Elevated Serum Elaidic Acid Predicts Risk of Repeat Revascularization After Percutaneous Coronary Intervention in Japan

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Background: Trans-fatty acid (TFA) intake increases the risk of coronary artery disease (CAD). Our previous cross-sectional survey showed that middle-aged patients with CAD in Japan have elevated serum TFA. In this study, we longitudinally investigated whether elevated TFA is a risk factor in the secondary prevention of CAD for the same-age patients.

Methods and Results: A total of 112 patients (age, 21–66 years) who underwent percutaneous coronary intervention were followed up for up to 2 years. Serum elaidic acid was measured using gas chromatography/mass spectrometry as a marker of TFA intake and divided into quartiles. The primary endpoint was ischemia-driven target lesion revascularization (TLR). The hazard ratio (HR) for TLR increased significantly with higher serum elaidic acid ($P<0.01$). The significant positive trend remained unchanged after adjusting for conventional lipid profile and bare-metal stent usage. In contrast, although triglycerides and low-density lipoprotein cholesterol were positively correlated with elaidic acid, they were not associated with TLR. On multivariable Cox proportional hazard analysis, elevated elaidic acid was independently associated with TLR risk after adjusting for conventional coronary risks (HR, 10.7, $P<0.01$).

Conclusions: Elevated elaidic acid is associated with higher TLR rate in middle-aged patients with CAD, suggesting that excessive TFA intake is becoming a serious health problem in Japan.

Key Words: Coronary artery disease; Elaidic acid; Target lesion revascularization; Trans-fatty acid

Trans-fatty acids (TFA) are defined as unsaturated fatty acids with at least 1 non-conjugated double bond in the trans configuration. While small amounts of TFA are found in ruminant dairy and meat products, the main dietary sources of TFA are industrially produced by partial hydrogenation of vegetable oils, a process that converts unsaturated oils into semisolid fats.¹ Accumulated epidemiological studies have demonstrated that excessive TFA intake is associated with an increased risk of coronary artery disease (CAD) and sudden cardiac death.^{2–5} Therefore, the World Health Organization (WHO) recommended that the dietary intake of industrial TFA should be $<1\%$ of the daily energy intake.⁶ Moreover, the WHO has recently issued a statement that industrially produced TFA from the global food supply should be eliminated.⁷ Elaidic acid (trans-9 C18:1) is the trans-isomer of oleic acid (cis-9 C18:1) and the main component of TFA derived from industrial products. Circulating elaidic acid

level can be used as an indicator of oral TFA intake because it cannot be synthesized by the body.⁸ Recently, we showed that middle-aged patients with CAD and/or metabolic syndrome have elevated serum elaidic acid compared with those without such conditions in Japan.⁹ Additionally, elevated serum elaidic acid concentration is associated with higher prevalence of vulnerable coronary plaque¹⁰ and insulin resistance.¹¹ Although the Japanese diet is assumed to contain lesser fat including TFA than the Western diet,^{12,13} our previous study alarmingly suggested that excessive TFA intake is becoming a public health problem in Japan as well.⁹

Given, however, that our previous studies were cross-sectional surveys, the causal relationship between serum elaidic acid concentration and CAD in Japan was not determined. In the previous survey, we found that serum elaidic concentration was elevated in patients with CAD aged ≤ 66 years.⁹ In the present study, we investigated

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Table 1. Baseline Subject Characteristics vs. Serum Elaidic Acid

Variables	Serum elaidic acid quartiles ($\mu\text{mol/L}$)				P-value
	Q1 [≤ 10.18] (n=28)	Q2 [10.19–13.63] (n=28)	Q3 [13.64–16.99] (n=28)	Q4 [≥ 17.0] (n=28)	
Age (years)	58.0 $\pm$ 5.7	60.9 $\pm$ 5.4	57.8 $\pm$ 7.3	56.5 $\pm$ 8.3	0.14
Male	27 (96.4)	22 (78.6)	26 (92.9)	22 (78.6)	0.09
Weight (kg)	70.0 $\pm$ 12.1	67.1 $\pm$ 11.1	68.6 $\pm$ 13.7	73.8 $\pm$ 8.3	0.21
BMI (kg/m^2)	24.8 $\pm$ 3.4	25.0 $\pm$ 3.8	24.8 $\pm$ 3.8	26.8 $\pm$ 4.0	0.15
Metabolic syndrome	13 (46.4)	13 (46.4)	11 (39.3)	17 (60.7)	0.32
Hypertension	21 (75.0)	22 (78.6)	24 (85.7)	23 (82.1)	0.78
Dyslipidemia	22 (78.6)	24 (85.7)	22 (78.6)	24 (85.7)	0.87
Diabetes mellitus	17 (60.7)	13 (46.4)	14 (50.0)	16 (57.1)	0.73
Smoking	25 (89.3)	22 (78.6)	22 (78.6)	24 (85.7)	0.52
Family history	6 (21.4)	15 (53.6)	5 (17.9)	8 (28.6)	0.02
Laboratory data					
Elaidic acid ($\mu\text{mol/L}$)	8.8 (8.0–9.5)	11.5 (10.8–12.2)	14.8 (14.1–15.7)	20.2 (18.8–25.8)	<0.001
T-cho (mg/dL)	170.5 $\pm$ 30.8	171.4 $\pm$ 47.7	186.5 $\pm$ 37.5	205.8 $\pm$ 37.3	<0.001
HDL-C (mg/dL)	48.4 $\pm$ 15.9	44.7 $\pm$ 10.9	49.9 $\pm$ 16.4	41.5 $\pm$ 14.8	0.13
LDL-C (mg/dL)	93.1 $\pm$ 21.9	100.5 $\pm$ 42.0	105.8 $\pm$ 30.3	126.3 $\pm$ 33.9	<0.01
TG (mg/dL)	119.5 (100.5–142.0)	132.0 (107.5–162.5)	147.5 (114.0–211.0)	195.0 (137.0–262.0)	<0.001
HbA1c (%)	6.3 $\pm$ 1.6	6.5 $\pm$ 1.1	6.9 $\pm$ 1.5	7.0 $\pm$ 1.0	0.26
hs-CRP (mg/dL)	0.04 (0.02–0.09)	0.06 (0.03–0.19)	0.05 (0.03–0.14)	0.06 (0.03–0.16)	0.57
Medication at discharge					
Statin	15 (53.6)	19 (67.9)	15 (53.6)	14 (50.0)	0.55
Dual antiplatelet therapy	28 (100)	28 (100)	28 (100)	28 (100)	1.00
EPA	3 (10.7)	3 (10.7)	2 (7.1)	3 (10.7)	0.96
Fibrates	0 (0)	0 (0)	1 (3.6)	1 (3.6)	0.35
Ezetimibe	1 (3.6)	1 (3.6)	0 (0)	2 (7.1)	0.56
Anti-diabetic agent	12 (42.9)	8 (28.6)	7 (25.0)	12 (42.9)	0.35
Stent					
Bare-metal stent	1 (3.6)	3 (10.7)	0 (0)	2 (7.1)	0.15
Drug-eluting stent	27 (96.4)	25 (89.3)	28 (100)	26 (92.9)	0.42
Total no. stents	2.4 $\pm$ 1.3	2.1 $\pm$ 1.0	1.8 $\pm$ 1.1	1.9 $\pm$ 1.1	0.21
Total stent length (mm)	52.6 $\pm$ 38.3	50.9 $\pm$ 35.3	39.4 $\pm$ 28.0	45.1 $\pm$ 36.5	0.58
Maximum stent diameter (mm)	3.4 $\pm$ 0.2	3.4 $\pm$ 0.3	3.2 $\pm$ 0.5	3.3 $\pm$ 0.4	0.48
Stent overlap	20 (71.4)	19 (67.9)	14 (50.0)	14 (50.0)	0.24

Data given as mean $\pm$ SD, median (IQR) or n (%). Stent overlap was defined as the presence of two or more stents within a treated lesion and an overlapping stent zone. BMI, body mass index; EPA, purified eicosapentaenoic acid; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Q, quartile; T-cho, total cholesterol; TG, triglycerides.

whether elevated serum elaidic acid could be a predictive risk factor for repeat revascularization after percutaneous coronary intervention (PCI) in the same-age patients with CAD in Japan.

Methods

Subjects

The Kobe Cardiovascular Marker Investigation (CMI) registry is a single-center registry of patients with cardiovascular disease (CVD) who were referred to Kobe University Hospital. It started in 2008 to identify blood-based biomarkers that are used in predicting CVD.

Based on the Kobe CMI registry, we enrolled consecutive patients aged between 21 and 66 years from our previous study,⁹ and those who underwent successful PCI with stent

placement at Kobe University Hospital because of stable angina or inducible myocardial ischemia between July 2008 and February 2013.

Exclusion criteria were defined as follows: (1) emergency admission; (2) acute heart failure (New York Heart Association functional class IV) and pulmonary hypertension; (3) occurrence of cancer in the last 5 years; (4) serum triglyceride >400 mg/dL; (5) kidney failure (serum creatinine concentration >2.0 mg/dL or hemodialysis); (6) familial hypercholesterolemia; or (7) active inflammation (serum C-reactive protein [CRP] concentration >1 mg/dL).⁹ Patients who were lost to follow-up were also excluded from the analysis.

Ethics

The study protocol was in accordance with the ethics

Figure 1. Distribution of serum elaidic acid concentration (n=112).

guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Review Committee at Kobe University (Japan) and was registered in the UMIN Clinical Trials Registry with identification number 000030297. Written informed consent was obtained from all patients prior to enrollment in this study.

Study Design

Blood samples were collected in the morning prior to cardiac catheterization after overnight fasting and stored at -80°C until measurement of elaidic acid concentration. The participants were followed up for up to 2 years after PCI. Clinical outcome data were obtained by reviewing outpatient records or telephone interviews for death, myocardial infarction, stroke, and target lesion revascularization (TLR). The primary outcome was ischemia-driven TLR during the follow-up period.

Serum Elaidic Acid Concentration

Serum elaidic acid was measured using gas chromatography/mass spectrometry (GC-MS QP2010; Shimadzu, Kyoto, Japan) at the Integrated Center for Mass Spectrometry of Kobe University Graduate School of Medicine according to the procedure described in our previous report.⁹ A quality control sample ($10\mu\text{mol/L}$ elaidic acid standard) was measured in each analytical batch to calibrate serum elaidic acid concentration. The coefficient of variation was $<2\%$. Serum elaidic acid concentration was divided into quartiles as follows: Q1, $\leq 10.18\mu\text{mol/L}$; Q2, $10.19\text{--}13.63\mu\text{mol/L}$; Q3, $13.64\text{--}16.99\mu\text{mol/L}$; and Q4, $\geq 17.0\mu\text{mol/L}$.

Other Clinical Variables

Serum total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), hemoglobin A1c (HbA1c), and high-sensitivity CRP (hs-CRP) were measured using the standard methods. HbA1c (%) is expressed as the National Glycohemoglobin Standardization Program (NGSP) equivalent, using the formula $\text{HbA1c (\%)} = \text{HbA1c (Japan Diabetes Society (\%))} + 0.4\%$.¹⁴ Hypertension was defined as blood

pressure $\geq 140/90\text{ mmHg}$ or treatment with antihypertensive drugs. Diabetes mellitus was defined as $\text{HbA1c} \geq 6.5\%$ and fasting serum glucose $\geq 126\text{ mg/dL}$ or non-fasting serum glucose $\geq 200\text{ mg/dL}$, or treatment with anti-diabetic drugs. Dyslipidemia was defined according to the Japan Atherosclerosis Society Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases¹⁵ or treatment with anti-hyperlipidemia drugs. Metabolic syndrome was defined according to the Japan Atherosclerosis Society guidelines.¹⁶

Statistical Analysis

All statistical analysis was performed using Stata 14.2 (StataCorp, College Station, TX, USA). Two-sided $P \leq 0.05$ was considered statistically significant in all analyses. Categorical variables are expressed as numbers and percentage. Continuous variables are expressed as mean $\pm$ SD, unless otherwise specified. Variables with skewed distribution were normalized using natural logarithmic transformation. The trends in baseline characteristics across serum elaidic acid quartiles were tested using one-way analysis of variance (ANOVA). Moreover, the relationships between 2 numerical variables were investigated on linear regression analysis. The incidence rates of TLR were calculated using the person-year method. Hazard ratio (HR) with 95% CI of serum elaidic acid levels for TLR was assessed using the Cox proportional hazards model. The trends in the risk of TLR across serum elaidic acid level were tested with the Cox proportional hazards model by assigning ordered numbers (i.e., 1, 2, 3, and 4) to the quartiles. Multivariate analysis was performed to explore the influence of independent variables on TLR.

Results

Subject Baseline Characteristics

From the Kobe CMI registry between July 2008 and February 2013, a total of 112 patients were enrolled according to the inclusion and exclusion criteria. Median serum elaidic acid concentration in all participants; in men ($n=97$); and in women ($n=15$), was $13.6\mu\text{mol/L}$ (IQR, $10.2\text{--}17.0\mu\text{mol/L}$), $13.4\mu\text{mol/L}$ (IQR, $10.0\text{--}16.4\mu\text{mol/L}$), and $13.7\mu\text{mol/L}$ (IQR, $11.5\text{--}20.2\mu\text{mol/L}$), respectively. **Table 1** lists the baseline subject characteristics according to serum elaidic acid level. A higher prevalence of positive family history of CAD was seen in patients with lower elaidic acid level. Serum total cholesterol, LDL-C, and TG significantly increased with higher elaidic acid. In contrast, mean serum HDL-C was lower in patients with higher elaidic acid, but, the trend across the quartiles was not statistically significant.

Serum Elaidic Acid and Conventional Biomarkers

We assessed the relationship between serum elaidic acid level and other biomarkers including conventional lipid profiles. Given that the distribution of serum elaidic level was skewed to the left (**Figure 1**), we normalized serum elaidic acid level by logarithmic transformation prior to analysis. Serum elaidic acid was positively associated with TG, total cholesterol, and LDL-C (**Figure 2A–C**). In contrast, no significant relationship was noted between serum elaidic acid and HDL-C, HbA1c, and hs-CRP (**Figure 2D–F**).

Serum Elaidic Acid and Risk of TLR

During the median follow-up period of 2.0 years (IQR,

Figure 2. Ln(elaidic acid) vs. (A) ln(triglycerides [TG]), (B) total cholesterol (T-cho), (C) low-density lipoprotein cholesterol (LDL-C), (D) high-density lipoprotein cholesterol (HDL-C), (E) hemoglobin A1c (HbA1c), and (F) ln(high-sensitivity C-reactive protein [hs-CRP]). Ln, natural logarithm.

0.7–2.0 years), 30 patients (26.8%) required TLR. The incidence rates and HR for TLR according to serum elaidic acid quartile are given in **Table 2**. The HR for TLR increased significantly with higher serum elaidic acid ($P < 0.01$). In the multivariable model adjusted for conventional lipid profiles, the positive relationship between serum elaidic acid and TLR was also observed. Furthermore, the significant association remained unchanged even after adjusting for the use of bare-metal stents (BMS). In contrast, although TG and LDL-C were positively correlated with elaidic acid level, no significant difference was observed in the HR for TLR according to TG and LDL-C quartile (**Table 3**). Finally, we found that serum elaidic

acid was the only factor associated with the incidence of TLR (**Table 4**). On multivariate Cox proportional hazard analysis, elaidic acid remained independently associated with TLR after adjusting for traditional coronary risk factors, conventional lipid profiles, and stent parameters (number, length, diameter, and overlap), according to the previous reports (**Table 4**).^{17,18} In contrast, we omitted the use of anti-platelet medication and BMS from the Cox proportional hazard model, because all participants underwent dual anti-platelet therapy after PCI, and no patients with BMS implantation required TLR in the present study.

Table 2. Serum Elaidic Acid Level and Risk of TLR

Elaidic acid quartiles	No. TLR/ subjects	Incidence rate (per 10 ² person years)	HR (95% CI)	P-value	Multivariable-adjusted model 1		Multivariable-adjusted model 2	
					HR (95% CI)	P-value	HR (95% CI)	P-value
Q1	3/28	5.6	1.0 (Ref.)		1.0 (Ref.)		1.0 (Ref.)	
Q2	6/28	14.8	2.6 (0.6–10.4)	0.16	3.6 (0.7–17.6)	0.11	4.5 (0.9–23.6)	0.08
Q3	10/28	30.2	5.1 (1.4–18.7)	<0.01	5.3 (1.3–21.5)	0.02	5.2 (1.3–21.0)	0.02
Q4	11/28	33.3	5.6 (1.5–20.1)	<0.01	8.0 (1.7–37.5)	<0.01	8.2 (1.8–38.2)	<0.01
P for trend				<0.01		<0.01		<0.01

Multivariable model 1 was adjusted for HDL-C, LDL-C, ln(TG). Multivariable model 2 was adjusted for the variables in model 1+stent type (bare-metal stent). ln, natural logarithm; TLR, target lesion revascularization. Other abbreviations as in Table 1.

Table 3. Conventional Lipid Profiles and Risk of TLR

TG quartiles	No. TLR/ subjects	Incidence rate (per 10 ² person years)	HR (95% CI)	P-value	LDL-C quartiles	No. TLR/ subjects	Incidence rate (per 10 ² person years)	HR (95% CI)	P-value
Q1	5/28	11.4	1.00 (Ref.)		Q1	7/28	16.2	1.00 (Ref.)	
Q2	7/28	16.0	1.4 (0.43–4.3)	0.60	Q2	8/28	17.0	1.0 (0.37–2.8)	0.97
Q3	8/28	20.4	1.7 (0.57–5.3)	0.34	Q3	9/28	25.9	1.5 (0.57–4.1)	0.39
Q4	10/28	29.9	2.4 (0.84–7.3)	0.10	Q4	6/28	18.1	1.1 (0.35–3.1)	0.92
P for trend				0.08	P for trend				0.66

Serum TG: Q1, ≤109 mg/dL; Q2, 110–137 mg/dL; Q3, 138–196 mg/dL; Q4, ≥197 mg/dL. LDL-C: Q1, ≤81 mg/dL; Q2, 82–108 mg/dL; Q3, 109–130 mg/dL; Q4, ≥131 mg/dL. Abbreviations as in Tables 1,2.

Table 4. Indicators of TLR

Variable	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
Ln(elaidic acid)	4.17	1.76–9.88	0.001	10.7	2.11–54.2	0.004
Age (years)	1.01	0.96–1.07	0.71			
Male gender	0.94	0.33–2.69	0.91			
Smoking at baseline	1.54	0.47–5.08	0.48			
Metabolic syndrome	0.84	0.41–1.78	0.67			
Diabetes mellitus	0.72	0.33–1.58	0.42			
Hypertension	1.77	0.62–5.07	0.29			
Ln(TG)	1.68	0.72–3.91	0.23			
HDL-C (mg/dL)	1.01	0.99–1.03	0.22			
LDL-C (mg/dL)	1.00	0.99–1.01	0.71			
HbA1c (%)	1.11	0.83–1.48	0.47			
Ln(hs-CRP)	1.18	0.88–1.59	0.28			
Total no. stents	1.10	0.82–1.51	0.48			
Total stent length (mm)	1.00	0.99–1.02	0.25			
Maximum stent diameter (mm)	0.77	0.28–2.09	0.60			
Stent overlap	2.03	0.87–4.73	0.10			

Abbreviations as in Tables 1,2.

Discussion

In our previous cross-sectional survey, serum TFA was found to be elevated in middle-aged patients with CAD and/or metabolic syndrome in Japan.⁹ In the present longitudinal study, we showed that elevated serum elaidic acid was associated with the incidence of TLR in the same-age Japanese generation with CAD. In contrast, although serum elaidic acid level was positively associated with LDL-C and TG, they were not associated with TLR.

TFA are known to worsen the lipid profile by increasing

LDL-C and TG and reducing HDL-C.^{19–22} In the present study, we also found that elaidic acid level was positively associated with LDL-C and TG. The present findings might also reflect excessive calorie intake in patients with higher elaidic acid. In contrast, serum elaidic acid level was associated with the incidence of TLR, independent of both LDL-C and TG. Particularly, because TFA are components of TG, it is undeniable that elaidic acid merely reflects circulating TG. In contrast to elaidic acid, the HR for TLR according to TG quartile did not show a significant positive trend. And also, we did not observe an inverse relationship

between elaidic acid and HDL-C. Therefore, the mechanisms underlying the relationship between elevated circulating elaidic acid and the incidence of TLR cannot be attributed to the lipid profile alone.

Excessive TFA intake is also assumed to induce abnormal glucose metabolism^{11,23,24} and elevated inflammatory biomarkers.^{25,26} But while mean HbA1c increased in patients with higher elaidic acid, no significant difference was noted in elaidic acid quartiles. In addition, serum hs-CRP was not associated with elaidic acid level. Because approximately half of the present participants were prescribed medications including statins and anti-diabetic agents at the time of enrollment (Table 1), effects of TFA on glucose metabolism and systemic inflammation might be counteracted by medical treatment.

In contrast, it has been assumed that TFA exert direct adverse effects on the arterial wall beyond their impact on the plasma lipid profile. We have recently shown that excessive TFA intake accelerates atherosclerosis by inducing inflammation and oxidative stress in mice.²⁷ TFA was also found to induce vascular inflammation and reduce vascular nitric oxide production in endothelial cells.²⁸ We have shown that TFA also promote thrombus formation by aggravating anti-thrombogenic endothelial functions via Toll-like receptors 2 and 4 in mice.²⁹ It was also recently suggested that lipid rafts might be a platform for Toll-like receptors for TFA to activate pro-inflammation signaling on cell membranes.³⁰ In contrast, TFA promote pro-inflammatory signaling and cell death by stimulating the apoptosis signal-regulating kinase 1/p38 pathway.³¹

In the present study, we demonstrated the relationship between circulating elaidic acid level and the incidence of TLR. Elaidic acid is a typical industrial TFA, produced by partial hydrogenation of vegetable oil. It has also been shown that different TFA subtypes are associated with the risk of CVD.³¹ Because linoleic acid has 2 double bonds in the cis configuration at the 9- and 12- positions, several trans isomers should exist (i.e., trans-9/trans-12 [linoelaidic acid], cis-9/trans-12 and trans-9/cis-12). Of the trans isomers of linoleic acid, circulating linoelaidic acid level has been shown to be associated with total mortality, mainly due to the increased risk of CVD.³² Further studies are required to elucidate the impact of each TFA subtype on CVD.

Study Limitations

Several limitations of the present study should be noted. First, cardiovascular events, such as sudden cardiac death, myocardial infarction, and stroke, did not occur during the follow-up period in the present study, which might be due to the small number of participants and relatively short observation period. Larger prospective studies are required to confirm whether elevated circulating elaidic acid concentration is associated with increased risk of CVD in Japan. Second, the higher prevalence of positive family history of CAD in patients with lower elaidic acid might be due to the conscious maintenance of a proper diet. Given, however, that a questionnaire survey regarding dietary habits was not conducted in the present study, we cannot discuss what kinds of food have particularly created an impact on serum elaidic acid concentration. Third, given that serum elaidic acid was measured only at baseline, changes in circulating elaidic acid during the follow-up period were not considered. Finally, the sample size was too small to conclude that there is a relationship between prescribed medication and serum elaidic acid level. A prospective interventional

trial is warranted to address this issue.

Conclusions

Elevated circulating elaidic acid is associated with the need for repeat revascularization after PCI in middle-aged patients with CAD. This suggests that excessive TFA intake is becoming an alarmingly serious health problem in Japan, as well as in Western countries.

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Disclosures

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Author Contributions

T.O. contributed to the study design, data collection and analysis, and manuscript writing. R.T. planned and supervised the study, provided logistic support, and edited the manuscript. M.S. and Y.I. measured serum elaidic acid and provided technical support. K.M., M.N., T.H. and H.O. provided logistic support. T.I. and K.H. provided logistic support and supervised the study. R.T. is the guarantor and is responsible for the overall content of the study.

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