Trans-octadecenoic Acid Positional Isomers Have Different Accumulation and Catabolism Properties in Mice

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Abstract: *Trans* fatty acids (TFA) are considered risk factors for cardiovascular disease (CVD), while the details of distribution and metabolism of the individual isomers are not clear. Here we investigated the accumulation and catabolic rate of TFA positional isomers of octadecenoic acid (18:1) in mice. ICR mice were fed deuterium- and [¹³C] stable isotope-labeled *trans*-9-18:1 (9*t*-18:1*), *trans*-10-18:1 (10*t*-18:1*), or *trans*-11-18:1 (11*t*-18:1*) for 2 or 4 weeks, or a TFA mixture (9*t*-18:1*, 10*t*-18:1*, and 11*t*-18:1*) for 3 weeks. Analysis of whole-body tissues by gas chromatography-chemical ionization mass spectrometry revealed the highest 9*t*-18:1* levels in the heart. Significant differences in the accumulation of the respective *trans*-18:1 were observed in the heart and erythrocytes, where 9*t*- > 11*t*- > 10*t*-18:1*, but no significant difference was observed in the liver or white adipose tissue (WAT). Mice fed on 11*t*-18:1 demonstrated accumulation of endogenously synthesized conjugated linoleic acid in the liver, WAT, and heart, but any other metabolites were not found in other groups. Furthermore, we analyzed catabolic rates of single-dose-administered *trans*-18:1* isomers into [¹³C]-labeled CO₂ using isotope-ratio mass spectrometry, and the 10*t*-18:1* catabolic rate was significantly higher than those of 9*t*- and 11*t*-18:1*. We found that the accumulation and catabolism of *trans*-18:1 positional isomers varied in these mice. Differential accumulation in tissues suggests that individual TFA positional isomers may play different roles in human health.

Key words: accumulation, catabolism, [¹³C]-labeled carbon dioxide, stable isotope label, *trans* fatty acid isomers

1 INTRODUCTION

A large body of evidence has indicated that *trans* fatty acids (TFA), the geometrical isomers of unsaturated fatty acids possessing at least one double bond in the *trans* configuration, have multiple adverse effects on cardiovascular disease (CVD), including dyslipidemia, endothelial dysfunction, and inflammation^{1, 2)}. TFA occur naturally in some meat and dairy products from ruminant animals and are found industrially in processed, partially hydrogenated vegetable oil. Catalytic hydrogenation has been used in food manufacturing to turn liquid oils into a solid form of fat and to add stability against autoxidation. Consumption of industrial TFA, mainly composed of elaidic acid (*trans*-

9-octadecenoic acid, 9*t*-18:1) and *trans*-10-octadecenoic acid (10*t*-18:1), has been shown to increase the ratio of

Abbreviations: AOCS, American Oil Chemists' Society; CI/MS, chemical ionization mass spectrometry; CLA, conjugated linoleic acid; ¹³CO₂, [¹³C]-labeled CO₂; CVD, cardiovascular disease; FID, flame ionization detector; GC, gas chromatography; HDL, high-density lipoprotein; ICR, imprinting control region; IRMS, isotope-ratio mass spectrometry; LDL, low-density lipoprotein; OLA, oleic acid; 9*t*-18:1*, *trans*-9-octadecenoic acid-[1-¹³C]-[9,10-d2]; 10*t*-18:1*, *trans*-10-octadecenoic acid-[1-¹³C]-[10,11-d2]; 11*t*-18:1*, *trans*-11-octadecenoic acid-1-[¹³C]-[11,12-d2]; TFA, *trans* fatty acids; VPDB, Vienna Pee Dee Belemnite; WAT, white adipose tissue

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low-density lipoprotein (LDL) or total cholesterol concentration to high-density lipoprotein (HDL) cholesterol in blood and to increase the risk for CVD^{3,4)}.

Fats from ruminants, such as cows, sheep, and goats, contain 2–9% TFA, and ruminant TFA are produced by bacterial metabolism of polyunsaturated fatty acids in the rumen. While industrial TFA have been linked to CVD, the relationship between naturally occurring ruminant TFA, which predominantly contain vaccenic acid (*trans*-11-octadecenoic acid, 11*t*-18:1), and heart disease is not clear. While evidence from human studies has shown no association between consumption of ruminant TFA and CVD risk^{5,6)}, a non-significant inverse and a non-significant positive association between the consumption of ruminant TFA and risk of CVD have been reported^{6–9)}. These indistinct results have concerned many people, leading officials to take measures against TFA consumption in many countries. Some countries and local agencies have implemented a ban on TFA in foodstuffs, with some calling for the elimination of TFA, whereas other countries that have a large-scale dairy farming industry, such as Denmark, have banned the use of industrial TFA in foods, but the ban excludes TFA from ruminant animal products.

Total TFA in foods are mainly composed of *trans*-octadecenoic acid (18:1) (approximately 80–90%)¹⁰⁾. Industrial and ruminant TFA reportedly consist of exactly the same individual *trans*-18:1 isomers¹¹⁾, although the relative proportion is significantly different between these sources. In industrial TFA, 9*t*- and 10*t*-18:1 are predominant with smaller proportions of 6-, 8-, 11-, and 12*t*-18:1^{11–13)}, whereas the major isomer of ruminant TFA is 11*t*-18:1, with lower proportions of 13-/14-, 9-, 10-, and 12*t*-18:1^{11,14)}. However, most previous studies have evaluated the increased risk of CVD caused by total TFA from industrial and/or ruminant fats, but did not account for the presence of specific isomers. The differential effects of industrial and ruminant TFA on CVD risk could be due to the presence and properties of specific harmful or beneficial isomers since individual TFA isomers could potentially have different roles in the etiology of heart disease. In addition, 11*t*-18:1 can be metabolized to conjugated linoleic acid (CLA) by $\Delta 9$ -desaturase in animals and humans¹⁵⁾, which has been reported to have beneficial effects on CVD in animal studies^{16,17)}, although it is still not clear whether CLA exhibits beneficial effects in humans^{18–20)}. Therefore, studies comparing the effects of individual TFA isomers are needed, rather than solely comparing the sources of TFA. Moreover, *in vivo* studies are needed to clarify the biological availability and fate of individual TFA isomers so that appropriate TFA policies can be determined.

In the present study, we investigated the differential distribution of the three most abundant industrial and natural TFA positional isomers (9*t*-, 10*t*- and 11*t*-18:1) into tissues and erythrocytes of imprinting control region (ICR) mice.

To compare the propensity of each isomer's accumulation in tissues, we also fed mice with a diet containing equal proportions of these TFA isomers. In addition, to clarify the differential effects of double-bond position on fatty acid metabolism, we determined the catabolic rate of these TFA isomers through beta-oxidation and catabolism to CO₂ using isotope-ratio mass spectrometry (IRMS)^{21,22)}. Analysis of [¹³C]-labeled CO₂ (¹³CO₂), as an indicator of the metabolic rate of [¹³C]-labeled TFA, revealed a significant difference in metabolic rate among the three evaluated isomers. Here, our findings provide evidence that individual TFA isomers have differential propensities for metabolism in mice.

2 EXPERIMENTAL

2.1 Chemicals and materials

Individual TFA ethyl esters containing both deuterium (d) and the stable isotope [¹³C], *trans*-9-octadecenoic acid-[1-¹³C]-[9,10-d₂] (9*t*-18:1*) ethyl ester, *trans*-10-octadecenoic acid-[1-¹³C]-[10,11-d₂] (10*t*-18:1*) ethyl ester, and *trans*-11-octadecenoic acid-1-[¹³C]-[11,12-d₂] (11*t*-18:1*) ethyl ester were obtained from Tsukishima Foods Industry (Tokyo, Japan). The purity of these fatty acid ethyl esters was greater than 99% as determined by gas chromatographic analysis. Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Animal care and diet

Animals were handled in accordance with the guidelines established by the Institutional Animal Care and Use Committee of the Tokyo University of Marine Science and Technology. Eight-week-old male ICR mice were purchased from Japan SLC (Shizuoka, Japan). The ICR mouse is a phenotypically normal and multipurpose research animal model that is used in such fields as toxicology, pharmacology, and immunology. We considered that the ICR strain is a suitable model for the purpose of this lipid metabolism study. Six mice were housed per cage under standard conditions with a 12:12-h light-dark cycle at 22 ± 1°C and 50% humidity. The animals had free access to food and tap water. Mice were fed rodent chow MF (Oriental Yeast, Tokyo, Japan) and switched to experimental diets after acclimation for one week. To elucidate the distribution of TFA isomers into tissues, individual TFA ethyl esters were mixed into powdered rodent chow MF and fed to mice at concentrations of 1% TFA ethyl ester by energy content (kcal%) for 2 or 4 weeks. In addition, a TFA ethyl ester mixture (9*t*-18:1*, 10*t*-18:1*, and 11*t*-18:1*; 1 kcal% each) was fed at a concentration of 3 kcal% for 3 weeks to compare the propensity for accumulation in tissues between the three isomers. Mice fed a diet supplemented with 9-*cis*-octadecenoic acid (oleic acid: OLA, 9*c*-18:1)

ethyl ester at concentrations of 1 or 3 kcal% were used as a control group. The fatty acid composition of the experimental diets was analyzed by gas chromatography (GC) (Table 1).

At the end of experiment, blood samples were collected in tubes with K3-EDTA from the cervical vein of animals under diethyl ether anesthesia. After euthanasia by cervical dislocation, the liver, epididymal white adipose tissue (WAT), and heart were collected and stored at -60°C until lipid analysis.

2.3 Blood sample preparation

Red blood cells were isolated by adding 1.5 mL of whole blood to 5 mL of saline, and then cells were centrifuged at $1,000 \times g$ for 10 min at 4°C . The supernatant with plasma and platelets was then removed. This procedure was repeated two times. The erythrocytes were burst with pure water and membranes were separated by centrifugation at $15,000 \times g$ for 20 min at 4°C .

2.4 Fatty acid analysis

Total lipids were extracted according to the method of Folch *et al.*⁽²³⁾. Fatty acids were then transmethyated in the presence of boron trifluoride in methanol as established by the American Oil Chemists' Society (AOCS) official method, Ce 1b-89⁽²⁴⁾. Fatty acid methyl esters were extracted with *n*-hexane, and then the hexane phase was collected and the solvent evaporated under nitrogen gas. The fatty acid composition was analyzed with a GC-flame ionization detector (GC-FID) system (TRACE GC Ultra, Thermo Fisher Scientific, Waltham, MA) equipped with a capillary column (SLB-IL111, 100 m $\times$ 0.25 mm ID, Sigma-Aldrich Japan K.K., Tokyo, Japan). The temperature of the injection port and detector was 250°C . The initial column temperature was 160°C for 35 min, followed by an increase of $15^{\circ}\text{C}/\text{min}$ up to 240°C and holding for 10 min. Helium

was used as the carrier gas at a flow rate of 1.2 mL/min. Individual fatty acids were identified by comparison with the retention times of standards (Supelco 37 Component FAME Mix, Sigma-Aldrich Japan K.K.). Heptadecanoic acid was added prior to lipid extraction from tissues as an internal standard for the quantification of fatty acids⁽²⁵⁾. Analyzed results reflect total amount of fatty acid including both free and esterified fatty acids.

Labeled fatty acids were quantified using GC-chemical ionization mass spectrometry (GC-CI/MS; GC: TRACE GC Ultra, CI/MS: ITQ1100, Thermo Fisher Scientific). The methyl-esterified sample lipid was injected into the GC-CI/MS using the splitless mode. The injection port and detector temperature was 250°C . The initial column temperature was held at 180°C for 30 min and subsequently increased at a rate of $10^{\circ}\text{C}/\text{min}$ to a maximum of 240°C and then held for 15 min. The carrier gas was helium, provided at a flow rate of 1.0 mL/min. GC separation was achieved using an SP-2560 capillary column with 0.25- μm film thickness (100 m $\times$ 0.25 mm ID, Sigma-Aldrich Japan K.K.). The mass spectrum was obtained with 70 eV chemical ionization (methane as reagent gas) and detected with scan mode (m/z 50–650). The target compounds were detected at m/z 297⁺ ($M+1^{+}$, unlabeled 9*t*-, 10*t*-, and 11*t*-18:1), 300⁺ ($M+4^{+}$, labeled *trans* 9-, 10-, and 11-18:1), 295⁺ ($M+1^{+}$, unlabeled CLA), and 298⁺ ($M+4^{+}$, labeled CLA).

2.5 Analysis of the catabolic rates of respective TFA

Respective TFA emulsions were prepared as described in our previous work⁽²⁶⁾. In brief, individually labeled TFA ethyl esters were mixed with triolein, Triton X-100, and distilled water. The mixture was then emulsified using a Sonifier 250 ultrasonic homogenizer (Branson Japan, Kanagawa, Japan).

Fifteen ICR mice were divided equally into 3 groups after acclimation for one week. Individual TFA emulsions

Table 1 Fatty acid composition of experimental diets (mol%).

Group	Single TFA isomer supplement				TFA mixture supplement	
	Control	9 <i>t</i> -C18:1	10 <i>t</i> -C18:1	11 <i>t</i> -C18:1	Control	Mix
16:0	14.6	14.7	14.7	14.5	12.4	12.3
18:0	1.6	1.6	1.5	1.6	1.3	1.3
9 <i>c</i> -C18:1	30.4	20.5	20.5	20.6	41.6	17.1
9 <i>t</i> -C18:1	—	9.7	—	—	—	8.2
10 <i>t</i> -C18:1	—	—	9.7	—	—	8.3
11 <i>t</i> -C18:1	—	—	—	9.6	—	7.9
18:2	49.3	49.3	49.3	49.5	41.3	41.1
18:3	3.3	3.4	3.4	3.4	2.8	2.8
20:5	0.5	0.5	0.5	0.5	0.4	0.5
20:6	0.4	0.4	0.4	0.4	0.3	0.3

of 10 mL/kg (TFA dose = 400 mg/kg) were administered orally to the animals. Each mouse was then placed in a 450-mL plastic vessel for 5 min, and a sample of expired gas was collected in a 50-mL syringe. The gas was divided into three 12-mL evacuated vials (Exetainer, Labco, High Wycombe, UK). The expired gas was collected at 0 (before administration), 15, 30, 45, 60, 75, 90, 105, 120, 240, and 360 min after administration. The mice were fasted during sample collection.

The actual ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in the expired CO_2 gas was analyzed using IRMS (ANCA-GSL, SerCon, Cheshire, UK). First, the natural abundance of ^{13}C and ^{12}C in G1-grade CO_2 manufactured by Taiyo Nippon Sanso (Tokyo, Japan) was analyzed by SI Science (Sugito, Japan, $\delta^{13}\text{C}$ -Vienna Pee Dee Belemnite (VPDB) = -29.6%). The CO_2 was diluted 10 times with nitrogen gas, and the mixed gas was packed into a 12-mL evacuated vial. The mixed gas was used as a standard to adjust the measured value using IRMS. Next, the actual ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ in the expired CO_2 gas from the mice was analyzed. The following parameters were used: injection time, 12 s; column temperature, 100°C ; carrier gas, helium; carrier gas flow rate, 60 mL/min; ionization method, electron ionization. The $\delta^{13}\text{C}$ values in each sample of expired gas were calculated using Equation 1²⁷⁾:

$$\delta^{13}\text{C}(\text{‰}) = \left(\left(\frac{^{13}\text{C}/^{12}\text{C}}{^{13}\text{C}/^{12}\text{C}} \right)_{\text{sample}} / \left(\frac{^{13}\text{C}/^{12}\text{C}}{^{13}\text{C}/^{12}\text{C}} \right)_{\text{standard}} - 1 \right) \times 1000$$

Equation 1

where $(^{13}\text{C}/^{12}\text{C})_{\text{sample}}$ denotes the ratio of ^{13}C to ^{12}C in the expired CO_2 , and $(^{13}\text{C}/^{12}\text{C})_{\text{standard}}$ represents the ratio of ^{13}C to ^{12}C in VPDB (0.0112372).

$\delta^{13}\text{C}$ values at each sampling time were adjusted by subtracting the $\delta^{13}\text{C}$ at 0 h using Equation 2, and the resulting values were reported as $\Delta^{13}\text{C}$.

$$\Delta^{13}\text{C}(\text{‰}) = \delta^{13}\text{C}_t - \delta^{13}\text{C}_0$$

Equation 2

where $\delta^{13}\text{C}_t$ represents the $\delta^{13}\text{C}$ value at time t after sample administration, and $\delta^{13}\text{C}_0$ represents the $\delta^{13}\text{C}$ value before sample administration.

2.6 Chemicals and materials

Statistical analysis

Statistical analysis was performed using an ANOVA with Tukey-Kramer test. The difference was considered significant when p was less than 0.05.

3 RESULTS

3.1 Distribution of individual TFA isomers in tissues

9*t*-18:1* is generally considered the primary cause of CVD among all TFA isomers, because it is typically found

Fig. 1 Distribution of 9*t*-18:1* in mice tissues. ICR mice were fed 9*t*-18:1* at a concentration of 1 kcal% for 1 week. Each value represents the mean $\pm$ standard error. Different letters indicate significant differences at $p < 0.05$.

in the highest proportion in industrial TFA. Therefore, we initially investigated the distribution of 9*t*-18:1* in whole-body tissues in order to identify the specific tissues in which 9*t*-18:1* predominantly accumulates. Consumption of 9*t*-18:1* at 1 kcal% was distributed in different proportions to all tissues except for the brain (Fig. 1). The highest proportion of 9*t*-18:1* among whole-body tissues was observed in the heart. Based on our results, we selected the liver, WAT and heart, which are the major organs for lipid metabolism, as target organs for this study. In addition, we also investigated the erythrocyte membrane because *trans*-18:1 is known to accumulate primarily in the phospholipid fraction of plasma as well as the TAG fraction of organs¹⁵⁾.

Next, we fed mice with individual isomers for 2 or 4 weeks to identify the differences in accumulation in tissues. The content of stable isotope-labeled TFA and 9*c*,11*t*-CLA* (thought to be formed from 11*t*-18:1*) in the total lipid from each tissue is shown in Table 2. Although there was no detectable TFA in the control group, enrichment of each isomer in respective tissues was observed for all TFA-treated groups. When compared to the amounts of respective isomers, 10*t*-18:1 was present at relatively low levels in the liver and WAT. The accumulated amount in the heart was significantly different, with the relative content of isomers found to be 9*t*->11*t*->10*t*-18:1. The relative content of respective isomers in the erythrocytes was 9*t*-, 11*t*->10*t*-18:1 at 2 weeks. Such a trend was observed at 4 weeks, but not significant. GC-MS analysis revealed that endogenously synthesized CLA from ingested TFA was observed only in the 11*t*-18:1* group. The relative content of 9*c*,11*t*-CLA* in respective tissues was WAT>liver>heart at 4 weeks, but was not detected in erythrocytes. No other CLA thought to be formed from 9*t*- or 10*t*-18:1* was detected in this study.

Table 2 TFA content in total lipid from tissues (g/100 g lipid).

Group	2 weeks				4 weeks			
	Control	9 <i>t</i> -C18:1	10 <i>t</i> -C18:1	11 <i>t</i> -C18:1	Control	9 <i>t</i> -C18:1	10 <i>t</i> -C18:1	11 <i>t</i> -C18:1
(Liver)								
9 <i>t</i> -C18:1	—	0.51 ± 0.06	—	—	—	0.50 ± 0.04	—	—
10 <i>t</i> -C18:1	—	—	0.39 ± 0.05	—	—	—	0.45 ± 0.02	—
11 <i>t</i> -C18:1	—	—	—	0.52 ± 0.09	—	—	—	0.49 ± 0.04
9 <i>c</i> ,11 <i>t</i> -CLA	—	—	—	0.08 ± 0.01	—	—	—	0.16 ± 0.03
(WAT)								
9 <i>t</i> -C18:1	—	0.91 ± 0.19	—	—	—	1.56 ± 0.18	—	—
10 <i>t</i> -C18:1	—	—	0.61 ± 0.10	—	—	—	1.11 ± 0.14	—
11 <i>t</i> -C18:1	—	—	—	0.61 ± 0.06	—	—	—	1.64 ± 0.42
9 <i>c</i> ,11 <i>t</i> -CLA	—	—	—	0.12 ± 0.02	—	—	—	0.30 ± 0.03
(Heart)								
9 <i>t</i> -C18:1	—	1.69 ± 0.05 ^a	—	—	—	1.91 ± 0.05 ^a	—	—
10 <i>t</i> -C18:1	—	—	0.24 ± 0.06 ^b	—	—	—	0.45 ± 0.07 ^b	—
11 <i>t</i> -C18:1	—	—	—	0.84 ± 0.19 ^c	—	—	—	1.02 ± 0.04 ^c
9 <i>c</i> ,11 <i>t</i> -CLA	—	—	—	N.D.	—	—	—	0.02 ± 0.01
(Erythrocyte)								
9 <i>t</i> -C18:1	—	0.22 ± 0.03 ^a	—	—	—	0.58 ± 0.16	—	—
10 <i>t</i> -C18:1	—	—	0.08 ± 0.02 ^b	—	—	—	0.21 ± 0.05	—
11 <i>t</i> -C18:1	—	—	—	0.19 ± 0.01 ^a	—	—	—	0.68 ± 0.18
9 <i>c</i> ,11 <i>t</i> -CLA	—	—	—	N.D.	—	—	—	N.D.

Different letters show significant difference between treatments at $p < 0.05$

N. D.: Not Detected

3.2 Comparison of TFA isomer accumulation in mice fed TFA mixture

Several kinds of *trans*-18:1 isomers are present in food, including meats, dairy products, and industrially processed, partially hydrogenated vegetable oil. To clarify whether *trans*-18:1 isomers influence each other's uptake into organs, we fed mice a TFA mixture containing equal amounts of 9*t*, 10*t*, and 11*t*-18:1* for 3 weeks. Separation of 9*t*, 10*t*, and 11*t*-18:1* was achieved using a GC-FID equipped with a SLB-IL111 column, as previously described²⁸. **Figure 2** shows the labeled-TFA content in lipids from each tissue. A significant difference was observed in the heart, but not in the WAT, liver, or erythrocytes. The relative content of the respective labeled TFA isomers was 9*t* > 11*t* > 10*t*-18:1*, and this trend was identical to those of mice fed TFA isomers individually. The 9*t*-18:1* content in the heart was 4.5- and 2.0-fold higher than 10*t*- and 11*t*-18:1*, respectively.

3.3 Differential catabolic rates for TFA isomers

Dietary fatty acids are catabolized through beta-oxidation in the mitochondria to acetyl-CoA, which enters the

citric acid cycle to be oxidized to CO₂. Whole-body oxidation of fatty acids has been measured by the administration of [¹³C]-labeled fatty acids followed by measurement of expired ¹³CO₂^{29,30}. To elucidate whether the metabolic rate of each TFA isomer depended upon double-bond position, we fed mice with individual isomers and calculated Δ¹³C values from the carbon isotope ratios in expired CO₂ gas samples using an IRMS system^{26,27}. Δ¹³C values rapidly increased in the first 90 min of labeled-TFA ingestion and peaked during 75–105 min in all test groups (**Fig. 3**). The metabolic rate was significantly higher in the 10*t*-18:1* group than that of 9*t*- and 11*t*-18:1* during 75–120 min and 105–120 min, respectively. The greater Δ¹³C values rapidly decreased to the same level of other isomers by 240 min. There was no difference in the metabolic rate between 9*t*- and 11*t*-18:1* during the experiment, and their Δ¹³C values gradually decreased from 120 min until the end of the experiment. These results indicate that 10*t*-18:1* was the preferred substrate for beta-oxidation and was most effectively catabolized into ¹³CO₂ via the citric acid cycle among the three isomers.

Fig. 2 TFA content in tissues of mice fed the TFA mixture. ICR mice were fed a TFA mixture that contained an equal proportion of 9*t*-, 10*t*-, and 11*t*-18:1* at a total of 3 kcal% for 3 weeks. Each value represents the mean $\pm$ standard error. Different letters indicate significant differences at $p < 0.05$.

Fig. 3 $\Delta^{13}\text{C}$ changes in expired CO_2 gas. [^{13}C]-labeled 9*t*-18:1*, 10*t*-18:1*, or 11*t*-18:1* at a dose of 10 mg/kg was administered orally to the ICR mice. Each value represents the mean $\pm$ standard error. Different letters indicate significant differences at $p < 0.05$ at each sampling time.

4 DISCUSSION

Previous reports have demonstrated the incorporation and accumulation of dietary TFA into blood, organs, and tissues^{31–33}. Our results are generally consistent with reports showing that dietary 9*t*-18:1 is incorporated into various tissues, and its proportion to total lipid was different depending on the tissue (Fig. 1). Further, we found that each isomer differentially accumulates in different

tissues. Although no difference was observed in the liver and WAT, the proportion of 9*t*-18:1* to total lipid in the heart was the highest, followed by that of 11*t*-18:1* and 10*t*-18:1* (Table 2). Such a difference was also observed in the mice fed the labeled-TFA mixture (Fig. 2). It appears that TFA isomers did not interfere with each other's incorporation. In this study, we did not examine the dose or time effect of TFA accumulation. However, we found that feeding the TFA mixture for 3 weeks led to accumulation of each isomer at the same levels as 4 weeks of feeding with the individual isomers, suggesting that steady-state pharmacokinetics are achieved by 3 weeks of feeding under these experimental conditions. Our results clearly show that the difference in accumulation of individual isomers can be attributed to their physical and structural properties.

It is well known that *trans*-18:1 accumulates primarily in the phospholipid and TAG fractions of plasma and tissues. In the present study, a difference in the accumulation was observed in the heart and erythrocytes, which have a large proportion of phospholipids among total lipids. It has been reported that *trans*-18:1 positional isomers are positively incorporated into the 1-acyl position of PtdCho and are strictly excluded from the 2-acyl position relative to OLA³⁴. Moreover, according to that report, the incorporation of 10*t*-18:1 into human plasma 1-acyl PtdCho is approximately 50% lower than 9*t*- and 11*t*-18:1, and 10*t*-18:1 and 11*t*-18:1 are strictly excluded from the 2-acyl position of PtdCho compared to 9*t*-18:1. *In vitro* studies have demonstrated that PtdCho acyltransferases distinguish differences in the structures of isomers 18:1 and C18:2³⁵. Therefore, we considered that PtdCho acyltransferases are sensitive to the number and position of double bonds, and this specificity results in differences in accumulation of respective isomers in the phospholipids. However, further analysis of the respective lipid fractions is required to clarify the differences in the specificity of acyltransferases, including PtdCho, TAG, and cholesteryl ester transferases, among *trans*-18:1 isomers in different tissues.

Consistent with a previous study³⁶, we observed a time-dependent formation of 9*c*,11*t*-CLA from ingested 11*t*-18:1* (Table 2). Conversion of 11*t*-18:1 to 9*c*,11*t*-CLA is catalyzed by $\Delta 9$ -desaturase, which inserts a *cis*-double bond at the $\Delta 9$ position of the fatty-acyl chain. *In vitro* studies have suggested that each positional isomer is desaturated at a unique rate, and that 8*t*-, 9*t*-, and 10*t*-18:1 have low affinity for the enzyme^{37, 38}. These reports support our current results showing that there were no other metabolized labeled fatty acids in any tissue in mice fed 9*t*- and 10*t*-18:1*.

We further showed differences in catabolic rates between *trans*-18:1 isomers by measurement of expired $^{13}\text{CO}_2$ derived from administered [^{13}C]-labeled fatty acids. A higher catabolic rate for 10*t*-18:1* relative to those for 9*t*-

and 11*t*-18:1* leads us to presume that the low accumulation of 10*t*-18:1* into the tissues was partly due to a high catabolic rate through beta-oxidation as compared to other isomers. Previous *in vitro* experiments have shown that even-numbered isomer positions were catabolized at rates higher than or equal to the next lower odd-numbered isomer positions by comparing beta-oxidation of the 4*t*- to 16*t*-18:1 acyl-CoA esters in rat liver and heart mitochondria³⁹. Furthermore, Du *et al.* showed a lower catabolic rate for 9*t*-18:1 than 11*t*-18:1 in liver cells, homogenates, and mitochondria⁴⁰. Such a difference between even- and odd-numbered *trans* 18:1 isomers may be inferred from the beta-oxidation process^{39, 41}, in which odd-numbered 18:1 isomers need an additional modification to move the double bond from the 3-position to the 2-position by Δ^3 -*cis*- Δ^2 -*trans*-enoyl-CoA isomerase for beta-oxidation to proceed, compared with the even-numbered 18:1 isomers and the intermediates produced by fatty acyl-CoA dehydrogenase. These previous reports support our *in vivo* study demonstrating that the lower accumulation of 10*t*-18:1* in mouse tissues, compared with the other isomers, was partly due to its propensity to be catabolized through beta-oxidation.

It has been reported that lipases in the gastrointestinal tract can discriminate against the fatty acid ethyl esters⁴², suggesting that differential hydrolytic rates between TFA isomers might affect some metabolic phenomena including accumulation and catabolic rate. Unfortunately, we did not examine digestive absorption and blood TFA levels, which could greatly influence the catabolic rate and accumulation in tissues. A more detailed kinetic analysis of individual TFA isomers is necessary for a better understanding of their metabolic rates.

5 CONCLUSION

In the present study, we first demonstrated that the type of TFA isomer does affect its accumulation and metabolism using an *in vivo* model. Differences in accumulation in tissues are in part attributable to their propensities for conversion to metabolites, or differences in catabolic rates of respective isomers. These results indicate that each isomer potentially has different effects on the development of CVD. The effect of individual TFA isomers on the ratio of LDL cholesterol to HDL cholesterol (LDL/HDL) in blood must be investigated to identify principal isomers that affect cholesterol metabolism. It is necessary to identify specific isomers that are associated with adverse heart events based on an evaluation of differences in their metabolic and physiological activities as well as clinical research.

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