9. The incidence of AEs in subjects

System organ class, Preferred term	Control ($n=8$)	MND-2119 2g ($n=8$)	MND-2119 4g ($n=8$)	EPA-E 1.8g ($n=8$)
Adverse Events (AEs), *n*	1	5	3	2
Nervous system disorders, *n*	0	1	0	0
Headache , *n*	0	1	0	0
Gastrointestinal disorders, *n*	1	3	3	2
Diarrhoea , *n*	1	2	2	1
Faeces hard , *n*	0	1	1	0
Abdominal pain , *n*	0	1	0	0
Change of bowel habit , *n*	0	0	0	1
Constipation , *n*	0	0	1	0
Investigations, *n*	0	1	0	0
Blood bilirubin increased, *n*	0	1	0	0

Dictionary for terms: MedDRA/J Ver.24.1.

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