Estimated Elovl6 and delta-5 desaturase activities might represent potential markers for insulin resistance in Japanese adults

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Abstract

Purpose Results from a recent study indicated that lower stearic acid/palmitic acid (SA/PA) and arachidonic acid/dihomo-γ-linolenic acid (AA/DGLA) ratios were associated with metabolically unhealthy obesity. However, this has not been extensively studied in the Japanese population.

Methods We recruited 291 Japanese subjects with serum free fatty acid profiles undergoing health examinations. Whole serum desaturase activity was estimated as the product: precursor ratio –SA/PA ratio for elongation of long-chain fatty acid family member 6 (Elovl6) and AA/DGLA for delta-5 desaturase (D5D). The determinants of Elovl6 and D5D activity were investigated using multiple regression analyses.

Results The Elovl6 and D5D activities exhibited a negative correlation with the logmatic-transformed TG/HDL-C ratio and TyG index. Multiple regression analyses revealed that the TG/HDL-C ratio and TyG index were negatively associated with Elovl6 and D5D activities. Most atherogenic markers were worse in the low Elovl6 or D5D activity group than in the high Elovl6 or D5D activity group. When study subjects were further stratified by TG levels, most atherogenic markers were the worst in the highest TG group in either the lowest Elovl6 or lowest D5D activity groups.

Conclusion The estimated Elovl6 and D5D activities might be useful markers of insulin resistance in Japanese subjects.

Keywords Elongation of long-chain fatty acids family member 6 activity · Delta-5 desaturase activity · Triglyceride to high-density lipoprotein cholesterol ratio · Fasting triglycerides-glucose index · Insulin resistance

Introduction

Previous epidemiologic studies have suggested that individuals with higher plasma concentrations of free fatty acids (FAs) are at increased risk of type 2 diabetes [1–3], and they have also been linked to peripheral (muscle) insulin

resistance (IR) [4]. Plasma free FA levels are chronically elevated in obese individuals [5]; therefore, it was hypothesized that increased free FA levels are an important feature of obesity-associated metabolic syndrome (MetS) and cardiovascular disease (CVD) [6].

The evaluation of individual serum FA levels is also important. For instance, a previous study indicated that higher serum total n–6 ($\varpi - 6$) polyunsaturated fatty acids (PUFAs), linoleic acid (LA), and arachidonic acid (AA) concentrations are associated with a lower risk of incident type 2 diabetes and higher γ-linolenic acid (GLA) and dihomo-γ-linolenic acid (DGLA) concentrations were associated with a higher risk [7]. Another study reported that a high serum DGLA level was associated with obesity, body fat accumulation, a high ALT level, and IR in patients with type 2 diabetes [8].

Free FA composition depends in part on the endogenous metabolism of free FAs via elongation and desaturation [9]. Many previous studies have shown that FA

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product-to-precursor ratios can be used to estimate elongase and desaturase activities and the association of their altered activity with the worsening of glycemia and incidence of type 2 diabetes [7, 10–12]. Higher estimated delta-5 desaturase (D5D) activity was associated with a lower risk of incident type 2 diabetes, and higher estimated delta-6 desaturase (D6D) activity was associated with a higher risk [7]. IR and its associated disorders, including type 2 diabetes, are associated with an increase in the estimated activity of the delta-9 desaturase (D9D) [13–17] and D6D [13, 14, 17], as well as a decrease in the activity of the D5D [13–17].

The results of a recent 10-year longitudinal Shanghai Diabetes Study (SHDS) [18] indicate that a higher baseline level of oleic acid/stearic acid (OA/SA) and lower levels of stearic acid/palmitic acid (SA/PA) and AA/DGLA ratios were associated with a higher rate of conversion between metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUO) conversion. This finding was validated in cross-sectional and interventional studies. Recently, we reported that the OA/SA ratio might be a useful marker for IR in non-obese Japanese subjects [19].

This study was designed to investigate whether the estimated elongation of long-chain fatty acid family member 6 (Elovl6) (SA/PA ratio) and D5D (AA/DGLA ratio) activities are associated with IR. The study also investigated whether the Elovl6 and D5D activities measure metabolic abnormalities in Japanese adults.

Materials and methods

Subjects

A total of 319 subjects, undergoing an anti-aging health examination at the Health Screening Center, Tokai University Tokyo Hospital in 2016, were included in this cross-sectional study. After excluding 28 subjects, for whom the serum FFA profiles were not analyzed, 291 subjects were included in the final analysis. Medical histories were obtained using self-administered questionnaires and interviews conducted by nurses.

Measurements

Waist circumference (WC) was measured at the level of the umbilicus during slight expiration, with the participant in a standing position. Blood pressure (BP) was measured on the upper right arm using an automatic BP monitor (TM-2655P; A&D, Tokyo, Japan) while the participant was seated. Blood samples were collected in heparin-coated tubes early in the morning following an overnight fast. Fasting plasma glucose (FPG) levels were measured with an L-type Glu 2 kit, using the hexokinase/glucose-6-phosphate dehydrogenase method

(Wako Pure Chemicals). The low-density lipoprotein cholesterol (LDL-C), HDL-C, and TG levels were measured using visible spectrophotometry (Determiner L LDL-C, Determiner L HDL-C, and Determiner L TG II, respectively; Kyowa Medex, Tokyo, Japan). Uric acid (UA) levels were measured with an L-Type UA M kit using the uricase-N-(3-sulfopropyl)-3-methoxy-5-methylaniline (Wako Pure Chemicals, Osaka, Japan). The serum free FA profile was measured by gas chromatography. TyG index was calculated as logmatic transformations (ln) [fasting triglycerides (mg/dL) × fasting glucose (mg/dL)/2] [20, 21].

All subjects provided written informed consent for the use of their health records for analysis. This study was approved by the Ethics Committee of Tokai University (No. 11R-125) and was conducted in accordance with the Declaration of Helsinki.

Statistical analyses

Data are expressed as mean ± standard deviation or median (interquartile range). The normality of data distribution was determined using the Kolmogorov–Smirnov test. Bonferroni's multiple comparison test was used to compare mean values across three or more groups. Student's t-test was used to compare the mean values between two groups. To compare various markers, the subjects were divided into three groups based on Elovl6 and D5D activities and into six groups based on the combinations of either Elovl6 activity and TG levels or D5D activity and TG levels. The determinants of Elovl6 and D5D activity were identified by multiple linear regression analysis. TG/HDL-C ratio and TyG index were used as markers for IR, as described previously [19]. Two sets of variables were considered: one set for TG/HDL-C ratio [sex, age, body mass index (BMI), WC, systolic and diastolic BP, FPG, TG/HDL-C ratio, LDL-C, UA], and the other set for TyG index (sex, age, BMI, WC, systolic and diastolic BP, TyG index, HDL-C, LDL-C, UA). The determinants of the upper tertiles of Elovl6 and D5D activities were identified through multiple logistic regression analyses using the same variables used in the multiple linear regression analysis, and a stepwise procedure was used to select variables for multiple regression analyses. All statistical analyses were performed using SAS Studio version 3.4 (SAS Institute, Cary, NC, USA). All p-values were two-tailed, and a p-value of < 0.05 was considered statistically significant.

Results

All the characteristics evaluated in this study are presented in Table 1. Of the 291 subjects, 115 (39.5%) were women. The mean age, BMI, FPG, median TG, mean TyG index, HDL-C levels, and median TG/HDL-C ratio were 54.4 years

Table 1 Characteristics of study subjects

Men/women (n)	176/115	
Age	54.4	± 13.9
BMI (kg/m^2)	22.4	± 3.1
Waist circumference (cm)	81.6	± 9.1
Systolic BP (mmHg)	122.1	± 16.4
Diastolic BP (mmHg)	77.9	± 11.5
FPG (mg/dL)	96.8	± 12.1
TG (mg/dL)	85.0	[57.5,119.5]
TyG index	8.30	± 0.57
HDL-C (mg/dL)	64.3	± 14.9
TG/HDL-C ratio	1.33	[0.81,2.11]
LDL-C (mg/dL)	119.4	± 31.3
UA (mg/dL)	5.6	± 1.3
SA (μg/mL)	202.5	± 54.8
PA (μg/mL)	48.6	± 22.8
Elovl6 activity	4.73	± 1.62
AA (μg/mL)	164.1	± 38.2
DGLA (μg/mL)	30.4	± 10.1
D5D activity	5.83	± 1.91

Variables are given as mean ± standard deviation or median [interquartile range]. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; TG, triglyceride; TyG index, triglyceride-glucose index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; SA, stearic acid; PA, palmitic acid; Elovl6, elongation of long-chain fatty acids family member 6; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; D5D, delta-5 desaturase

old, 22.4 kg/m^2, 96.8 mg/dL, 85.0 mg/dL, 8.30, 64.3 mg/dL, and 1.33, respectively. Estimated mean Elovl6 and D5D activities were 4.73 and 5.83, respectively.

Possible associations of TG/HDL-C ratio and TyG index with Elovl6 and D5D activities were investigated using Pearson's correlation coefficient. As shown in Fig. 1, both Elovl6 and D5D activities exhibited negative correlations with both ln(TG/HDL-C ratio) [r = -0.434, 95% confidence interval (CI) -0.523 to -0.336, p < 0.001; r = -0.400, 95% CI -0.493 to -0.299, p < 0.001, respectively; Fig. 1 (a) and (b), upper panel], and TyG index [r = -0.455, 95% CI -0.542 to -0.359, p < 0.001; r = -0.357, 95% CI -0.454 to -0.253, p < 0.001, respectively, Fig. 1 (a) and (b), lower panel].

Figure 2 shows the association of estimated Elovl6 and D5D activities using Pearson's correlation coefficient. Estimated Elovl6 activity exhibited positive correlation with D5D activity (r = 0.280, 95% CI 0.171 to 0.383, p < 0.001).

The determinants of Elovl6 and D5D activities were identified by multiple linear regression analysis (Table 2). Two sets of variables were considered: one set for the TG/HDL-C ratio and the other set for the TyG index. Among the variables included in the TG/HDL-C ratio (sex, age, BMI, WC, systolic and diastolic BP, FPG, TG/HDL-C ratio, LDL-C,

UA), two variables (WC and TG/HDL-C ratio) were selected for Elovl6 activity using a stepwise procedure [Table 2 (a)]. Among the variables included in the TyG index (sex, age, BMI, WC, systolic and diastolic BP, TyG index, HDL-C, LDL-C, UA), three variables (WC, TyG index, and LDL-C) were selected for Elovl6 activity using a stepwise procedure [Table 2 (b)]. The analysis revealed that WC, TG/HDL-C ratio, and TyG index were negatively associated with Elovl6 activity, while LDL-C was positively associated with Elovl6 activity. Among the variables included in the TG/HDL-C ratio, two variables (BMI and TG/HDL-C ratio) were selected for D5D activity using a stepwise procedure [Table 2 (c)]. Among the variables included in the TyG index, two variables (BMI and TyG index) were selected for D5D activity using a stepwise procedure [Table 2 (d)]. The analysis revealed that BMI, TG/HDL-C ratio, and TyG index were negatively associated with D5D activity.

Determinants of the upper tertile of Elovl6 activity were analyzed using multiple logistic regression analysis [Table 3 (a) and (b)]. When we analyzed the same variables included TG/HDL-C ratio in multiple linear regression analysis, two variables (TG/HDL-C ratio and UA) were selected using a stepwise procedure [Table 3 (a)]. When we analyzed the same variables included TyG index, two variables (TyG index and UA) were selected using a stepwise procedure [Table 3 (b)]. Determinants of the upper tertile of D5D activity were analyzed using multiple logistic regression analysis [Table 3 (c) and (d)]. When we analyzed the same variables included TG/HDL-C ratio in multiple linear regression analysis, two variables (TG/HDL-C ratio and BMI) were selected using a stepwise procedure [Table 3 (c)]. When we analyzed the same variables included TyG index, two variables (TyG index and HDL-C) were selected using a stepwise procedure [Table 3 (d)]. Taken together, the results of the analysis revealed that TG/HDL-C, UA, and TyG index were negatively associated with the upper tertile of Elovl6 activity. The TG/HDL-C ratio, BMI, and TyG index were negatively correlated, while HDL-C was positively associated with the upper tertile of D5D activity.

To evaluate the impact of Elovl6 activity on various markers, the subjects were divided into three Elovl6 groups. Table 4 (a) shows the characteristics of the study subjects stratified according to Elovl6 activity. BMI, WC, systolic BP, TG, TyG index, TG/HDL-C ratio, UA, PA, AA, and DGLA decreased as Elovl6 activity increased. In contrast, HDL-C and D5D activity increased as Elovl6 increased.

To evaluate the impact of D5D activity on various markers, the subjects were divided into three D5D groups. Table 4 (b) shows the characteristics of the study subjects stratified according to the D5D activity. BMI, WC, BP, FPG, TG, TyG index, TG/HDL-C ratio, LDL-C, PA, and DGLA decreased as D5D activity increased. In contrast, HDL-C and Elovl6 activity increased as D5D activity increased.

Fig. 1 Scatter plots and regression lines for the comparisons of ln(TG/HDL-C) or TyG index and Elovl6 (a) or D5D (b) activities. Pearson's correlation coefficients with 95% confidence intervals are shown in the graph. Ln(TG/HDL-C), logarithmically transformed triglyceride to high-density lipoprotein cholesterol ratio; TyG index, triglyceride-glucose index; Elovl6, elongation of long-chain fatty acids family member 6; D5D, delta-5 desaturase
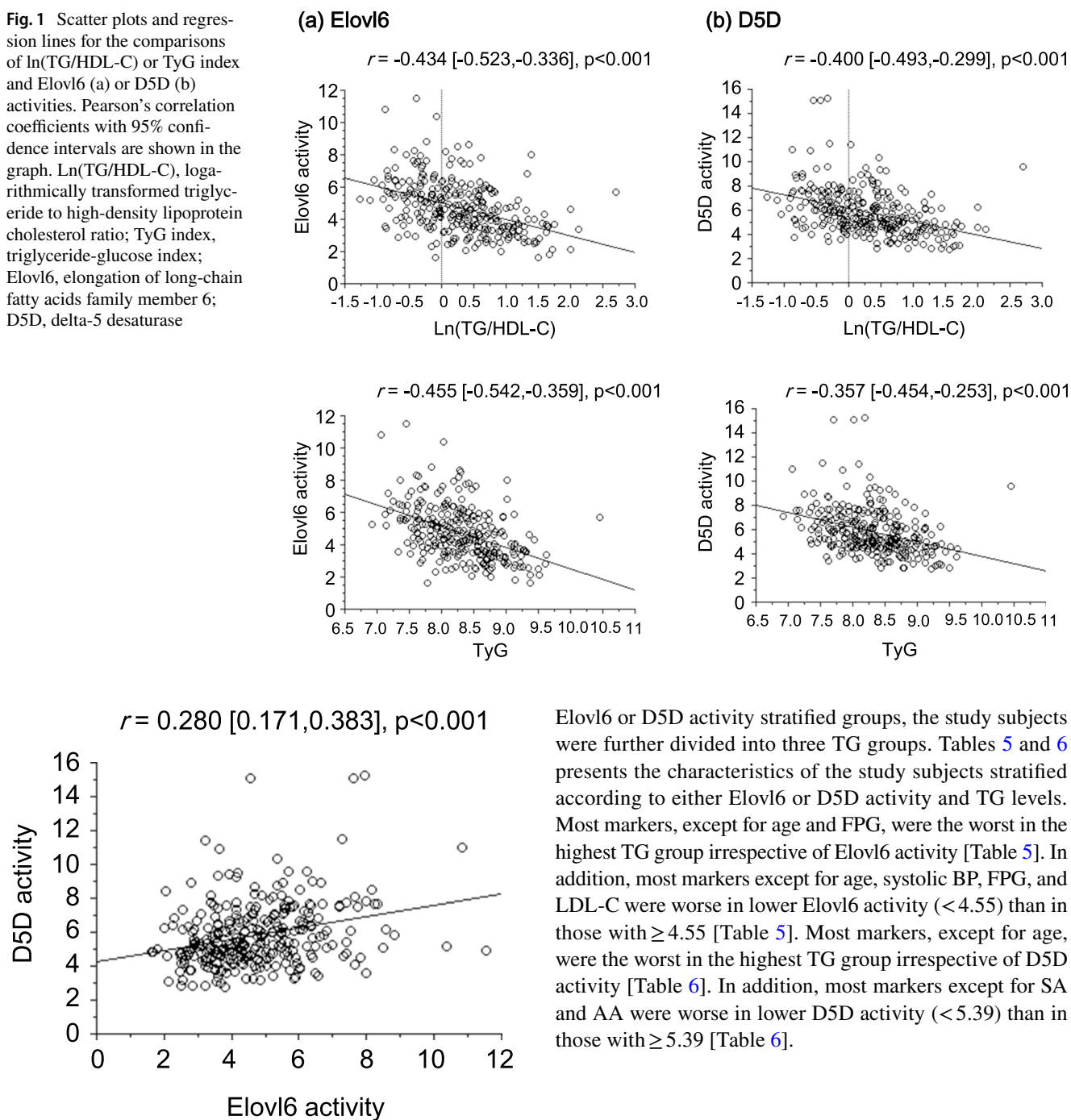

(a) Elovl6

$r = -0.434 [-0.523, -0.336]$, p<0.001

$r = -0.455 [-0.542, -0.359]$, p<0.001

(b) D5D

$r = -0.400 [-0.493, -0.299]$, p<0.001

$r = -0.357 [-0.454, -0.253]$, p<0.001

$r = 0.280 [0.171, 0.383]$, p<0.001

Fig. 2 Scatter plots and regression lines for the comparisons of estimated Elovl6 and D5D activities. Pearson's correlation coefficients with 95% confidence intervals are shown in the graph. Elovl6, elongation of long-chain fatty acids family member 6; D5D, delta-5 desaturase

PUFAs are known to suppress TG synthesis, resulting in decreased TG levels in the blood through sterol regulatory element-binding protein (SREBP)-1c suppression [22]. To evaluate the impact of TG levels on various markers in the

Elovl6 or D5D activity stratified groups, the study subjects were further divided into three TG groups. Tables 5 and 6 presents the characteristics of the study subjects stratified according to either Elovl6 or D5D activity and TG levels. Most markers, except for age and FPG, were the worst in the highest TG group irrespective of Elovl6 activity [Table 5]. In addition, most markers except for age, systolic BP, FPG, and LDL-C were worse in lower Elovl6 activity (<4.55) than in those with ≥4.55 [Table 5]. Most markers, except for age, were the worst in the highest TG group irrespective of D5D activity [Table 6]. In addition, most markers except for SA and AA were worse in lower D5D activity (<5.39) than in those with ≥5.39 [Table 6].

Discussion

In this study, we showed that ELOVL6 and D5D activities are associated with IR. Most atherogenic markers were worse in the low ELOVL6 or D5D activity group than in the high ELOVL6 or D5D activity group. When study subjects were further stratified by TG levels, most atherogenic markers were the worst in the highest TG group in either the lowest ELOVL6 or lowest D5D activity group. We concluded that the estimated ELOVL6 and D5D activities might be useful markers of IR in Japanese subjects.

Table 2 Multiple linear regression analyses

(a) Elovl6					(c) D5D				
	RC	SRC	t	P		RC	SRC	t	P
WC	-0.03960	-0.22303	-3.89	0.0001	BMI	-0.17457	-0.28339	-4.86	< 0.0001
TG/HDL-C ratio	-0.27885	-0.25929	-4.52	< 0.0001	TG/HDL-C ratio	-0.21741	-0.17104	-2.93	0.0036
(b) Elovl6					(d) D5D				
WC	-0.13242	-0.02351	-2.24	0.0256	BMI	-0.14128	-0.22934	-3.81	0.0002
TyG index	-1.230408	-0.42948	-7.14	< 0.0001	TyG index	-0.86101	-0.25425	-4.22	< 0.0001
LDL-C	0.00635	0.12291	2.26	0.0246					

Variable selection was made by a stepwise procedure. Elovl6, elongation of long-chain fatty acids family member 6; RC, regression coefficient; SRC, standardized regression coefficient; WC, waist circumference; D5D, delta-5 desaturase; BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; TyG index, triglyceride-glucose index; LDL-C, low-density lipoprotein cholesterol

Table 3 Multiple logistic regression analyses

(a) Elovl6						(c) D5D					
	RC	SE	OR	95% CI	P		RC	SE	OR	95% CI	P
TG/HDL-C ratio	-0.4584	0.1566	0.632	0.465–0.859	0.0030	TG/HDL-C ratio	-0.4374	0.1619	0.646	0.470–0.887	0.0069
UA	-0.3327	0.11600	0.717	0.571–0.900	0.0041	BMI	-0.1457	0.0532	0.864	0.779–0.959	0.0062
(b) Elovl6						(d) D5D					
TyG index	-1.1860	0.28950	0.305	0.173–0.539	< 0.0001	TyG index	-1.1015	0.31040	0.332	0.181–0.611	0.0004
UA	-0.2918	0.1171	0.747	0.594–0.940	0.0127	HDL-C	0.0286	0.0114	1.029	1.006–1.052	0.0120

Variable selection was made by a stepwise procedure. Elovl6, elongation of long-chain fatty acids family member 6; D5D, delta-5 desaturase; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; RC, regression coefficient; SE, standard error; OR, odds ratio; CI, confidence interval; TyG index, triglyceride-glucose index; UA, uric acid; BMI, body mass index

The function of Elovl6 gene was mainly investigated by mouse models. Elolv6 has been shown to be a target of SREBP-1 by microarray analysis of SREBP-1 transgenic mice, and it was predicted to be important for tissue FA composition [22]. A high-fat, high-sucrose diet induced IR and hyperglycemia are improved by the deletion of the Elovl6 gene in mice, suggesting that Elovl6 is a determinant of insulin sensitivity [23]. The same group later reported that Elovl6 expression is positively correlated with the severity of hepatosteatosis and liver injury in nonalcoholic steatohepatitis (NASH) patients [24]. However, another study indicated that when mice were fed a high-fat diet or Elovl6 was deleted in ob/ob mice, the absence of Elovl6 did not alter the development of obesity, fatty liver, hyperglycemia, or hyperinsulinemia [25]. Consistent with these results, inhibition of Elovl6 activity by compounds changed tissue fatty acid compositions, but they did not improve IR in genetically obese and diabetic animal mice [26].

Our results suggested that estimated low Elovl6 activity was associated with high IR. This is probably due to a protective role in β-cell function when Elovl6 gene expression was reduced. Deletion of Elovl6 gene limits the elongation of palmitate to stearate, which instead allows palmitate to be desaturated to palmitoleate, a potentially less lipotoxic FA

in mice. This leads to the attenuation of palmitate-induced endoplasmic reticulum stress and apoptosis in pancreatic β-cells [27].

Although there are a few studies describing estimated Elovl6 activity and IR in humans, estimated Elovl6 activity was a significant predictor of IR in children aged 9–12 years [28]. Cofounding factors such as alcohol intake, physical activity, diet, and fatty liver were not considered; transaminases in our study subjects were higher in the lower Elovl6 or D5D groups (data not shown). Moreover, estimated Elovl6 was associated with high IR, when the estimated Elovl6 activity was low. Taken together, lower Elovl6 activity might be ideal for protection against atherosclerosis and NASH. Since information on how diet intake and lifestyle habits (i.e., exercise, physical activity, alcohol drinking, and smoking) affect Elovl6 activity is limited, it would be interesting to investigate their relationship in human studies.

In contrast to Elovl6, estimated D5D activity has been well studied in clinical studies. Our findings on the associations between estimated D5D activity and risk factors are consistent with the results of most previous studies, where high estimated D5D activity has been favorably associated with risk factors. Higher estimated D5D activity has been associated with lower LDL-C [29], higher HDL-C [30],

Table 4 Characteristics of study subjects stratified by Elovl6 or D5D activities

(a)

	Elovl6					
	< 3.85 (n = 95)		3.85- < 5.33 (n = 99)		≥ 5.33 (n = 97)	
Men/women (n)	69/26		63/36		44/53	
Age	56.0	± 14.5	54.2	± 14.4	52.9	± 12.8
BMI (kg/m^2)	23.6	± 3.2	22.3	± 3.9*	21.4	± 2.9**
Waist circumference (cm)	85.2	± 8.8	81.3	± 8.8**	78.3	± 8.5**,#
Systolic BP (mmHg)	126.1	± 15.7	122.4	± 16.3	117.8	± 16.2**
Diastolic BP (mmHg)	79.9	± 12.0	77.9	± 11.2	75.9	± 11.2
FPG (mg/dL)	98.6	± 9.4	96.8	± 11.8	95.2	± 14.6
TG (mg/dL)	122.0	[87.0,163.0]	77.0	[59.0,112.0]**	66.0	[47.0,90.0]**
TyG index	8.67	± 0.50	8.25	± 0.48**	8.04	± 0.53**,#
HDL-C (mg/dL)	59.8	± 15.4	63.4	± 14.4	69.6	± 13.3**,##
TG/HDL-C ratio	2.04	[1.26,3.30]	1.31	[0.89,1.90]**	0.89	[0.65,1.36]**
LDL-C (mg/dL)	120.0	± 33.3	120.7	± 29.8	117	± 31.1
UA (mg/dL)	6.1	± 1.2	5.6	± 1.3*	5.1	± 1.2**,##
SA (µg/mL)	210.9	± 44.4	196.6	± 36.8	200.4	± 75.1
PA (µg/mL)	70.9	± 21.9	43.8	± 9.2**	31.6	± 14.1**,##
Elovl6 activity	3.08	± 0.53	4.53	± 0.42**	6.54	± 1.19**,##
AA (µg/mL)	176.0	± 39.8	162.5	± 33.7*	154.2	± 38.1**
DGLA (µg/mL)	35.9	± 11.0	29.9	± 8.5**	25.5	± 8.0**,##
D5D activity	5.25	± 1.66	5.75	± 1.70	6.49	± 2.15**,#

(b)

	D5D					
	< 4.84 (n = 96)		4.84- < 6.24 (n = 97)		≥ 6.24 (n = 98)	
Men/women (n)	66/30		59/38		51/47	
Age	55.9	± 13.4	51.7	± 14.1	55.5	± 13.9
BMI (kg/m^2)	23.8	± 3.2	22.3	± 2.8**	21.3	± 2.8**
Waist circumference (cm)	85.7	± 8.8	80.7	± 8.4**	78.4	± 8.6**
Systolic BP (mmHg)	126.5	± 17.0	120.3	± 14.4*	119.5	± 16.7*
Diastolic BP (mmHg)	80.8	± 11.9	76.5	± 11.3*	76.4	± 11.0*
FPG (mg/dL)	99.5	± 14.4	96.1	± 10.2	95.0	± 11.3*
TG (mg/dL)	110.0	[74.5,151]	84.0	[58.0,117.0]**	67.5	[47.0,98.0]**
TyG index	8.60	± 0.52	8.28	± 0.52**	8.07	± 0.53**,#
HDL-C (mg/dL)	58.7	± 15.0	63.5	± 13.4	70.5	± 14.0**,##
TG/HDL-C ratio	1.93	[1.17,3.17]	1.31	[0.86,1.98]**	0.93	[0.65,1.53]**
LDL-C (mg/dL)	127.9	± 31.4	116.3	± 31.5*	114.0	± 29.5**
UA (mg/dL)	5.8	± 1.3	5.6	± 1.2	5.4	± 1.4
SA (µg/mL)	213.4	± 44.0	194.7	± 34.2	199.5	± 75.7
PA (µg/mL)	56.5	± 24.2	46.4	± 20.4**	43.1	± 21.6**
Elovl6 activity	4.24	± 1.40	4.76	± 1.71	5.2	± 1.62**
AA (µg/mL)	158.2	± 35.5	163.7	± 34.0	170.4	± 43.7
DGLA (µg/mL)	39.2	± 9.2	30.0	± 6.4**	22.2	± 6.3**,##
D5D activity	4.09	± 0.57	5.48	± 0.45**	7.89	± 1.73**,##

Variables are given as mean ± standard deviation or median [inter-quartile range]. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; TG, triglyceride; TyG index, triglyceride-glucose index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; SA, stearic acid; PA, palmitic acid; Elovl6, elongation of long-chain fatty acids family member 6; AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; D5D, delta-5 desaturase

(a) **p < 0.01, *p < 0.05 (< 3.85 vs 3.85- < 5.33, < 3.85vs ≥ 5.33), ##p < 0.01, #p < 0.05 (3.85- < 5.33 vs ≥ 5.33) by Bonferroni's multiple comparison test

(b) **p < 0.01, *p < 0.05 (< 4.84 vs 4.84- < 6.24, < 4.84 vs ≥ 6.24), ##p < 0.01, #p < 0.05 (4.84- < 6.24 vs ≥ 6.24) by Bonferroni's multiple comparison test

Table 5 Characteristics of study subjects stratified by Elovl6 activity and TG

TG (mg/dL)	Elovl6 < 4.55						Elovl6 ≥ 4.55					
	<67 (n=27)		67-<108 (n=45)		≥108 (n=73)		<67 (n=66)		67-<108 (n=55)		≥108 (n=25)	
Men/women (n)	13/14		29/16		60/13		25/41		30/25		19/6	
Age	53.3	±15.9	57.2	±12.9	53.3	±13.7	53.0	±15.3	54.4	±12.7	57	±13.0
BMI (kg/m^2)	20.9	±2.7	23.0	±3.4**	24.3	±2.5**	20.6	±2.8	22.2	±2.7**	22.9	±2.8**
Waist circumference (cm)	77.3	±8.3	84.2	±9.1**	86.6	±7.6**	75.1	±8.1	81.4	±7.8**	84.3	±7.5**
Systolic BP (mmHg)	119.8	±14.1	123.1	±18.0	126.3	±14.1	116.3	±18.2	121.6	±16.0	126.4	±13.9*
Diastolic BP (mmHg)	75.4	±9.0	78.3	±11.6	81.0	±11.6	74.7	±12.7	77.0	±10.8	80.8	±9.4
FPG (mg/dL)	94.3	±8.6	99.6	±10.1	99.3	±11.0	92.6	±14.5	96.2	±11.2	100.1	±14.2
TG (mg/dL)	52.0	[47.0,60.0]	84.0	[72.0,92.0]**	142.0	[123.0,195.0]**,##	47.0	[38.0,56.0]	83.0	[72.0,93.0]**	125.0	[113.0,152.0]**,##
TyG index	7.80	±0.21	8.34	±0.20**	8.94	±0.30**,##	7.65	±0.28	8.28	±0.19**	8.85	±0.40**,##
HDL-C (mg/dL)	72.9	±15.2	64.0	±13.4*	54.5	±11.6**,##	74.1	±13.1	64.2	±12.3**	58.6	±14.6**
TG/HDL-C ratio	0.73	[0.57,0.91]	1.33	[1.15,1.54]*	2.83	[2.06,3.85]**,##	0.66	[0.49,0.76]	1.28	[1.06,1.64]**	2.19	[1.85,3.16]**,##
LDL-C (mg/dL)	109.5	±33.1	121.0	±33.7	124.3	±33.1	107.5	±25.8	122.0	±27.1*	138.1	±28.0**,#
UA (mg/dL)	5.3	±1.4	6.0	±1.2*	6.1	±1.2**	4.9	±1.2	5.4	±1.2*	6.0	±1.0**
SA (μg/mL)	176.3	±34.9	187.7	±28.7	226.7	±42.8**,##	177.5	±27.4	200.3	±26.9	257.8	±127.9**,##
PA (μg/mL)	49.1	±12.0	52.3	±13.2	74.0	±23.2**,##	29.2	±6.5	35.5	±7.3	47.5	±22.8
Elovl6 activity	3.70	±0.68	3.70	±0.61	3.21	±0.65**,##	6.32	±1.43	5.80	±1.02	5.54	±0.99*
AA (μg/mL)	154.0	±36.4	164.5	±35.9	182.4	±38.2*,#	148.7	±32.3	159.5	±33.0	171.9	±47.7*
DGLA (μg/mL)	25.1	±6.4	31.0	±7.8*	39.3	±10.6**,##	23.4	±7.3	27.5	±6.8**	33.9	±8.1**,##
D5D activity	6.33	±1.51	5.64	±1.85	4.84	±1.20**,#	6.88	±2.45	6.03	±1.53	5.34	±1.73**

Variables are given as means ± standard deviations or median [inter-quartile range]. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; TG, triglyceride; TyG index, triglyceride-glucose index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; SA, stearic acid; PA, palmitic acid; Elovl6, elongation of long-chain fatty acids family member 6; AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; D5D, delta-5 desaturase

** $p < 0.01$, *$p < 0.05$ (TG < 67 vs 67-<108, < 67 vs ≥ 108), ##$p < 0.01$, #$p < 0.05$ (TG 67-< 108 vs ≥ 108) by Bonferroni's multiple comparison test

Table 6 Characteristics of study subjects stratified by D5D activity and TG

	D5D < 5.39						D5D ≥ 5.39					
TG (mg/dL)	<67		67-<108		≥108		<67		67-<108		≥108	
	(n=27)		(n=50)		(n=68)		(n=66)		(n=50)		(n=30)	
Men/women (n)	11/16		31/19		58/10		27/39		28/22		21/9	
Age	53.7	±17.2	54.9	±12.8	54.9	±13.1	52.8	±14.7	56.4	±12.8	52.9	±14.6
BMI (kg/m^2)	21.1	±3.0	23.7	±3.1**	24.3	±2.6**	20.5	±2.7	21.5	±2.5	23.1	±2.6**,#
Waist circumference (cm)	78.0	±7.7	84.7	±8.8**	87.2	±7.8**	74.8	±8.2	80.6	±7.6**	83.4	±6.5**
Systolic BP (mmHg)	117.8	±17.3	124.6	±17.4	128.4	±13.7*	117.0	±17.2	120.0	±16.1	121.0	±13.4
Diastolic BP (mmHg)	74.2	±10.8	79.3	±11.5	82.3	±11.2**	75.2	±12.2	76.0	±10.7	77.5	±10.1
FPG (mg/dL)	94.0	±15.9	97.9	±11.3	99.8	±12.2	92.7	±11.8	97.6	±10.5	98.8	±11.2*
TG (mg/dL)	52.0	[44.0,59.0]	87.5	[73.0,94.0]**	142.5	[123.0,197.5]**,##	48.0	[42.0,56.0]	81.0	[72.0,92.0]**	124.5	[115.0,155.0]**,##
TyG index	7.76	±0.21	8.32	±0.20**	8.95	±0.30**,##	7.67	±0.28	8.29	±0.19**	8.86	±0.39**,##
HDL-C (mg/dL)	71.4	±14.7	61.3	±13.1**	53.7	±11.7**,##	74.7	±13.2	66.9	±11.8**	59.6	±13.3**,#
TG/HDL-C ratio	0.73	[0.51,0.93]	1.36	[1.14,1.77]*	2.84	[2.10,4.11]**,##	0.66	[0.53,0.76]	1.24	[1.03,1.53]*	2.22	[1.79,3.25]**,##
LDL-C (mg/dL)	114.3	±23.0	120.7	±30.9	129.5	±34.4	105.5	±29.5	122.5	±30.4**	124.0	±27.0*
UA (mg/dL)	4.9	±1.3	5.8	±1.2**	6.2	±1.1**	5.0	±1.2	5.6	±1.3	5.7	±1.2*
SA (µg/mL)	177.5	±21.7	187.6	±28.5	232.8	±38.5**,##	177.0	±32.4	201.7	±26.6	238.8	±123.5**,#
PA (µg/mL)	36.9	±14.2	43.4	±13.2	68.5	±25.2**,##	34.2	±11.6	42.7	±13.6*	64.3	±27.0**,##
Elovl6 activity	5.47	±2.08	4.65	±1.35	3.74	±1.21**,##	5.59	±1.58	5.07	±1.33	3.97	±1.37**,##
AA (µg/mL)	143.9	±22.5	147.3	±24.5	173.4	±37.2**,##	152.8	±36.8	176.2	±36.8**	194.0	±45.4**
DGLA (µg/mL)	30.9	±4.3	33.0	±6.5	41.5	±9.3**,##	21.1	±5.8	25.2	±6.2**	29.8	±7.1**,##
D5D activity	4.69	±0.55	4.53	±0.64	4.25	±0.70*	7.55	±2.11	7.18	±1.33	6.62	±1.01*

Variables are given as means ± standard deviations or median [inter-quartile range]. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; TG, triglyceride; TyG index, triglyceride-glucose index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; SA, stearic acid; PA, palmitic acid; Elovl6, elongation of long-chain fatty acids family member 6; AA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; D5D,delta-5 desaturase

** $p < 0.01$, * $p < 0.05$ (TG <67 vs 67-<108, <67 vs ≥108, 67-<108 vs ≥108), ## $p < 0.01$, # $p < 0.05$ (TG 67-<108 vs ≥108) by Bonferroni's multiple comparison test

lower blood pressure [31, 32], lower BMI [30, 33–35], and lower HOMA-IR [36]. *FADS1*, which encodes D5D, polymorphisms have been shown to associate, for example, with serum lipid levels and glucose metabolism [37], adding more evidence for the impact of D5D activity on these risk factors. Estimated D5D activity was independently associated with HOMA-IR in Japanese patients with type 2 diabetes [38]. In prospective cohort studies, D5D activity has been reported to be associated with incident type 2 diabetes [7, 11]. Not only estimated D5D activity, but also decreased D5D activity in obese patients who underwent subtotal gastrectomy was reported. In this study, 5D activity was measured using liver samples and negatively correlated with IR [39]. The linking low D5D activity to high IR may be due to an underlying inflammation, since previous studies indicated that low D5D activity were associated with high serum concentration of C-reactive protein [40] and has been associated with markers of IR and type 2 diabetes [41].

Knockdown of mouse *Fads1* resulted in a striking reorganization of both ω-6 and ω-3 polyunsaturated FA levels and their associated pro-inflammatory and pro-resolving lipid mediators in a highly diet-specific manner [42]. Therefore, it is possible that different amounts of precursor ω-6 or ω-3 FA intake can influence the harmony of specialized pro-resolving mediators. This may lead to differential phenotypic response to *Fads1* deletion. For this reason, information on dietary food and estimated FA intake should be considered for D5D activity in future studies.

Conditions such as obesity, IR as well as nonalcoholic fatty liver disease, a de novo lipogenesis has been found markedly induced, heavily contributing to liver fat deposition and changes in FA composition [43], resulting in disrupted homeostatic control of FA tissue concentrations [44]. Thus, it would be interesting to investigate whether estimated Elovl6 and D5D activities are associated with serum concentrations of transaminases and liver fat deposition in the future study.

This study has several limitations. Desaturase activities are commonly estimated from phospholipid or cholesterol ester FAs, not from whole serum FAs. However, the direct measurement of enzyme activity is not realistic in clinical settings. D5D activity estimated from whole serum has been shown to be strongly associated with a known intron variant of the *FADS1* gene, which provides indirect validation for the use of whole serum FAs as well to estimate desaturase activity [45]. The cross-sectional design of this study was its major limitation, as it hindered the determination of a causal relationship between Elovl6, D5D activity, and IR. The data regarding fasting immunoreactive insulin levels are not available in this study, and therefore, the IR measured by TG/HDL-C and TyG index was not compared with the HOMA-IR. In addition, information on dietary FA intake was not considered. All the participants in this study were

middle-aged and Japanese; thus, we were not able to determine whether the relationship between the Elovl6 and D5D activities and clinical markers reported here was affected by ethnicity. Finally, our dataset was small, and our findings may not apply to all Japanese individuals.

Conclusions

Our results indicate that the estimated Elovl6 and D5D activities might be useful markers of insulin resistance in Japanese adults.

Declarations

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