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Comparison of plasma levels of different species of trans fatty acids in Japanese male patients with acute coronary syndrome *versus* healthy men

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HIGHLIGHTS

- There are several differences in *trans*-C18:1 positional isomers between acute coronary syndrome (ACS) and control.
- Palmitelaidic acid, ruminant-derived TFA were lower in ACS patients, especially middle-aged patients.
- Linoleic trans isomers, industrially-produced TFA (IP-TFAs) were higher in ACS.
- IP-TFA to arachidonic acid (AA) ratio was significantly higher in ACS, especially elder ACS patients.
- Palmitelaidic acid was significantly directly associated with HDL-C, EPA + DHA, and EPA + DHA/AA ratios.

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ABSTRACT

Background and aims: It remains unclear how *trans* fatty acid (TFA) at low-level intake affect lipid levels and the development of acute coronary syndrome (ACS). The study aimed to investigate how plasma TFA composition differs between male patients with ACS and healthy men.
Methods: Plasma fatty acid (FA) composition (as determined by gas chromatography) was analyzed in ACS patients on hospital admission and compared to that of age-adjusted healthy men.
Results: Total FA and TFA levels were similar between ACS and control subjects. Palmitelaidic acid, ruminant-derived TFA (R-TFA), levels were lower in ACS patients (0.17 ± 0.06 *vs.* 0.20 ± 0.06 of total FA, in ACS and control, respectively, $p < 0.01$), and were significantly directly associated with HDL cholesterol (HDL-C) (rho = 0.269) and n-3 polyunsaturated FA (n-3 PUFA) (rho = 0.442). Linoleic *trans* isomers (total C18:2 TFA), primary industrially-produced TFA (IP-TFAs), were significantly higher in ACS patients (0.68 ± 0.17 *vs.* 0.60 ± 0.20 of total FA, in ACS and control, respectively). Total *trans*-C18:1 isomers were comparable between ACS and control. Differences between ACS and controls in C18:1 trans varied by specific C18:1 trans species. Absolute concentrations of *trans*-C18:2 isomers were significantly directly associated with LDL-C and non-HDL-C in ACS men. The ACS patients showed significantly lower levels of both n-6 and n-3 PUFA (i.e., eicosapentaenoic, docosahexaenoic and arachidonic acids).
Conclusions: There were several case-control differences in specific TFA that could potential affect risk for ACS. Japanese ACS patients, especially middle-aged patients, may consume less R-TFA.

1. Introduction

Fatty acids (FA) are biologically-active molecules with a wide array of effects [1]. FA are classified as saturated or unsaturated on the basis of the absence or presence of double bonds. Monounsaturated FA (MUFA) have one double bond; polyunsaturated FA (PUFA) have more than one double bond. UFA usually occur in the *cis* configuration, and *trans* FA (TFA) are UFA containing at least one double bond in *trans* configuration. Because humans cannot synthesize TFA, the plasma concentration of TFA is regulated by dietary TFA intake. TFAs are

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produced either by industrial partial hydrogenation of vegetable or fish oils or by biohydrogenation in ruminant animals [1]. Dietary recommendations for prevention of coronary heart diseases (CHD) include decreasing the intake of saturated FA (SFA) and TFA and replacing them with UFA [1].

Brouwer et al. reported that all TFA raised the ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C), and presumably the risk of CHD [2]. There is some evidence that ruminant-derived TFA (R-TFA) do not increase risk for disease but industrially-produced TFA (IP-TFA) do [3,4]. The prospective cohort study of Norwegian patients with suspected CHD failed to show the association between plasma concentrations of TFA (palmitelaidic acid, an R-TFA; and *trans* C18:1 isomers; primarily IP-TFA) and incidence of acute myocardial infarction (AMI) after multivariate adjustments [5]. The prospective cohort study of German patients with suspected CHD showed that higher levels of palmitelaidic acids in erythrocyte membranes were associated with lower risk for sudden cardiac death, and that three *trans* isomers of C18:2n6 were not related to fatal cardiovascular outcomes [6]. The cross-sectional study of Japanese patients undergoing coronary angiography (CAG) failed to show differences in levels of elaidic acid and linoelaidic acids (the two major IP-TFA, although the latter can be formed by frying in non-hydrogenated vegetable oils [7]) between patients with and without CHD [8]. They showed significantly higher elaidic acid levels in younger patients with CHD (≤66 years) compared with elder CHD patients, and/or patients with metabolic syndrome compared with patients without metabolic syndrome [8]. In that study, all subjects had undergone CAG and thus subjects without CHD were not generally healthy individuals. It remains controversial how R-TFA and IP-TFA are associated with acute coronary syndrome (ACS).

The consumption of TFA is currently decreasing in many countries. According to the report of the total diet study (market basket method) from the Ministry of Agriculture, Forestry and Fisheries in Japan, estimated daily intake of TFA in the Japanese is 0.92–0.96 g, or 0.44–0.47% of the total energy intake [9]. This is lower than the < 1% of energy target recommended by the World Health Organization [9] and is much lower than the average consumption in the Western countries [10]. It remains unclear how low intakes of TFA affect lipid levels and the development of ACS. On the other hand, the Japanese dietary style has markedly changed from the 1960s, and fish to meat ratios in food consumption are decreasing in the younger generation, while the ratios in the Western countries stayed the same or slightly increased [11,12]. The age profile of the fish/meat > 1.0 was ≥40 years in 2000, ≥50 years in 2005 and 2010, and ≥60 years in 2015 in Japan. The study aim was to compare plasma TFA composition such as R-TFA and twelve kinds of *trans*-C18 isomers (IP-TFA) in male patients with ACS and healthy men, and to investigate their difference by age.

2. Patients and methods

2.1. Subjects

This study enrolled patients with ACS that followed successful percutaneous coronary intervention (PCI) at Showa University Hospital between July 2013 and December 2014. Controls were healthy, non-smoking males aged 40–80 years who were not receiving any pharmacological treatment. The fifty-five healthy men were recruited by advertisement among acquaintances of the research team, and six men were excluded because of their fasting blood glucose levels ≥126 mg/dL. Patients aged above 80, and those undergoing hemodialysis were excluded. The diagnoses of ACS were based on electrocardiographic changes and CAG findings. Serum and plasma samples were collected immediately before the emergency CAG on admission of ACS, and samples from the control subjects were collected after an overnight fast. The institutional review board of Showa University (1535) and Showa Women's University (13–02, 15–02, 17–12) approved this protocol. The investigation conformed to the principles of the Declaration of Helsinki, and the written informed consent was obtained from all subjects.

2.2. Baseline evaluation

Serum concentrations of total protein, albumin, total bilirubin, creatinine, total cholesterol, triglyceride, and HDL-C were measured using standard laboratory procedures. LDL-C levels were measured with a direct homogenous assay of the serum using detergents (Sekisui Medical, Tokyo, Japan). Non-HDL-C was estimated by subtracting the HDL-C concentration from the total cholesterol concentration.

The diagnosis of hypertension was based on a history of hypertension or blood pressure > 140 mmHg systolic or > 90 mmHg diastolic [9]. Diabetes mellitus was diagnosed as a fasting serum glucose value greater than 126 mg/dL, hemoglobin (Hb) A1c levels greater than 6.5%, or treatment with either oral hypoglycemic agents or insulin [13]. Dyslipidemia was defined as the current use of lipid-lowering medications and/or meeting the criteria of the Japan Atherosclerosis Society for fasting serum lipid levels, i.e., LDL-C ≥140 mg/dL, HDL-C < 40 mg/dL, or TG ≥ 150 mg/dL [9]. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Patients with a reported smoking habit of at least one cigarette per day on admission were classified as current smokers.

2.3. Measurement of fatty acid composition

Plasma FA levels were measured at Omegaquant, LLC (Sioux Falls, SD, USA). The Plasma samples were stored at −80 °C until the assay. After thawing, an internal standard (C23:0 in the triglyceride form) was added to an aliquot of plasma which was then combined (1:40 parts) with the methylating mixture (boron trifluoride in methanol [14%], toluene, and methanol [35/30/35 v/v]), shaken at 100 °C for 45 min. After cooling, 40 parts of both hexane and distilled water were added. After briefly vortexing, the samples were spun to separate layers, and an aliquot of the hexane layer that contained the FA methyl esters was analyzed by gas chromatography as previously described [14,15]. Identification, precision, and accuracy were evaluated with model mixtures of known FA methyl esters and an established in-house quality-control pool. The chromatographic conditions used in this study were sufficient to isolate the C16:1, C18:1, and C18:2 *trans* isomers. FA concentrations are expressed as percentages of total FAs by weight and/or as μg/dl. Total *trans*-C18:1 isomers are calculated as the sum of nine kinds of C18:1 *trans* isomers. Total *trans*-C18:2 isomers are calculated as the sum of three *trans*-linoleic isomers. Total *trans* C18 FA are the sum of total *trans* C18:1 isomers and *trans* C18:2 isomers. The sum of palmitelaidic acids and *trans*-C18:1 isomer (11-t, Vaccenic) are termed R-TFA, while the sum of the other C18:1 and C18:2 *trans* species are IP-TFA. Total TFA are calculated as the sum of total R-TFA and IP-TFA.

2.4. Statistical analysis

Statistical analysis was performed using the IBM SPSS Statistics for Macintosh, Version 23.0. (IBM Corp. Armonk, NY, USA). Baseline characteristics were compared between control and ACS patients using unpaired *t*-test for parametric variables, and Wilcoxon tests for non-parametric variables. Comparisons among the groups based on age and ACS or control were performed by one-way analysis of variance (ANOVA) with Tukey's honest significant difference test to identify differences among the four groups. Categorical variables were compared with chi-square tests. Correlation coefficients between lipid levels and fatty acid compositions among subjects who did not take any lipid-lowering drugs, were determined by Spearman's rank analyses. All the statistical analyses were two tailed. $p < 0.05$ was considered statistically significant.

Table 1
Comparison of clinical characteristics between control and ACS patients.

	Control (n = 49)	ACS (n = 66)	*p*
Age, years	61.5 ± 10.1	62.2 ± 11.5	0.483
BMI, kg/cm^2	24.6 ± 3.4	24.4 ± 4.2	0.892
AMI/UAP	0	57/9	< 0.0001
Prior MI	0	2	0.327
CVD	0	3	0.185
Prior coronary revascularization	0	3	0.185
Risk factors			
Smoker (current/former), n	0	34/19	< 0.0001
Hypertension, n (%)	0	38 (58%)	< 0.0001
Diabetes, n (%)	0	18 (27%)	< 0.0001
Dyslipidemia, n (%)	22 (45%)	50 (76%)	0.001
Prior medication, n (%)			
Prior any medication	0	31 (47%)	< 0.0001
Blood pressure lowering	0	21 (32%)	< 0.0001
Anti-diabetic therapy	0	10 (15%)	0.003
Beta blocker	0	5 (8%)	0.058
Anti-thrombotic drugs	0	7 (11%)	0.018
Lipid-lowering drugs	0	15 (23%)	< 0.0001
Statin	0	10 (15%)	0.003
Ezetimibe	0	1 (2%)	0574
Fibrate	0	1 (2%)	0.574
Omega-3 fatty acid	0	4 (6%)	0.104
Laboratory findings			
Total protein, g/dL	7.4 ± 0.2 (25)	7.1 ± 0.7	0.006
Albumin, g/dL	4.7 ± 0.2 (25)	4.1 ± 0.5	< 0.0001
Total bilirubin,	0.6 ± 0.3 (25)	0.8 ± 0.4	0.028
Creatinine, mg/dL	0.88 ± 0.13	1.02 ± 0.54	0.943
Glucose, mg/dL	91.2 ± 16.6	148.5 ± 60.9	< 0.0001
Triglyceride, mg/dL	125.8 ± 79.8	111.3 ± 69.3	0.299
Non-HDL-C, mg/dL	148.1 ± 33.8	143.8 ± 35.8	0.507
LDL-C, mg/dL	123.4 ± 29.5	123.1 ± 34.7	0.954
HDL-C, mg/dL	59.8 ± 15.0	43.7 ± 11.2	< 0.0001

Data are expressed as mean ± SD, or number (%). The number in parenthesis indicates the actual number of analyzed cases.
AMI = acute myocardial infarction; BMI = body mass index; CVD = cerebral vascular disease; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Non-HDL-C = non-high-density lipoprotein cholesterol; PCI = percutaneous coronary intervention; UAP = unstable angina pectoris.

3. Results

Table 1 compares the general characteristics and serum biomarkers between control and ACS subjects. Age, BMI, LDL-C, non-HDL-C, and triglyceride were comparable between the groups. Albumin and HDL-C were significantly lower in ACS patients compared with controls.

Table 2 compares TFA between control subjects and ACS patients. Total TFA, total *trans*-C18 isomers, total *trans*-C18:1 isomers, total R-TFA isomers, and total IP-TFA isomers were comparable between the groups. Palmitelaidic acid was significantly lower and total *trans*-C18:2 isomers were significantly higher in ACS patients. R-TFA/arachidonic acid (AA) were similar, while IP-TFA/AA were significantly higher in ACS patients. When the absolute concentrations of TFA were compared, palmitelaidic acid and R-TFA were significantly lower and 18:2 n6tt were significantly higher in ACS patients (Supplementary Table 1). These results were constant when patients treated with lipid-lowering, patients who took ezetimibe and/or n3 PUFA, and those with glucose ≥140 mg/dl were excluded (Supplementary Table 2).

Spearman correlation coefficients were computed between TFA concentration and LDL-C, non-HDL-C, HDL-C, LDL-C to HDL-C ratios, or FA concentrations in 100 subjects not receiving lipid-lowering therapy. Palmitelaidic acid was significantly directly associated with HDL-C and significantly inversely associated with LDL-C/HDL-C ratio (Table 3). Total C18:1 *trans* isomers were significantly inversely associated with LDL-C and non-HDL-C, and total C18:2 *trans* isomers were significantly inversely associated with non-HDL-C. On the other hand, absolute concentrations of total C18:2 TFA were significantly directly associated with LDL-C and non-HDL-C in ACS men. Absolute concentrations of palmitelaidic acids and total C18:1 TFA were significantly positively associated with each FA concentration. Significantly positive association between palmitelaidic acids (either percentage of TFA or absolute levels) and total n-3 PUFA, eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), or EPA plus DHA/AA were observed in all subjects, controls, and ACS men.

Table 2
Comparison of plasma TFA composition between control and ACS men.

	Control (n=49)	ACS men (n=66)	p
Total fatty acids, mg/L	3640.9 ± 1096.9	3329.2 ± 1062.1	0.138
Total TFAs, %	1.67 ± 0.34	1.70 ± 0.32	0.622
16:1 7t (palmitelaidic), %	0.20 ± 0.06	0.17 ± 0.06	0.011
Total C18 trans isomers, %	1.47 ± 0.32	1.53 ± 0.30	0.393
Total 18:1 TFAs, %	0.87 ± 0.19	0.85 ± 0.22	0.449
Total 18:2 TFAs, %	0.60 ± 0.20	0.68 ± 0.17	0.015
Total R-TFA, %	0.30 ± 0.08	0.28 ± 0.09	0.178
Total IP-TFA, %	1.37 ± 0.30	1.41 ± 0.28	0.483
Oleic trans Isomers			
18:1 4t, %	0.09 ± 0.05	0.07 ± 0.05	0.008
18:1 5t. %	0.08 ± 0.04	0.07 ± 0.04	0.152
18:1 (6-8)t, %	0.05 ± 0.02	0.05 ± 0.02	0.821
18:1 9t (elaidic), %	0.10 ± 0.04	0.11 ± 0.05	0.366
18:1 10t, %	0.12 ± 0.05	0.12 ± 0.04	0.422
18:1 11t (vaccenic), %	0.10 ± 0.05	0.11 ± 0.06	0.306
18:1 12t, %	0.05 ± 0.03	0.07 ± 0.03	0.004
18:1 13-14t, %	0.16 ± 0.06	0.14 ± 0.06	0.164
18:1 16t, %	0.11 ± 0.06	0.10 ± 0.05	0.065
Linoleic trans isomers			
18:2 n6tt, %	0.18 ± 0.09	0.23 ± 0.01	0.019
18:2 n6ct, %	0.24 ± 0.10	0.26 ± 0.07	0.042
18:2 n6tc, %	0.18 ± 0.07	0.19 ± 0.08	0.293
TFA / AA			
Total R-TFA / AA, %	5.18 ± 1.93	5.46 ± 2.07	0.409
Total IP-TFA / AA, %	23.38 ± 6.01	27.03± 7.39	0.004

Data are expressed as mean ± SD, and FA are presented as a percent of total plasma FA. Pink rows mean increase in ACS, and blue ones mean decrease in ACS.

Table 4 compares other FA compositions between the control subjects and ACS patients. ACS patients had significantly higher levels of saturated FA, mainly myristic and palmitic acids, and MUFA, mainly oleic acid, and lower levels of n-3 PUFA, mainly EPA and DHA, and AA, n-6 PUFA.

Table 5 compares FA composition and blood parameters between control subjects and ACS patients separated by age. In subjects < 60 years old, palmitelaidic acid and certain *trans*-C18:1 isomers (4t and 5t) were significantly lower in ACS patients. In elder groups, IP-TFA/AA were significantly higher in ACS patients. Both total n-3 PUFA and EPA plus DHA were significantly lower in ACS patients and further lower in ACS patients < 60 years old.

4. Discussion

To the best of our knowledge, this is the first study to compare various TFA isomers between patients with ACS and age-matched healthy men in Japanese subjects. There are four novel findings in this report. First, palmitelaidic acid, a major R-TFA, was significantly lower in ACS patients, especially middle-aged ACS patients than in healthy controls, and was inversely associated with LDL-C to HDL-C ratios. Second, *trans*-C18:2 isomers (IP- or frying-derived TFA) and IP-TFA/AA ratios were significantly higher in ACS patients, especially elder ACS patients, than healthy men. Third, absolute concentrations of *trans*-C18:2 isomers were significantly directly associated with LDL-C and

Table 3
Comparisons of correlation coefficients between TFA and various lipid levels based on TFA as percentage of total fatty acids or absolute amount.

	16:1 n7t	Total C18:1 TFA	Total C18:2 TFA
TFA as percentage of total fatty acids			
All subjects (n = 100), %	0.20 ± 0.06	0.86 ± 0.21	0.64 ± 0.19
LDL-C (mg/dl)	−0.081	−0.333***	−0.151
Non-HDL-C (mg/dl)	−0.062	−0.363***	−0.330**
HDL-C (mg/dl)	0.269**	0.039	−0.172
LDL-C/HDL-C	−0.201*	−0.172	0.063
Total SFA (%)	−0.033	0.126	−0.091
Total oleic *cis* isomers (%)	−0.305**	−0.184	−0.127
Total n-3 PUFA (%)	0.442***	0.170	−0.181
EPA plus DHA (%)	0.458***	0.185	−0.163
Total n-6 PUFA (%)	0.136	−0.183	0.230*
AA (%)	−0.039	0.019	0.108
EPA plus DHA/AA	0.474***	0.189	−0.199
Healthy men (n = 49), %	0.20 ± 0.06	0.87 ± 0.19	0.60 ± 0.19
LDL-C (mg/dl)	0.167	−0.261	−0.260
Non-HDL-C (mg/dl)	0.163	−0.324*	−0.406**
HDL-C (mg/dl)	0.142	0.175	−0.101
LDL-C/HDL-C	0.037	−0.194	−0.003
Total SFA (%)	−0.179	−0.021	0.055
Total oleic *cis* isomers (%)	−0.180	−0.337*	−0.255
Total n-3 PUFA (%)	0.310*	0.144	−0.155
EPA plus DHA (%)	0.321*	0.172	−0.135
Total n-6 PUFA (%)	−0.094	0.012	0.241
AA (%)	−0.219	0.120	0.361*
EPA plus DHA/AA	0.385**	0.140	−0.266
ACS men (n = 51), %	0.16 ± 0.05	0.86 ± 0.23	0.67 ± 0.17
LDL-C (mg/dl)	−0.222	−0.446**	−0.120
Non-HDL-C (mg/dl)	−0.228	−0.431**	−0.310*
HDL-C (mg/dl)	0.122	−0.179	−0.027
LDL-C/HDL-C	−0.218	−0.195	−0.104
Total SFA (%)	0.172	0.254	−0.310*
Total oleic *cis* isomers (%)	−0.244	−0.007	−0.219
Total n-3 PUFA (%)	0.448**	0.191	−0.020
EPA plus DHA (%)	0.466**	0.191	−0.002
Total n-6 PUFA (%)	−0.230	−0.332*	0.266
AA (%)	−0.032	−0.049	−0.031
EPA plus DHA/AA	0.477***	0.229	0.008
TFA as absolute amount			
All subjects (n = 100) μg/ml	6.30 ± 2.64	29.8 ± 10.3	21.4 ± 5.8
LDL-C (mg/dl)	0.136	0.007	0.192
Non-HDL-C (mg/dl)	0.296**	0.189	0.194
HDL-C (mg/dl)	0.216*	0.086	−0.196
LDL-C/HDL-C	−0.094	−0.071	0.210*
Total SFA (μg/ml)	0.611***	0.601***	0.221*
Total oleic *cis* isomers (μg/ml)	0.473***	0.505***	0.313**
Total n-3 PUFA (μg/ml)	0.654***	0.545***	0.117
EPA plus DHA (μg/ml)	0.620***	0.505***	0.069
Total n-6 PUFA (μg/ml)	0.487***	0.431***	0.259**
AA (μg/ml)	0.340**	0.327**	0.136
EPA plus DHA/AA	0.475***	0.365***	−0.016
Healthy men (n = 49) μg/ml	7.13 ± 2.86	30.7 ± 7.9	20.5 ± 4.7
LDL-C (mg/dl)	0.332*	0.129	0.025
Non-HDL-C (mg/dl)	0.481***	0.316*	0.070
HDL-C (mg/dl)	0.044	0.020	−0.278
LDL-C/HDL-C	0.130	0.025	0.204
Total SFA (μg/ml)	0.624***	0.637***	0.256
Total oleic *cis* isomers (μg/ml)	0.610***	0.635***	0.319*
Total n-3 PUFA (μg/ml)	0.637***	0.574***	0.160
EPA plus DHA (μg/ml)	0.599***	0.518***	0.110
Total n-6 PUFA (μg/ml)	0.559***	0.576***	0.249
AA (μg/ml)	0.380**	0.424**	0.310*
EPA plus DHA/AA	0.447**	0.371**	−0.054
ACS men (n = 51), μg/ml	5.51 ± 2.16	29.1 ± 12.1	22.3 ± 6.5
LDL-C (mg/dl)	0.048	−0.094	0.311*
Non-HDL-C (mg/dl)	0.188	0.075	0.305*
HDL-C (mg/dl)	0.156	0.001	−0.033
LDL-C/HDL-C	−0.073	−0.081	0.126
Total SFA (μg/ml)	0.588***	0.551***	0.206
Total oleic *cis* isomers (μg/ml)	0.384**	0.412**	0.305*
Total n-3 PUFA (μg/ml)	0.624***	0.486***	0.245
EPA plus DHA (μg/ml)	0.588***	0.453**	0.177
Total n-6 PUFA (μg/ml)	0.403**	0.253	0.305*
AA (μg/ml)	0.157	0.122	0.024
EPA plus DHA/AA	0.449**	0.351*	0.085

Data are expressed as Spearman's Rho between TFA levels (% or μg/ml) and LDL-C, non-HDL-C, HDL-C, LDL-C/HDL-C ratio, total SFA, total oleic *cis* isomers, total n-3 PUFA, EPA plus DHA, total n-6 PUFA, AA, and EPA plus DHA/AA ratio in subjects who did not take any lipid-lowering drugs.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 4
Comparison of plasma fatty acid composition between the groups.

	Control (n=49)	ACS men (n=66)	*p*
Total FAs, mg/L	3640.9 ± 1096.9	3329.2 ± 1062.1	0.138
Total saturated FAs (6 FAs), %	30.17 ± 0.23	30.88 ± 0.24	0.041
14:0 (myristic), %	0.70 ± 0.20	0.85 ± 0.35	0.025
16:0 (palmitic), %	21.91 ± 1.37	22.68 ± 1.67	0.021
18:0 (stearic), %	7.06 ± 0.63	6.90 ± 0.64	0.203
20:0 (arachidic), %	0.12 ± 0.04	0.12 ± 0.05	0.870
22:0 (behenic), %	0.15 ± 0.05	0.17 ± 0.06	0.156
24:0 (lignoceric), %	0.22 ± 0.09	0.16 ± 0.06	<0.0001
Total *cis*-monounsaturated FAs (9 FAs), %	25.16 ± 0.41	27.53 ± 0.34	<0.0001
16:1 (palmitoleic), %	1.87 ± 0.60	2.09 ± 0.70	0.148
Total oleic *cis* isomers, %	22.80 ± 0.39	25.02 ± 0.31	<0.0001
18:1 9c (Oleic), %	20.27 ± 2.75	22.52 ± 2.47	<0.0001
18:1 11c, %	2.00 ± 0.25	2.00 ± 0.33	0.671
18:1 12c, %	0.14 ± 0.07	0.12 ± 0.06	0.306
18:1 13c, %	0.13 ± 0.07	0.11 ± 0.05	0.169
18:1 14c, %	0.16 ± 0.07	0.17 ± 0.07	0.246
18:1 15c, %	0.10 ± 0.05	0.10 ± 0.05	0.946
20:1 (eicosenoic) n9, %	0.15 ± 0.04	0.16 ± 0.04	0.145
24:1 (nervonic) n9, %	0.34 ± 0.19	0.25 ± 0.14	0.010
n-3 polyunsaturated FAs (4 FAs), %	8.62 ± 0.40	6.66 ± 0.31	<0.0001
18:3 (alpha-linolenic) n3, %	0.66 ± 0.24	0.73 ± 0.26	0.188
20:5 (EPA) n3, %	2.43 ± 1.30	1.73 ± 1.23	0.001
22:5 (DPA) n3, %	0.66 ± 0.25	0.59 ± 0.24	0.065
22:6 (DHA) n3, %	4.87 ± 1.48	3.61 ± 1.24	<0.0001
n-6 polyunsaturated FAs (7 FAs), %	34.39 ± 0.57	33.24 ± 0.44	0.107
18:2 (linoleic) n6, %	26.62 ± 4.03	25.93 ± 3.49	0.320
18:3 (gamma-linoleic) n6, %	0.29 ± 0.15	0.25 ± 0.15	0.089
20:2 (eicosadienoic) n6, %	0.18 ± 0.03	0.19 ± 0.03	0.045
20:3 (DGLA) n6, %	1.04 ± 0.23	1.15 ± 0.34	0.045
20:4 (AA) n6, %	6.01 ± 1.14	5.45 ± 1.17	0.008
22:4 (docosatetraenoic) n6, %	0.12 ± 0.03	0.13 ± 0.04	0.040
22:5 (DPA) n6, %	0.13 ± 0.04	0.12 ± 0.04	0.086
EPA plus DHA, %	7.30 ± 2.64	5.35 ± 2.32	<0.0001
EPA plus DHA / AA	1.26 ± 0.51	1.02 ± 0.51	0.016

Data are expressed as mean ± SD, and FAs are presented as a percent of total plasma FA. Pink rows mean increase in ACS, and blue ones mean decrease in ACS.
AA = arachidonic acid; DGLA = dihomo-gamma linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid.

non-HDL-C in ACS men. Forth, both proportional and absolute concentrations of palmitelaidic acid were significantly directly associated with HDL-C, EPA + DHA, and EPA + DHA/AA ratios.

Higher TFA levels have been reported to be associated with increased risk for CHD but this has mostly been observed in Western populations [3]. In the Nurses' Health Study, increases in total TFA, *trans*-C18:1 isomers, and *trans*-C18:2 isomers in erythrocyte membrane were significantly associated with CHD [16]. In the Cardiovascular Health study, a prospective cohort of older US adults, neither plasma *trans*-C18:1 isomers nor palmitelaidic acids, but linoelaidic acid (*trans*-C18:2 isomers), were significantly associated with fatal CHD [15] and total mortality, mainly due to cardiovascular disease and increased risk of CHD [17]. In recent studies comparing TFA levels between US and Japan, both we and others have observed much lower levels in Japan, consistent with lower CHD rates [18.19]. Our recent study with Japanese and American older men (> age 50) showed markedly lower levels of elaidic and linoelaidic acids (IP-TFA) and significantly higher levels of palmitelaidic acids (R-TFA), compared with American men [18]. The Hawaii-Los Angeles-Hiroshima Study reported that serum elaidic acid concentrations in the native Japanese living in Hiroshima were significantly lower than those in the Japanese-Americans living in Los Angeles [19]. In a German cohort with relatively low TFA levels, there was no association of IP-TFA with CHD outcomes [6]. Therefore, we undertook this study to see if in Japan, another country with relatively low TFA levels, there was a relationship of TFA with CHD risk. We found that not *trans*-C18:1 isomers but *trans*-C18:2 isomers were significantly higher in ACS, that was in good agreement with previous reports [20,21]. In the Costa Rican population, not *trans*-C18:1 isomers but high *trans*-C18:2 isomers in adipose tissue were significantly associated with increased risk of non-fatal AMI [20]. Two population-based case-control studies in the US reported that neither total TFA nor *trans*-C18:1 isomers but high *trans*-C18:2 isomers in erythrocyte membrane were significantly associated with cardiac arrest [15,21]. Our present results showed *trans*-C18:2 isomers and IP-TFA were higher, and IP-TFA/AA were significantly higher in ACS patients. These results support that IP-TFA is associated with increased risk of CHD even in Japan.

The previous cohort studies showed that higher plasma levels or higher intake of palmitelaidic acid was significantly associated with lower risk for sudden cardiac death [6] and diabetes [22,23]. Our finding of slightly but significantly lower palmitelaidic acid in ACS patients is generally consistent with these findings. The major sources of palmitelaidic acid are ruminant meat and milk, and it has been reported that the consumption of dairy products may help reduce risk for CHD and diabetes [24–26].

It has been well accepted that higher dietary intake of TFA raises LDL-C and decreases HDL-C, which results in an elevated risk of CHD [3,27]. However, some studies pointed out that the dose-response relations between TFA and lipid levels were not observed when the dietary intake of TFAs was low [9,28,29]. This is consistent with a TFA intervention study in young Japanese women, which showed no relationship between TFA and LDL-C or HDL-C [10]. The TRANSFAIR study, a cross-sectional study among middle-aged men and women in eight European countries, showed no associations between total TFA intake and LDL-C, HDL-C, or LDL-C/HDL-C ratio [30]. In addition, the TRANSFAIR study reported two relationships between TFA intake and serum lipid levels: *trans*-C18:1 isomers or *trans*-C18:2 were significantly inversely associated with total cholesterol or LDL-C, and palmitelaidic acids were inversely associated with HDL-C and were positively associated with LDL-C/HDL-C ratio [30]. On the other hand, the Cardiovascular Health study [22] showed that palmitelaidic acid was positively associated with HDL-C and inversely associated with triglyceride. The reason of this discrepancy remains unclear. Our present results support the inverse association between *trans*-C18:1 isomers or *trans*-C18:2 and non-HDL-C or LDL-C in the TRANSFAIR study [30]; the direct association between palmitelaidic acids and HDL-C in the Cardiovascular Health study [22]; and the direct association between palmitelaidic acids and LDL-C in the previous intervention studies [31]. In addition, our present results showed inconsistent relationship between TFA and lipid parameters based on the proportional and absolute concentration of TFA. The absolute concentration of *trans*-C18:2 was significantly directly associated with LDL-C or non-HDL-C in ACS patients. On the other hand, both proportional and absolute concentration of palmitelaidic acids was significantly directly associated with HDL-C, n-3 PUFA, EPA + DHA or EPA + DHA/AA. Therefore, dietary patterns rich in EPA and DHA may be associated with higher intake of palmitelaidic acids, and these lifestyles were less observed in ACS patients, especially middle-aged patients. The present study supports that R-TFA is cardioprotective, although further studies are needed [6,22,23].

Although it is likely that most of TFA are of dietary origin [10], we have very recently reported that various food intake except preference drinks such as tea or coffee, was not associated with plasma TFA levels in Japanese healthy old men [32]. A meta-analysis of seven cohorts with genome-wide association studies has not identified a significant genetic control for TFA, including palmitelaidc acids, *trans*-C18:1 isomers, *trans/trans*-C18:2, and *trans/cis*-C18:2 isomers [33]. Gotoh et al. have very recently reported the distribution of *trans*-C18:1 positional isomers in various foods consumed in Japan [34]. They showed that

Table 5
Comparison of plasma TFA composition between control and ACS men by age.

	Middle-age, < 60 years		Elder ≥60 years	
	Control (n = 24)	ACS men (n = 25)	Control (n = 25)	ACS men (n = 41)
Age, years	52.5 ± 3.2	50.0 ± 7.4	70.1 ± 6.0###	69.6 ± 5.6###
BMI, kg/m^2	24.6 ± 3.0	26.3 ± 4.4	24.6 ± 3.0	23.2 ± 3.8*
Diabetes, %	0	16	0	34
Lipid-lowering, %	0	12	0	29
Total FA, mg/L	3772.3 ± 1201.3	3530.7 ± 1412.6	3514.7 ± 994.8	3206.3 ± 771.4
Total TFAs, %	1.71 ± 0.31	1.64 ± 0.29	1.63 ± 0.36	1.73 ± 0.33
16:1 7t, %	0.21 ± 0.06	0.16 ± 0.05**	0.18 ± 0.05	0.18 ± 0.06
Total 18:1 TFAs, %	0.91 ± 0.18	0.81 ± 0.23	0.83 ± 0.20	0.87 ± 0.21
Total 18:2 TFAs, %	0.59 ± 0.20	0.67 ± 0.17	0.61 ± 0.19	0.68 ± 0.17
Total R-TFA, %	0.32 ± 0.08	0.27 ± 0.08	0.28 ± 0.08	0.29 ± 0.10
Total IP-TFA, %	1.40 ± 0.29	1.37 ± 0.24	1.35 ± 0.32	1.44 ± 0.30
18:1 4t, %	010 ± 0.05	0.06 ± 0.03*	0.08 ± 0.05	0.08 ± 0.05
18:1 5t. %	0.09 ± 0.05	0.06 ± 0.02*	0.07 ± 0.03	0.08 ± 0.04
18:1 (6–8)t, %	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.02
18:1 9t, %	0.11 ± 0.05	0.12 ± 0.06	0.09 ± 0.04	0.11 ± 0.04
18:1 10t, %	0.13 ± 0.06	0.12 ± 0.04	0.10 ± 0.05	0.12 ± 0.04
18:1 11t, %	0.11 ± 0.04	0.12 ± 0.06	0.09 ± 0.05	0.11 ± 0.05
18:1 12t, %	0.05 ± 0.02	0.07 ± 0.04	0.05 ± 0.03	0.07 ± 0.03
18:1 13-14t, %	0.15 ± 0.07	0.12 ± 0.05	0.17 ± 0.05	0.16 ± 0.06
18:1 16t, %	0.10 ± 0.05	0.10 ± 0.06	0.12 ± 0.06	0.10 ± 0.05
18:2 n6tt, %	0.17 ± 0.09	0.21 ± 0.09	0.19 ± 0.08	0.23 ± 0.11
18:2 n6ct, %	0.23 ± 0.10	0.27 ± 0.06	0.25 ± 0.11	0.26 ± 0.08
18:2 n6tc, %	0.19 ± 0.07	0.19 ± 0.08	0.17 ± 0.06	0.19 ± 0.08
Total R-TFA/AA, %	5.7 ± 2.0	5.0 ± 1.9	4.7 ± 1.8	5.7 ± 2.1
Total IP-TFA/AA, %	24.4 ± 6.0	24.9 ± 6.7	22.4 ± 6.0	28.4 ± 7.6**
Total SFA, %	30.0 ± 1.7	30.9 ± 2.1	30.5 ± 1.5	30.8 ± 1.9
Total MUFA, %	25.8 ± 3.6	27.6 ± 2.7	24.6 ± 2.0	27.5 ± 2.9**
Total n3PUFA, %	8.0 ± 2.9	5.4 ± 1.8**	9.2 ± 2.6	7.5 ± 2.6*##
Totaln6PUFA, %	34.7 ± 4.6	34.5 ± 3.7	34.1 ± 3.4	32.5 ± 3.3
AA, %	5.85 ± 1.09	5.73 ± 1.18	6.16 ± 1.19	5.27 ± 1.15*
EPA + DHA, %	6.7 ± 2.8	4.2 ± 1.7**	7.9 ± 2.4	6.0 ± 2.4*#
EPA + DHA/AA	1.19 ± 0.58	0.74 ± 0.27**	1.32 ± 0.44	1.20 ± 0.55##
Total protein, g/dL	7.4 ± 0.2	7.2 ± 0.7	7.4 ± 0.3	7.1 ± 0.7
Albumin, g/dL	4.8 ± 0.2	4.3 ± 0.5*	4.6 ± 0.2	4.0 ± 0.5**
Total bilirubin,	0.6 ± 0.3	0.9 ± 0.5	0.6 ± 0.2	0.8 ± 0.4
Creatinine, mg/dL	0.86 ± 0.07	0.79 ± 0.15	0.92 ± 0.18	1.16 ± 0.64##
Glucose, mg/dL	89.4 ± 18.2	138.1 ± 58.9**	92.9 ± 15.1	154.9 ± 61.9***
Triglyceride, mg/dL	140.0 ± 101.1	114.9 ± 74.6	112.3 ± 50.5	109.2 ± 66.7
Non-HDL-C, mg/dL	150.0 ± 37.1	158.4 ± 40.3	121.9 ± 29.4	114.0 ± 29.7
LDL-C, mg/dL	125.1 ± 30.2	138.0 ± 37.7	121.9 ± 29.4	114.0 ± 29.7#
HDL-C, mg/dL	61.2 ± 16.4	41.4 ± 9.5***	58.5 ± 13.8	45.1 ± 12.1**

Data are expressed as mean ± SD, and FA are presented as a percent of total plasma FA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* non-ACS counterpart; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ *vs.* younger counterpart by one-way ANOVA with Tukey's honest *post hoc* test.

some foods contained ≥1.0 g TFA/100 g food, and high content of *trans*-C18:2 in foods was attributed to the heating of oil. In addition, they described that difference between monoene-rich and polyene-rich TFA was attributed to diverse TFA formation mechanisms [34]. Further studies are required to investigate both dietary intake and plasma levels of TFA.

We also found case-control differences in other plasma FA in this study, in particular, for palmitic, oleic, EPA, DHA and AA. According to a meta-analysis of prospective cohort studies investigating plasma FA and CHD outcome, relative risk and 95% of confidence interval (CI) for CHD for these five FAs was (respectively): 1.15 (CI 0.96–1.37), 1.09 (CI 0.97–1.23), 0.78 (CI 0.65–0.94), 0.79 (CI 0.67–0.93), and 0.83 (CI 0.74–0.92) [35]. Our results are consistent with the statistically significant protective associations with EPA, DHA and AA, and with the trends toward adverse relationships with palmitic and oleic. The role of oleic acid in CHD is controversial [36]. Our finding of adverse associations for oleic acid between cases and controls is consistent with a recent report from the Multi-Ethnic Study of Atherosclerosis. In this prospective cohort study from the US with 6568 men and women aged 45–84 years without clinical evidence of cardiovascular disease, the top quartile of plasma oleic acid was linked to a significantly greater risk for all-cause mortality, cardiovascular disease, and heart failure after adjusting for typical cardiovascular risk factors, as well as plasma n-3 PUFA [37]. More studies are clearly needed to better understand the role of oleic acid in CHD prevention. The favorable association with n-6 PUFA, AA was perhaps unexpected given the popular view that this is a "proinflammatory" PUFA. Our findings, along with those of the Chowdhury meta-analysis cited above, indicate that this view needs to be re-considered. As noted above, the ACS patients, especially middle-aged patients, showed significantly lower levels of EPA and DHA, the two PUFAs coming from fish. It is well accepted that n3-PUFA has various beneficial effects in the prevention of CHD [38,39], but these effects have mostly been observed in Western populations. Our findings suggest that even in Japan, where average EPA and DHA levels are much higher than in the US [40], higher levels of the marine n-3 PUFA are associated with lower cardiovascular disease risk. However, the Japanese dietary style has changed markedly in the younger generation since 1990 [11,12]. Lack of fish intake and excessive oils and meat and poultry intakes have been recognized in subjects < 60 years old at the subject recruitment of 2013–2014. Our present results show that palmielaidic acid and n-3 PUFA are markedly lower in middle-aged ACS patients.

The major limitation of the present study is the single center cross sectional case-control analysis with very small sample size, and like all

cross-sectional study, causal relationships cannot be established. Second, the intakes of dietary TFA and FA compositions were not measured. Although we had been able to do so, and the ACS patients were in fact eating more 18:2t (perhaps more fried foods), our conclusions would not have changed. Third, our control subjects were not randomly selected from either the population or patients admitted to the hospital for non-cardiac diagnoses. Fourth, the setting for collecting blood samples was different between controls and ACS patients, i.e., fasting *vs*. possibly non-fasting. Whether 18:2t levels in the cases were higher because of a recent meal cannot be excluded. Fifth, we could not measure TFA in erythrocyte membranes and/or lipoprotein fractions. These factors should be investigated in future studies.

In conclusion, the present study demonstrated three findings. First, IP-TFA intake (estimated from plasma levels) is low in Japan, and accordingly, there is little difference in IP-TFA levels between Japanese ACS patients and healthy controls. Second, R-TFA intakes appear to be lower in ACS patients, possibly helping explain their increased risk for cardiac disease. Third, the association of TFA and CHD might differ depending on the TFA species, and the present study was too small to definitively answer this question. Future studies should be conducted to evaluate these issues in a larger number of patients.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

SK wrote the article, conducting the data analysis, contributed to study design, data interpretation, and critical revision of the article. TT, FS, MO, YI, YY, FF, FT, and MS contributed to acquisition of data and data analysis. WSH contributed to data analysis, drafting of the article, data interpretation, and critical revision of the article. AT contributed to study design, drafting of the article, data interpretation, and critical revision of the article.

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Appendix A. Supplementary data

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