Effect of 6,9,12,15-Hexadecatetraenoic Acid (C16:4n-1)-Ethyl Ester on Lipid Content and Fatty Acid Composition in the Blood and Organs of Mice

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Abstract: The effects of 6,9,12,15-hexadecatetraenoic acid (C16:4n-1, HDTA), an n-1 polyunsaturated fatty acid (FA), on plasma and liver lipid content and distribution in blood and tissues were investigated. Mice were fed experimental diets containing 10% HDTA or eicosapentaenoic acid in ethyl ester form based on corn oil for four weeks. Dietary HDTA intake lowered plasma triacylglycerol content without affecting plasma total cholesterol content. HDTA barely accumulated in the epididymal white adipose tissue (eWAT), while C18:4n-1, an HDTA metabolite, was detected in small amounts (< 1% of total FAs) in the plasma, liver, and eWAT.

Key words: 6,9,12,15-hexadecatetraenoic acid, n-1 polyunsaturated fatty acid, eicosapentaenoic acid, fish oil, mice

1 Introduction

Fish oil is rich in n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The intake of EPA and DHA has beneficial effects on human health, including anti-arrhythmic1, anti-inflammatory2, and anti-hypertriglyceremic3 effects. In addition to EPA and DHA, fish oil is composed of uncommon n-3 PUFA such as 6,9,12,15-octadecatetraenoic acid (C18:4n-3) and 4,7,10,13-hexadecatetraenoic acid (C16:4n-3)4, which are physiologically relevant. Previous studies reported that C18:4n-3 has inhibitory effects on platelet aggregation7 and the metabolism of C18:4n-3 in cultured NIH-3T3 cells8, while C16:4n-3 and C18:4n-3 have been reported to inhibit the production of leukotriene B4, leukotriene C4, and 5-hydroxyeicosatetraenoic acid in mouse mast cells9. A trace amount of C16:4n-3 induced by platinum-based chemotherapy was resistant to antitumor activity in mice, and fish oil containing C16:4n-3 neutralized the antitumor activity of platinum-based chemotherapy in various mouse models10. Fish oils derived from sardine and herring contain approximately 1% to 3% of 6,9,12,15-hexadecatetraenoic acid (C16:4n-1, HDTA), an isomer of C16:4n-311. HDTA is uniquely characterized by the presence of a double bond at the terminal carbon in its chemical structure (Fig. 1A). A previous study reported the isolation of HDTA from a fatty acid (FA)-methyl ester (ME) prepared from fish oil using high-speed counter-current chromatography12. Thus far, only one patent report has investigated the physiological effects of HDTA and described anti-inflammatory effects of HDTA against colitis, carrageenan-induced edema, and arthritis in a mouse model13. The metabolism and health-promoting functions of the n-1 PUFA are relatively unknown to date and would constitute an interesting area of research with substantial implications for advancing oleochemistry. This study evaluated the effect of dietary HDTA-ethyl ester (EE) on lipid content and FA composition in the blood and organs of mice compared with EPA-EE, a major n-3 PUFA found in fish oil. In this study, the HDTA-EE was concentrated to approximately 10% by precision distillation of anchovy oil-EE and 98% by preparative-scale high-performance liquid chromatography.

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2 Experimental Procedures

2.1 Materials

HDTA-EE (98%) and EPA-EE (98%) were provided by Bizen Chemical Co., Ltd. (Akaia, Japan). Corn oil-EE was prepared by NaOH-ethanol solution from triacylglycerol (TAG)-form corn oil (Merck KGaA, Darmstadt, Germany). The experimental diet components were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan) and Fujifilm Wako Pure Chemical Co. (Osaka, Japan). All other reagents were of reagent grade, and were obtained from Merck KGaA and Nacalai Tesque, Inc. (Kyoto, Japan).

2.2 Analysis of HDTA-EE

HDTA-EE was dissolved in n-hexane (2%, w/v) and analyzed via gas chromatography/mass spectrometry (GC/MS) to confirm its chemical structure. GC/MS (GCMS-QP2010PARVUM2, Shimadzu Co., Kyoto, Japan) system equipped with an Omegawax® column (30 m × 250 μm × 0.25 μm; Merck KGaA). The GC system parameters were set as follows: injector and interface temperatures of 250°C, column temperature of 120°C, gradual heating to 240°C at a rate of 2°C/min, held at 240°C for 10 min, and 1 mL/min helium as a carrier gas. MS system parameters were set as follows: ion source temperature, 200°C; electronic ionization (EI); 70 eV; full scan m/z 45–350 amu. Detailed total-ion chromatograms and EI mass spectra of HDTA-EE are shown in Figs. 1B and 1C.

2.3 Animal diet and care

All animal experiments were conducted in accordance with the Guide for the Care and Use of Experimental Animals issued by the Prime Minister’s Office of Japan and were reviewed and approved by the Animal Ethics Committee of Kansai University (Approval No. 1704).

The experimental diet was prepared based on the AIN-93G composition so that HDTA-EE and EPA-EE each had an FA composition of 10 mol% (HDTA and EPA diets, respectively). Corn oil-EE was added to the control diet to adjust the EE content to be approximately equal to that in the HDTA and EPA diets. Details of the ingredients and FA compositions of the experimental diets are shown in Table 1.

Four-week-old male C57BL/6J mice obtained from Japan SLC, Inc. (Hamamatsu, Japan) were kept in individual cages, housed at 21–23°C with a 12 h light-dark cycle (lights on: 08:00-20:00), and allowed free access to tap water and the prepared diets. Following an acclimatization period of seven days, 18 mice were divided into three dietary groups: control, HDTA, and EPA. Food consumption, water intake, and body weight (BW) were recorded every two days. After four weeks, the mice were fasted overnight, weighed, and euthanized using isoﬂurane (Intervet K.K., Osaka, Japan) between 09:00 and 11:00. Blood was collected from the inferior vena cava with anticoagulant, and plasma and red blood cells (RBC) were separated by centrifugation (2,000 × g) for 15 min. The liver, kidney, spleen, heart, brain, interscapular brown adipose tissue (BAT) and abdominal white adipose tissue (WAT) from the epididymis, mesentery, and perinephric and inguinal regions were quickly removed, weighed, and rinsed with cold saline. The tissues were frozen in liquid nitrogen and stored at −80°C until further analyses. A portion of the liver was stored in RNA-Later Storage Solution (Merck KGaA) for quantitative real-time PCR.

2.4 Analysis of lipid parameters in plasma and liver

Plasma biochemical parameters: aspartate aminotransferase [AST], alanine aminotransferase [ALT], urea nitrogen [UN], TAG, phospholipids [PL], total cholesterol, high-density lipoprotein [HDL] cholesterol, and non-HDL cholesterol were measured using an Olympus AU5431 automatic analyzer (Olympus Co., Tokyo, Japan) by a com-
Table 1 Ingredients and fatty acid compositions of the experimental diets.

| Ingredients compositions (g/kg) | Experimental diets |
|---------------------------------|--------------------|
|                                 | Control | HDTA  | EPA  |
| Casein                          | 200     | 200   | 200  |
| L-Cystine                       | 3       | 3     | 3    |
| Corn starch                     | 397.486 | 397.486 | 397.486 |
| Dextrinized corn starch          | 132     | 132   | 132  |
| Sucrose                         | 100     | 100   | 100  |
| Corn oil                        | 61.39   | 61.56 | 61.36 |
| Corn oil-EE                     | 8.61    |       |      |
| HDTA-EE                         |         | 8.44  |      |
| EPA-EE                          |         |       | 8.64 |
| Cellulose                       | 50      | 50    | 50   |
| AIN-93G mineral mixture          | 35      | 35    | 35   |
| AIN-93 vitamin mixture           | 10      | 10    | 10   |
| Choline bitartrate              | 2.5     | 2.5   | 2.5  |
| tert-Butylhydroquinone           | 0.014   | 0.014 | 0.014 |

Fatty acid composition (mol%)

| Fatty acid composition (mol%) | Control | HDTA  | EPA  |
|------------------------------|---------|-------|------|
| C16:0                        | 11.8    | 10.5  | 11.0 |
| C16:4n-1 (HDTA)              |         | 10.5  |      |
| C18:0                        | 1.8     | 1.5   | 2.0  |
| C18:1n-9                     | 31.7    | 28.1  | 27.6 |
| C18:1n-7                     | 0.8     | 0.7   | 1.1  |
| C18:2n-6                     | 52.3    | 47.4  | 45.8 |
| C18:3n-3                     | 1.6     | 1.3   | 1.9  |
| C20:5n-3 (EPA)               |         |       | 10.7 |

Diets were prepared on the basis of the AIN-93G composition.
AIN, American Institute of Nutrition; EE, ethyl ester; EPA, eicosapentaenoic acid; HDTA, 6,9,12,15-hexadecatetraenoic acid.

2.5 Analysis of fatty acid composition

The total lipids from the experimental diets, plasma, RBC, liver, epididymal WAT (eWAT), and brain were extracted using the chloroform/methanol/water extraction method described above\textsuperscript{12}. The FA compositions of the total lipids were determined using a GC system (GC-2014; Shimadzu Co.) with an Omegawax\textsuperscript{®} column after the methylation of FA with boron-trifluoride-methanol as described in our previous report\textsuperscript{10}. Identification of each FA was carried out using a standard mixture of FA-ME (Supelco 37 Component FAME Mix, Merck KGaA).

2.6 RNA isolation and real-time quantitative reverse transcription PCR

Liver samples, which were stored in RNA-Later Storage Solution, were homogenized using a bead beater-type homogenizer (MicroSmash MS-100R, TOMY SEIKO CO., LTD., Tokyo, Japan). Total RNA was isolated and purified using the TRIzol\textsuperscript{®} reagent (Thermo Fisher Scientific Inc, Massa-
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Table 2  Growth parameters, organ weights, plasma biochemical parameters, and liver lipid contents.

| Experimental groups | Control     | HDTA        | EPA         |
|---------------------|-------------|-------------|-------------|
| Growth parameters   |             |             |             |
| Initial BW (g)      | 19.3 ± 0.2  | 19.3 ± 0.3  | 19.2 ± 0.5  |
| Final BW (g)        | 24.1 ± 0.3  | 24.3 ± 0.4  | 23.9 ± 0.5  |
| BW gain (g/day)     | 0.16 ± 0.01 | 0.17 ± 0.01 | 0.15 ± 0.01 |
| Food intake (g/day) | 2.5 ± 0.1   | 2.7 ± 0.1   | 2.6 ± 0.1   |
| Organ weights (g/100 g BW) | | | |
| Liver               | 3.63 ± 0.09 | 3.66 ± 0.07 | 3.77 ± 0.15 |
| Kidney              | 1.13 ± 0.02 | 1.18 ± 0.04 | 1.16 ± 0.02 |
| Spleen              | 0.27 ± 0.01 | 0.26 ± 0.01 | 0.29 ± 0.01 |
| Heart               | 0.55 ± 0.02 | 0.59 ± 0.02 | 0.54 ± 0.03 |
| Brain               | 2.04 ± 0.05 | 2.00 ± 0.06 | 2.04 ± 0.05 |
| Epididymal WAT      | 1.56 ± 0.13 | 1.32 ± 0.05 | 1.55 ± 0.12 |
| Mesentery WAT       | 0.63 ± 0.07 | 0.58 ± 0.06 | 0.65 ± 0.05 |
| Perirenal WAT       | 0.92 ± 0.05 | 0.93 ± 0.12 | 1.12 ± 0.06 |
| Inguinal WAT        | 0.77 ± 0.08 | 0.62 ± 0.06 | 0.78 ± 0.14 |
| Interscapular BAT   | 0.51 ± 0.05 | 0.41 ± 0.03 | 0.43 ± 0.04 |
| Plasma biochemical parameters | | | |
| AST (IU/L)          | 40.2 ± 2.1  | 39.8 ± 1.9  | 40.8 ± 3.7  |
| ALT (IU/L)          | 12.5 ± 0.8  | 11.2 ± 0.8  | 12.5 ± 0.6  |
| UN (mg/dL)          | 28.2 ± 1.7  | 34.7 ± 2.4  | 34.3 ± 2.6  |
| TAG (mg/dL)         | 40.7 ± 5.8b | 25.7 ± 2.1a | 33.5 ± 2.9ab|
| PL (mg/dL)          | 209 ± 6b    | 222 ± 8b    | 141 ± 3a    |
| Total cholesterol (mg/dL) | 96 ± 4b  | 102 ± 5b    | 61 ± 1b     |
| HDL cholesterol (mg/dL) | 81.8 ± 3.4b | 88.8 ± 4.4b | 53.8 ± 0.9b |
| Non-HDL cholesterol (mg/dL) | 14.3 ± 1.5b | 13.5 ± 1.0b | 7.3 ± 0.7a |
| Liver lipid contents (mg/g) | | | |
| TAG                 | 35.8 ± 5.9b | 38.3 ± 5.3b | 16.5 ± 2.3a |
| PL                  | 27.7 ± 1.0  | 28.6 ± 1.7  | 25.7 ± 1.5  |
| Cholesterol         | 5.54 ± 0.28 | 6.20 ± 0.92 | 4.43 ± 0.22 |

Data are shown as mean ± SEM (n = 6). Values in the same row not sharing a common superscript are significantly different at \( p < 0.05 \), using Tukey’s multiple comparison test.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BAT, brown adipose tissue; BW, body weight; HDL, high-density lipoprotein; PL, phospholipid; SEM, standard error of the mean; TAG, triacylglycerol; UN, urea nitrogen; WAT, white adipose tissue.

chusetts, USA) according to the manufacturer’s protocol. The content and purity of total RNA were determined by measuring absorbance at 260 and 280 nm wavelengths by UV spectroscopy (UV-1800, Shimadzu Co.) using Hellma® TrayCell™ (Hellma Analytics, Müllheim, Germany). cDNA was then synthesized using the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA), and mRNA expression levels were measured using a Thermal Cycler Dice® Real Time System (Takara Bio Inc., Kusatsu, Japan) and GoTaq® qPCR Master Mix (Promega Co.). All primers were designed using Primer3Plus (http://primer3plus.com/), and the primer sequences are shown in Table S1.
Gene expression levels were standardized to the expression level of glyceraldehyde-3-phosphate dehydrogenase (Gapd) and expressed as the fold-change in gene expression relative to the control group.

2.7 Statistical analysis
Data are presented as mean values and standard errors of the mean (SEM). The differences between multiple groups were evaluated using analysis of variance (ANOVA) and Tukey’s multiple comparison test. Statistical significance and tendency were set as $p<0.05$, and $0.05 \leq p < 0.10$, respectively. Analyses were performed using GraphPad Prism7 (ver. 7.0d, GraphPad Software, California, USA).

3 Results

3.1 Growth parameters, organ weights, and biochemical parameters in plasma and liver
Table 2 shows growth parameters, organ weights, plasma biochemical parameters, and liver lipid contents. There were no significant differences in growth parameters and organ weights among the three groups.
HDTA intake decreased plasma TAG content compared to the control group. The EPA group showed reduced plasma PL, total cholesterol, HDL cholesterol, and non-HDL cholesterol contents compared to the control group. The EPA diet significantly reduced liver TAG content compared to the HDTA group, whereas it tended to be lower than that in the control group ($p = 0.06$). There were no significant differences in plasma AST, ALT, UN, liver PL, or cholesterol levels among the experimental groups.

3.2 Identifications of two unknown peaks in the HDTA group
Two unknown FA peaks (retention times (RTs) 32.8 and 42.7 min, respectively) in the eWAT were detected only in mice fed the HDTA diet and not in mice fed the control or EPA diets (Fig. 2A). Figure 2B shows the mass spectrum of the unknown FA at an RT of 32.8 min. This unknown FA was identified as HDTA, using RT and mass spectrum of standard HDTA-ME prepared from HDTA-EE. Figure 2C shows the mass spectrum of the unknown FA at an RT of 42.7 min. This unknown FA mass spectrum showed a molecular weight peak at $m/z = 290$ and fragment ions at $m/z = 222$ and 249, respectively. The mass spectrum at an RT of 42.7 min was very similar to the previously reported mass spectrum of C18:4n-1 ME [16]. From these results, we identified this unknown FA as C18:4n-1.

3.3 Fatty acid composition in the blood and organs
Figure 3 shows the FA composition in the total lipid content of the plasma, RBC, liver, eWAT, and brain. In the HDTA group, HDTA was detected only in eWAT, whereas C18:4n-1 was detected in the plasma, liver, and eWAT. In the HDTA group, HDTA and C18:4n-1 were not detected in the RBC and brain.
In the plasma, the EPA diet decreased the C16:1n-9,
C18:1n-7, C18:3n-6, and C20:4n-6 proportions and increased the C18:2n-6, C20:5n-3, C22:5n-3, and C22:6n-3 proportions compared to the control and HDTA diets. The HDTA diet increased the C18:3n-6 proportion compared to the control and EPA diets. In RBC, EPA intake increased the C16:0, C20:5n-3, and C22:6n-3 proportions and decreased the C18:1n-7, C20:3n-6, and C20:4n-6 proportions compared to the control and HDTA diets. In the liver, the EPA group exhibited higher C16:0, C20:5n-3, and C22:6n-3 proportions than the control and HDTA groups and lower C20:4n-6 proportion than the control group. The HDTA group showed higher C16:1n-7, C18:1n-9, and C18:1n-7 proportions than the EPA group. In the eWAT, the HDTA diets increased the C16:1n-9, C16:1n-7, and
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C18:1n-9 proportions compared to the control and EPA groups. The EPA diet decreased the C18:3n-3 and C20:4n-6 proportions compared to the control and HDTA diets, as well as the C18:1n-7 and C20:1n-9 proportions compared to the control diet. Moreover, the C20:5n-3 and C22:6n-3 proportions in the EPA group were higher than those in the control and HDTA groups. In the brain, the EPA group had a higher C20:3n-6 proportion than the HDTA group, which lowered the C20:4n-6 proportion compared to the control and HDTA groups.

3.4 Relative mRNA expression level in liver

Figure 4 shows the relative expression levels of genes related to FA metabolism in the liver. The expression of stearoyl-coenzyme A desaturase-1 (Scd-1), fatty acid synthase (Fas), acetyl-coenzyme A carboxylase alpha (Acaca), acyl-coenzyme A oxidase 1 (Acox1), carnitine palmitoyltransferase 1a (Cpt1a), fatty acid desaturase 1 (Fads1), Fads2, or elongation of very long-chain fatty acids (Elovl)2, Elovl3, Elovl5, Elovl6, and Elovl7 among the three groups.

4 Discussion

In this study, HDTA intake decreased plasma TAG content compared to the control group. The reduction in plasma TAG content in the HDTA group was notably more substantial than that in the EPA group (Table 2). EPA intake has a lowering effect on plasma TAG content due to its suppression of FA synthesis and the enhancement of FA oxidation through regulation of liver X receptor and peroxisome proliferator-activated receptor-α. Thus, EPA is offered as a pharmaceutical compound for patients with hypertriglyceridemia. HDTA intake was observed to have a more potent plasma TAG-lowering effect than EPA intake under these experimental conditions. As this study was conducted using normal mice, further studies should evaluate this effect using a mouse model of hyperlipidemia. Conversely, TAG content in the liver was increased in the HDTA group compared to that in the EPA group; however, it was not significantly different from that in the control group (Table 2). To elucidate the mechanism of HDTA-induced decrease in plasma TAG content, mRNA expression levels of genes related to FA metabolism in the liver were measured; however, there were no significant changes in the expression levels of genes associated with FA synthesis and oxidases (Scd1, Fas, Acaca, Acox1, and Cpt1a). A previous study reported that fasting time significantly affected lipid metabolism, especially through regulation of enzymes involved in liver FA biosynthesis in rodents; the results indicated that in order to elucidate the mechanisms of lipid reduction induced by dietary food components, rodents should not be fasted on the last day before dissection. In our study, we dissected the mice after a period of overnight fasting to assess plasma biochemical parameters and organ FA compositions, which were not affected by diet. This study therefore did not allow us to evaluate the effect of HDTA on FA metabolism in the liver, and further studies should be conducted in non-fasting conditions.

To evaluate the distribution of HDTA and its metabolites in the blood and organs, we measured FA compositions of the total lipids in the plasma, RBC, liver, eWAT, and brain (Fig. 3). We detected two unknown FA peaks (RTs 32.8 and 42.7 min, respectively) that were detected only in mice.
fed the HDTA diet and not in mice fed the control and EPA diets (Fig. 2A). From the GC/MS data, two unknown peaks were identified as HDTA (C16:4n-1) and C18:4n-1 (Figs. 2B and 2C, respectively). HDTA was detected only in 0.6 ± 0.0 mol% of eWAT in the HDTA group (Fig. 3D). In addition, the C18:4n-1 was detected in 0.3 ± 0.1, 0.9 ± 0.1, and 0.5 ± 0.1 mol% of plasma, liver, and eWAT in the HDTA group, respectively. However, EPA in the plasma, RBC, liver, and eWAT in the EPA group were 6.1 ± 1.5, 5.6 ± 0.3, 4.7 ± 0.5, and 1.0 ± 0.1 mol%, respectively. EPA was not detected in the brains of mice in the EPA group. The mammalian central nervous system is very low in EPA, which suggests that in the brain EPA may be a precursor to DHA synthesis. In the plasma, RBC, liver, and eWAT, HDTA proportions in the HDTA group were much lower than EPA proportions in the EPA group. HDTA intake accumulates in small amounts (<1% of total FAs) in the plasma, liver, and eWAT as HDTA and/or C18:4n-1. Dietary palmitic acid (C16:0), which has the same carbon chain length as HDTA, is incorporated in the eWAT TAG of rats. In addition, the intake of diet containing about 17% (% of total FAs in diet) palmitoleic acid (C16:1n-7) was accumulated in 3.5% (% of total FAs) of the liver in low-density lipoprotein receptor-deficient mice. Previous studies have administered unusual FAs to laboratory animals and investigated their distribution. Diet containing about 7% (% of total FAs in diet) conjugated linoleic acid administration was found to be incorporated in various tissues (kidney, brain, testis, liver, lung, spleen, and eWAT) to reach a proportion of 0.5–5.5% (% of total FAs) in rats, while the intake of about 10% (% of total FAs in diet) scidadeic acid, a non-methylene-interrupted PUFA, accumulated in 5.3% and 2.1% (wt% of total FAs) in the serum and liver TAG of rats. Odd-numbered long-chain FAs are more likely to accumulate in the mammalian body than even-numbered long-chain FAs, whereas dietary medium-chain FAs (MCFA) are absorbed from the intestinal tract to the portal vein, from where they are taken into the liver and rapidly undergo β-oxidation. Previous reports showed that mice fed a diet containing lauric acid (3 wt% of diet) showed no lauric acid in liver TAG and mice fed a diet containing medium-chain triglyceride were decreased serum TAG content. Since there was little accumulation of HDTA in the plasma, liver, and eWAT even when the mice were fed a 10% HDTA diet (% of total FAs) in our study, it can be considered that HDTA undergoes β-oxidation as well as MCFA, which may have decreased the plasma TAG content. However, HDTA may not be directly β-oxidized because C18:4n-1, a 2-carbon chain elongation of HDTA, is detected as a metabolite of HDTA. HDTA has four double bonds for its carbon chain length (C16), and since mouse do not contain such FAs, HDTA may have rapidly elongated the carbon chain, which may affect the stability of the cell membrane if it is incorporated into PL. In addition, it is also possible that the absorption rate of HDTA in the gastrointestinal tract is low. HDTA, an n-1PUFA, may not be a substrate for cluster of differentiation 36, which is important for FA uptake in the apical side of enterocyte. Further studies are therefore necessary to clarify the metabolism and absorption of HDTA.

5 Conclusion

In this study, we investigated the effects of HDTA, which has a double bond at the n-1 position, on the lipid content and FA composition in the blood and organs of mice. HDTA intake has a potent plasma TAG-lowering function. In addition, HDTA hardly accumulated in the plasma, RBC, liver, and eWAT, and a proportion of the HDTA was metabolized to C18:4n-1. This study shows, for the first time, the effects of HDTA on the plasma and liver lipid contents and FA composition in vivo.

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Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

This material is available free of charge via the Internet at http://dx.doi.org/jos.70.10.5650/jos.ess21025

References

1. Tribulova, N.; Szeiffova Bacova, B.; Egan Benova, T.; Knezl, V.; Barancik, M.; Slezak, J. Omega-3 Index and Anti-Arrhythmic Potential of Omega-3 PUFAs. Nutrients 9, 1191 (2017).
2. Mullen, A.; Loscher, C.E.; Roche, H.M. Anti-inflammatory effects of EPA and DHA are dependent upon time and dose-response elements associated with LPS stimulation in THP-1-derived macrophages. J. Nutr. Biochem. 21, 444-450 (2010).
3. Skulas-Ray, A.C.; Wilson, P.W.F.; Harris, W.S.; Brinton, E.A.; Kris-Etherton, P.M.; Richter, C.K.; Jacobson, T.A.; Engler, M.B.; Miller, M.; Robinson, J.G.; Blum, C.B.; Rodriguez-Leyva, D.; de Ferranti, S.D.; Welty, F.K. Omega-3 Fatty Acids for the Management of Hypertri-
Effect of Hexadecatetraenoic Acid on Mouse Lipid Metabolism

J. Oleo Sci.

1. Nakajima, A.; Saito, H.; Shiroma, K.; Maeda-Yamamoto, M. Inhibition of icodan-19:4n-3 polyunsaturated fatty acids isolated from edible marine algae. *Biosci. Biotechnol. Biochem.* **52**, 163–166 (1998).

2. Ishihara, K.; Murata, M.; Kaneniwa, M.; Saito, H.; Shiroma, K.; Maeda-Yamamoto, M. Inhibition of icosanoid production in MC9 mouse mast cells by n-3 polyunsaturated fatty acids isolated from edible marine algae. *Biosci. Biotechnol. Biochem.* **62**, 1412–1415 (1998).

3. Roodhart, J.M.; Daenen, L.G.; Fkeischer, S.; Yamamoto, A. Two dimensional thin layer chromatography of spots. *Can. J. Biochem. Physiol.* **75**, 1-6 (2012).

4. Doisaki, N.; Furuhata, K.; Hata, K.; Takeo, J.; Miyahara, H.; Yamashita, S. Prophylactic or therapeutic agent for inflammatory disease. *PCT/JP2006/039298* (2006).

5. Reeves, P.G.; Nielsen, F.H.; Fahey, G.C. Jr. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123**, 1939-1951 (1993).

6. Bligh, E.G.; Dyer, W.J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911-917 (1959).

7. Rouser, G.; Fkelsicher, S.; Yamamoto, A. Two dimensional then layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* **5**, 494-496 (1970).

8. Kaneda, T.; Nakajima, A.; Fujimoto, K.; Kobayashi, T.; Kiriyama, S.; Ebihara, K.; Innami, T.; Tsuji, K.; Tsuji, E.; Kinumaki, T.; Shinma, H.; Yoneyama, S. Quantitative analysis of cholesterol in foods by gas-liquid chromatography. *J. Nutr. Sci. Vitaminol.* **26**, 497-505 (1980).

9. Hosomi, R.; Matsudo, A.; Sugimoto, K.; Shimono, T.; Kanda, S.; Nishiyama, T.; Yoshida, M.; Fukunaga, K. Dietary fat influences the expression of genes related to sterol metabolism and the composition of cecal microbiota and its metabolites in rats. *J. Oleo Sci.* **68**, 1133-1147 (2019).

10. LIPID MAPS® Lipidomics Gateway. [Internet]. https://www.lipidmaps.org/resources/lipidweb/index.php?page=ms/methesters/me-arch/me_pufa/M0773.htm. Accessed 16 January 2021.

11. Forman, B.M.; Chen, J.; Evans, R.M. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 4312-4317 (1997).

12. Ou, J.; Tu, H.; Shan, B.; Luk, A.; DeBoose-Boyd, R.A.; Bashmakov, Y.; Goldstein, J.L.; Brown, M.S. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 6027-6032 (2001).

13. Ikeda, I.; Metoki, K.; Yamahira, T.; Kato, M.; Inoue, N.; Nagao, K.; Yanagita, T.; Shirakawa, H.; Komai, M. Impact of fasting time on hepatic lipid metabolism in nutritional animal studies. *Biosci. Biotechnol. Biochem.* **78**, 1584-1591 (2014).

14. Berman, D.R.; Mozurkewich, E.; Liu, Y.; Shangguan, Y.; Barks, J.D.; Silverstein, F.S. Docosahexaenoic acid augments hypothermic neuroprotection in a neonatal rat asphyxia model. *Neonatology* **104**, 71-78 (2013).

15. Abe, K.; Imaizumi, K.; Sugano, M. Effects of different triglyceride saturated fatty acids on tissue lipid level, fatty acid composition, fecal steroid excretion, prosta-cyclin production, and platelet aggregation in rats. *Biosci. Biotechnol. Biochem.* **57**, 247-252 (1993).

16. Yang, Z.H.; Pryor, M.; Noguchi, A.; Sampson, M.; Johnson, B.; Pryor, M.; Donkor, K.; Amar, M.; Remaley, A.T. Dietary palmitoleic acid attenuates atherosclerosis progression and hyperlipidemia in low-density lipoprotein receptor-deficient mice. *Mol. Nutr. Food Res.* **63**, e1900120 (2019).

17. Sugano, M.; Akahoshi, A.; Koba, K.; Tanaka, K.; Okumura, T.; Matsuyama, H.; Goto, Y.; Miyazaki, T.; Murao, K.; Yamasaki, M.; Nonaka, M.; Yamada, K. Dietary manipulations of body fat-reducing potential of conjugated linoleic acid in rats. *Biosci. Biotechnol. Biochem.* **65**, 2535-2541 (2001).

18. Endo, Y.; Tsunokake, K.; Ikeda, I. Effects of non-methylen-interrupted polyunsaturated fatty acid, sciadonic (all-cis-5,11,14-eicosatrienoic acid) on lipid metabolism in rats. *Biosci. Biotechnol. Biochem.* **73**, 577-581 (2009).

19. Gotoh, N.; Moroda, K.; Watanabe, H.; Yoshinaga, K.; Tanaka, M.; Mizobe, H.; Ichikawa, K.; Tokairin, S.; Wada, S. Metabolism of odd-numbered fatty acids and even-
numbered fatty acids in mouse. *J. Oleo Sci.* **57**, 293-299 (2008).

26) Schönfeld, P.; Wojtczak, L. Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. *J. Lipid Res.* **57**, 943-954 (2016).

27) Saraswathi, V.; Kumar, N.; Gopal, T.; Bhatt, S.; Ai, W.; Ma, C.; Talmon, G.A.; Desouza, C. Lauric acid versus palmitic acid: Effects on adipose tissue inflammation, insulin resistance, and non-alcoholic fatty liver disease in obesity. *Biology* **9**, 346 (2020).

28) Liu, Y.; Xue, C.; Zhang, Y.; Xu, Q.; Yu, X.; Zhang, X.; Wang, J.; Zhang, R.; Gong, X.; Guo, C. Triglyceride with medium-chain fatty acids increases the activity and expression of hormone-sensitive lipase in white adipose tissue of C57BL/6J mice. *Biosci. Biotechnol. Biochem.* **75**, 1939-1944 (2011).

29) Ko, C.W.; Qu, J.; Black, D.D.; Tso, P. Regulation of intestinal lipid metabolism: Current concepts and relevance to disease. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 169-183 (2020).