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ORIGINAL ARTICLE

Change in fatty acid composition of plasma triglyceride caused by a 2 week comprehensive risk management for diabetes: A prospective observational study of type 2 diabetes patients with supercritical fluid chromatography/mass spectrometry-based semi-target lipidomic analysis

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Keywords
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ABSTRACT

Aims/Introduction: Hypertriglyceridemia is common in patients with diabetes. Although the fatty acid (FA) composition of triglycerides (TGs) is suggested to be related to the pathology of diabetes and its complications, changes in the fatty acid composition caused by diabetes treatment remain unclear. This study aimed to identify short-term changes in the fatty acid composition of plasma triglycerides after diabetes treatment.

Materials and Methods: This study was a sub-analysis of a prospective observational study of patients with type 2 diabetes aged between 20 and 75 years who were hospitalized to improve glycemic control (n = 31). A lipidomic analysis of plasma samples on the 2nd and 16th hospital days was conducted by supercritical fluid chromatography coupled with mass spectrometry.

Results: In total, 104 types of triglycerides with different compositions were identified. Most of them tended to decrease after treatment. In particular, triglycerides with a lower carbon number and fewer double bonds showed a relatively larger reduction. The inclusion of FA 14:0 (myristic acid), as a constituent of triglyceride, was significantly associated with a more than 50%, and statistically significant, reduction (odds ratio 39.0; $P < 0.001$). The total amount of FA 14:0 as a constituent of triglycerides also decreased significantly, and its rate of decrease was the greatest of all the fatty acid constituents.

Conclusions: A 2 week comprehensive risk management for diabetes resulted in decreased levels of plasma triglycerides and a change in the fatty acid composition of triglycerides, characterized by a relatively large reduction in FA 14:0 as a constituent of triglycerides.

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INTRODUCTION

Diabetes mellitus, caused by a relative or absolute deficiency of insulin, is a group of chronic metabolic disorders characterized by hyperglycemia. Patients with diabetes also develop a variety of metabolic abnormalities, such as amino acid and lipid metabolic disorders, in addition to hyperglycemia. Hypertriglyceridemia and low HDL cholesterol levels are common in patients with diabetes. Triglycerides (TGs) comprise three fatty acid (FA) molecules with highly diverse possible FA chains. Triglycerides with different fatty acid compositions in blood are reported to have varied degrees of association with incident type 2 diabetes[1,2]. Plasma TG(54:2), triglyceride with 54 acyl chain carbons and two double bonds, is associated with future cardiovascular disease[3], which is one of the major causes of mortality in patients with type 2 diabetes. These findings suggest that the fatty acid composition of plasma triglycerides is associated with the pathology of diabetes and its complications. However, the effect of diabetes treatment on the fatty acid composition remains unclear.

Intake of myristic acid, a long-chain saturated FA (14:0), was shown to be associated with a decreased risk of incident type 2 diabetes[4]. In contrast, another study reported that serum non-esterified FA 14:0 was positively associated with the prevalence and incidence of type 2 diabetes[5], and a recent meta-analysis of the association between circulating saturated fatty acids (plasma phospholipids, erythrocyte membranes fraction, or esterified or non-esterified fatty acids in serum or whole blood) and incident type 2 diabetes revealed that circulating FA 14:0 was associated with an increased risk of incident type 2 diabetes[6]. However, the association between FA 14:0 as a constituent of triglycerides and the incidence or prevalence of type 2 diabetes remains unknown.

In most of the previous clinical lipidomic studies, triglycerides were grouped by total acyl chain carbon number and the total number of double bonds (double bond content) without identifying the constituent fatty acids. However, triglycerides with different fatty acid compositions can have the same total acyl chain carbon number and double bond content. For example, both TG(14:0/16:1/18:0) and TG(16:0/16:0/16:1) have 48 acyl chain carbons and one double bond (labeled TG(48:1)). Lee, Bamba *et al.*[7] reported that each triglyceride was identified successfully by supercritical fluid chromatography coupled with mass spectrometry (SFC/MS); triglycerides with different fatty acid compositions were effectively separated.

In the present study, we aimed to identify short-term changes in the fatty acid composition of plasma triglycerides, including FA 14:0 as a constituent of triglycerides, following comprehensive risk management for diabetes using SFC/MS-based semi-target lipidomic techniques.

MATERIALS AND METHODS

Study design

The present study was a sub-analysis of a prospective observational study of patients with diabetes who were hospitalized because of problems with glycemic control to investigate short-term changes in metabolism, as described previously[8]. A lipidomic analysis of plasma samples on the 2nd and 16th hospital days with SFC/MS was conducted, and the fatty acid composition of triglycerides was compared between these 2 days. This study was approved by the Ethics Committee of Osaka University Hospital, Japan (approval number: 16374) and was conducted in accordance with the principles of the Declaration of Helsinki and current legal regulations in Japan.

Study population

Subjects were randomly recruited among patients with type 2 diabetes who were admitted to Osaka University Hospital to improve glycemic control and whose ages were between 20 and 75 years. Patients who did not receive intensive glycemic control due to severe retinopathy; patients with serum creatinine >176.80 μmoL/L (2.0 mg/dL), severe infection, or trauma; and patients in the pre- and postoperative periods were excluded. All participants were recruited between 2017 and 2019 and provided written informed consent after a full explanation of the study. Overall, 33 patients with diabetes were enrolled in this study[8].

Study protocol

A medical history was obtained from all participants. Participants received comprehensive risk management for diabetes, including intensive glycemic control, as well as blood pressure, dyslipidemia, and body weight control, in the hospital, according to the Japanese treatment guideline for diabetes[9]. Blood samples were collected in the morning after fasting for about 15 h, and vital signs and weight were measured on the 2nd and 16th hospital day (hereinafter referred to as the pre-treatment and post-treatment periods, respectively).

For lipidomic analysis, portions of the blood samples were cooled in a freezer at 4°C immediately after collection. The samples were then centrifuged (3000 *g*, 10 min), and the plasma was stored at −80°C within 4 h.

Biochemical measurement

Serum triglyceride levels were measured using enzymatic assays. Estimated glomerular filtration rate was calculated according to the Statement of the Japanese Society of Nephrology[10].

Lipidomic measurement

Plasma samples were prepared for lipid extraction using the Bligh and Dyer method[11], with minor modifications. Lipids were extracted from plasma (30 μL) with 470 μL of methanol containing 0.70 nmol TG 15:0/18:1 (d_7)/15:0 as the internal standard. The samples were then vigorously mixed for 1 min and centrifuged at 16,000 *g* for 5 min at 4°C. The resultant supernatant (400 μL) was collected. After mixing with chloroform (400 μL) and water (224 μL), the aqueous and organic layers were separated by mixing and centrifuging at 16,000 *g* at 4°C for 5 min. The organic layer (bottom, 300 μL), obtained by phase separation, was dried under a nitrogen stream, then stored at −80°C until analysis. Prior to the analysis, the dried

sample was reconstituted in methanol/chloroform (1/1, v/v, 200 μL).

The analytical conditions for supercritical fluid chromatography tandem mass spectrometry (SFC/MS/MS) analysis were performed as described previously[12,13]. The SFC (Nexera UC system, Shimadzu, Japan) conditions were as follows: column, Acquity UPC^2 HSS C18 SB column (3.0 mm i.d. × 100 mm, 1.7 μm particle size, Waters); injection volume, 2 μL; column temperature, 50°C; mobile phase A, supercritical carbon dioxide; mobile phase B (modifier) and make-up pump solvent; methanol/water (95/5, v/v) with 0.1% (w/v) ammonium acetate; flow rate of mobile phase, 1.0 mL/min; flow rate of make-up pump, 0.1 mL/min; and back pressure regulator, 10 MPa. The gradient conditions were as follows: 0–50% B, 0–25 min; 50% B, 25–28 min; and 0% B, 28.1–30 min. The triple quadrupole mass spectrometer (TQMS, LCMS-8060, Shimadzu, Japan) analysis conditions were as follows: polarity, positive and negative ionization; electrospray voltage, 4 kV in the positive ion mode for triglyceride analysis and −3.5 kV in the negative ion mode for FAs analysis; nebulizer gas flow rate, 3.0 L/min; drying gas flow rate, 10.0 L/min; desolvation line temperature, 250 °C; heat block temperature, 400 °C; and detector voltage, 2.16 kV. The multiple reaction monitoring (MRM) parameters per time period were as follows: limit on the number of MRM transitions, 150; dwell time, 2 ms; and pause time, 2 ms. Data processing was performed using LabSolution software version 5.99 SP2 (Shimadzu, Kyoto, Japan).

A reference sample was prepared by mixing equal amounts (10 μL each) of plasma extracts. The 20 FA constituents (i.e., FA 14:0, FA 16:0, FA 16:1, FA 18:0, FA 18:1, FA 18:2, FA 20:0, FA 20:1, FA 20:2, FA20:3, FA 20:4, FA 20:5, FA 22:0, FA 22:1, FA 22:4, FA 22:5, FA 22:6, FA 24:0, FA 26:0, and FA 28:0) of the triglycerides in plasma were determined by SFC/MS/MS analysis of the hydrolyzed reference sample[12]. To determine the target triglyceride molecules in plasma, a reference sample was analyzed using the in-house MRM library for 1,540 targeted triglyceride molecules[14]. Targeted quantitative analysis of 104 selected triglycerides in all the samples was performed using the selected MRM method[13,14].

Validation for this SFC/MS method is shown in Table S1 and Figure S1, indicating good repeatability and accuracy.

Statistical analysis

Clinical data are expressed as mean and standard deviation if normally distributed, or as median and interquartile range if log-normally distributed. Categorical variables are expressed as counts and percentages. Statistical significance was set at $P < 0.05$. Paired *t*-tests were used to compare data between pre- and post-treatment periods. If the variables were log-normally distributed, tests were used after log-transformation.

To investigate the overview of qualitative changes in triglyceride, we calculated the fold-change value (the median of the ratios between post-treatment value and pre-treatment value) of each triglyceride and assessed the association of the fold-change value with total acyl chain carbon number and double bond content (total number of double bonds).

To compare the fatty acid composition of triglycerides between the pre- and post-treatment periods, triglyceride values were compared using paired *t*-tests after log-transformation. To account for multiple testing, we used the Benjamini-Hochberg method; that is, we calculated the adjusted *P*-values (*q*-values) with the significance level set at 0.05. A volcano plot was constructed using the *q*-values and fold-change values. We then selected the triglycerides with significantly different changes upon paired *t*-tests and whose fold-change values were not above 50% nor below 150%.

Next, we calculated the value of each fatty acid as a constituent of triglycerides by summing up the products of each triglyceride value and the number of fatty acids that constitute the triglyceride. We then compared the values of each fatty acid as a constituent of triglyceride between the pre- and post-treatment periods, similar to each triglyceride value. Moreover, the same comparison was performed among the obese and non-obese patients.

Finally, Pearson's correlations between the differences of fatty acids as constituents of triglyceride between pre- and post-treatment and those in clinical factors were analyzed, for the purpose of evaluating associations between the changes in fatty acids as constituents of triglyceride and the clinical factors. In addition to fatty acids as constituents of triglyceride, we evaluated the associations of total triglyceride, which were calculated by summing all triglycerides.

RESULTS

Effect of comprehensive risk management for diabetes on clinical parameters

Two patients withdrew consent after participation; therefore, 31 patients were included in the final analysis. The baseline clinical characteristics of the study participants have been described previously[8]. Briefly, the age of the participants was 63.9 ± 10.5 (mean ± standard deviation) years, 41.9% of the participants were men, glycated hemoglobin (HbA1c) was 9.1 ± 2.1%, and the body mass index (BMI) was 26.7 ± 5.1 kg/m^2. Most of the patients were treated with insulin (77.4%) or biguanide (64.5%) after admission, and insulin users were increased (Table S2). Comprehensive risk management for diabetes resulted in a statistically significant reduction in fasting plasma glucose, HbA1c, body weight, aspartate aminotransferase, γ-glutamyl transpeptidase, highly sensitive C-reactive protein, total cholesterol, low-density lipoprotein-cholesterol, and high-density lipoprotein-cholesterol levels. Serum triglyceride levels, measured by the enzymatic assay, also significantly decreased from 1.41 (0.92–2.59) to 1.06 (0.79–1.45) mmol/L, as reported previously[8] (median with interquartile range).

Change in triglycerides assessed by lipidomic measurement

All 104 targeted triglycerides with different fatty acids compositions were identified using SFC/MS (Table S3). We identified

an association between the fold-change value in triglycerides and their total acyl chain carbon number ($r = 0.762$, $P < 0.001$; Figure 1a) and double bond content ($r = 0.474$, $P < 0.001$; Figure 1b). In other words, triglycerides with relatively lower carbon numbers and fewer double bonds decreased more drastically after treatment.

As shown in Figure 2, paired *t*-tests comparing the triglyceride values before and after treatment showed that most of the identified triglycerides tended to decrease after treatment. Among them, a total of 14 triglycerides showed a statistically significant decrease and more than a 50% reduction (dark blue points in Figure 2). Among them, 12 triglycerides (85.7% of them) had FA 14:0 as a constituent, and seven triglycerides (50% of them) had FA 16:0. On the other hand, among the other 90 triglycerides (light blue points in Figure 2), which did not show drastic reduction after treatment, only 12 triglycerides (13.3%) had FA 14:0. Notably, the inclusion of FA 14:0 as a constituent was significantly associated with statistically significant and more than 50% reduction in triglycerides (odds ratio 39.0; $P < 0.001$). In contrast, the inclusion of FA 16:0 was not (odds ratio 1.31; $P = 0.640$).

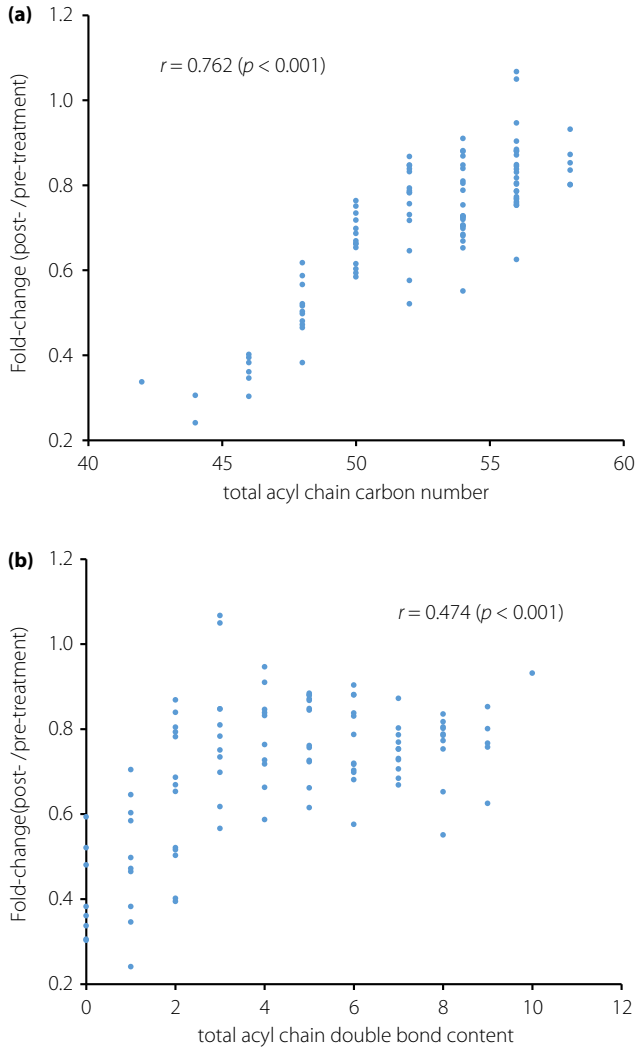

Figure 1 | Associations between the fold-change values (the medians of ratios between post-treatment value/pre-treatment value) in triglycerides (TGs), and (a) total acyl chain carbon number and (b) double bond content. The Spearman's rank correlations were used to calculate correlation coefficient *r*.

As shown in Figure 3, comparing the values of each fatty acid as a constituent of triglycerides between the pre- and post-treatment periods showed that FA 14:0 significantly decreased, and its decrease rate was the largest among all the fatty acid constituents. Other fatty acids in the *de novo* lipogenesis (DNL) pathway, such as FA 16:0, FA 18:0, and FA 16:1, also showed relatively large decrease rates. These DNL-related fatty acids showed a statistically significant decrease among the obese patients, while their decrease was not significant among the non-obese patients (Figure 4).

Associations between delta changes in fatty acids as constituents of triglycerides before and after treatment and those in clinical parameters are shown in Table 1. Changes in FA 16:0, FA 16:1, FA 18:0, FA 18:1, and FA 18:2 as constituents of triglycerides had a moderate association with those in HbA1c levels, while those in FA 14:0 had no statistically significant association.

DISCUSSION

In the present study, triglycerides with relatively lower carbon numbers and fewer double bonds (especially triglycerides with 42–48 total carbon numbers or 0–2 total double bonds) showed a larger reduction after comprehensive risk management for diabetes. This finding is consistent with that of previous studies. Schwab *et al.*[15] examined alterations in the fatty acid composition of plasma triglyceride induced by body weight reduction after diet therapy in nine individuals and showed that plasma triglycerides with fewer carbon numbers and those with lower double bond content had a relatively larger decrease after a 33 week intervention. Further, a decrease in plasma triglycerides containing relatively short-chain saturated fatty acids was correlated with increased insulin sensitivity[15]. Similarly, triglycerides with lower carbon number and lower double bond content strongly correlated with insulin resistance and an increased risk of diabetes, compared with triglyceride with a higher carbon number and a higher double bond content[16,17].

However, in these previous clinical lipidomic studies, triglycerides were grouped by the total acyl chain carbon number and the total number of double bonds (double bond content) without assessing fatty acid constituents. One of the strengths of the present study is that we identified triglycerides with different fatty acid compositions separately using SFC/MS-based semi-target lipidomic techniques[4] and assessed the change in each triglyceride separately.

The present study showed a relatively large reduction of FA 14:0, FA 16:0, FA 16:1, and FA 18:0 as constituents of triglycerides. Since these fatty acids belong to the DNL pathway, the

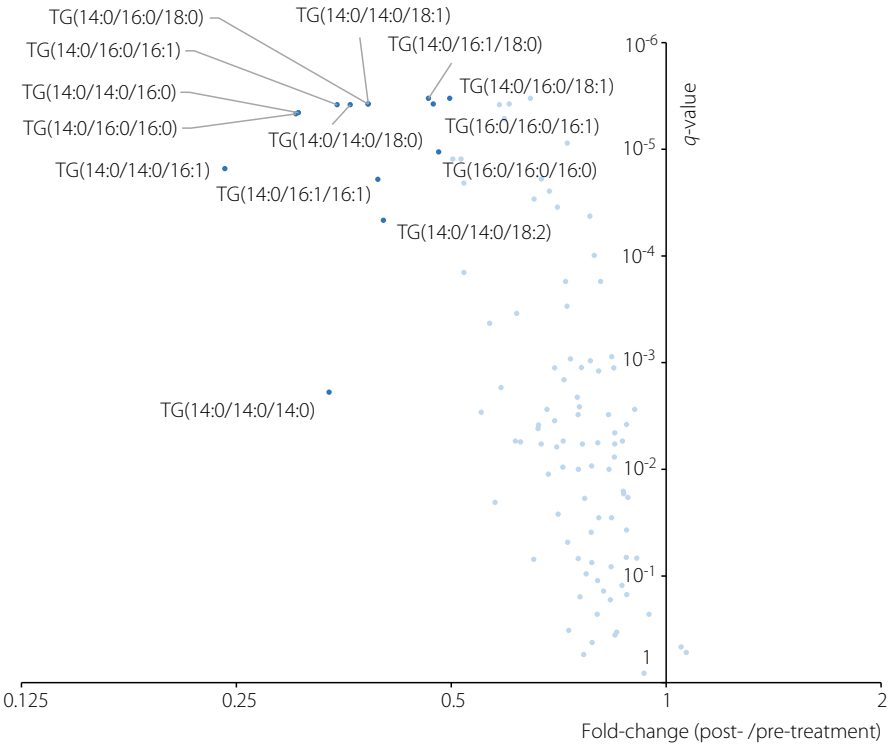

Figure 2 | Volcano plot showing the results of the paired *t*-tests comparing triglycerides (TG) values of the pre- and post-treatment. The *x*-axis represents the fold-change values (the medians of ratios between post-treatment value/pre-treatment value), whereas the *y*-axis represents the *q*-values (*P*-values adjusted using the Benjamini–Hochberg method). Dark blue points represent the TGs with a significant difference (*q*-value ≦ 0.05) and 50% change (fold-change≧1.5 or ≦0.5). Light blue points represent the other TGs.

larger reduction of triglycerides with relatively lower carbon numbers and fewer double bonds might be related to DNL. Notably, the decrease in these DNL-related fatty acids was statistically significant among the obese patients only. The lack of significance in the non-obese patients could be due to their small sample size, but obese patients tended to have a larger decrease rate of these triglycerides compared with the non-obese patients. In obese individuals, hepatic DNL is reported to contribute substantially to intrahepatic triglyceride than in lean individuals[18]. Weight reduction can improve peripheral insulin resistance, thus reducing circulating insulin. In effect, reduced circulating insulin decreases hepatic DNL, which might have contributed to the larger reduction of triglycerides with relatively lower carbon numbers and fewer double bonds, especially in the obese patients. However, the influence of anti-diabetic medications on the circulating insulin in the present study is unclear since the change of circulating insulin depends on the class of medications. Given the small sample size, we could not assess the associations between the class of medications and the change in the fatty acid composition of plasma triglycerides.

The present study also revealed that triglycerides containing FA 14:0 (myristic acid) as a constituent were drastically reduced after comprehensive risk management for diabetes. This finding is consistent with those of previous studies. For example, Schwab *et al.*[15] also reported that plasma levels of esterified FA 14:0 showed a relatively larger reduction after body weight reduction compared with that of other esterified fatty acids. Similarly, Guerendiain *et al.*[19] reported that body weight reduction induced a decrease in FA 14:0 in overweight adolescents and that subjects with greater weight reduction displayed a significant decrease in FA 14:0. They also revealed that a decrease in FA 14:0 is associated with a reduction in insulin levels[19]. While the decrease in most of the major fatty acid constituents of triglycerides was associated with improvement of glycemic control in the present study, that in FA 14:0 was not. Although the association between changes in FA 14:0 and that in the BMI was not statistically significant in the present study, this might be due to our small sample size. Considering the results of these previous studies, the decrease in FA 14:0 as a constituent of triglycerides may have been associated with body weight change. One possible mechanism is the involvement of adiponectin. A previous cross-sectional study revealed a negative association between serum adiponectin and FA 14:0 in serum phospholipids[20]. However, other clinical studies reported no associations between serum adiponectin and FA 14:0 derived from erythrocyte membrane[21], plasma esterified FA[22,23], and adipose tissue[23]. In the present study, no association was observed between the change of serum adiponectin and that of

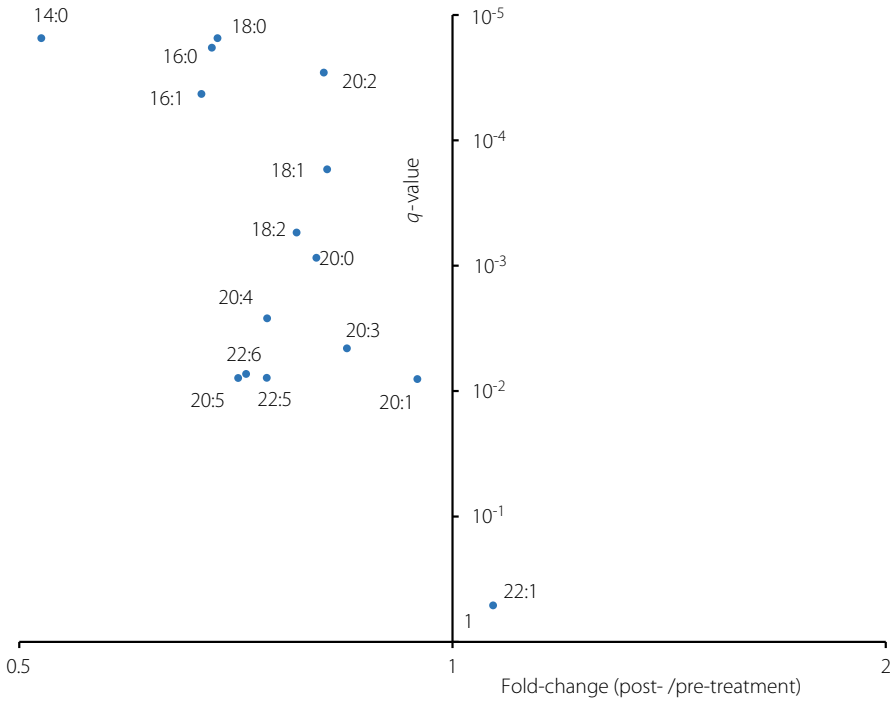

Figure 3 | Volcano plot showing the results of the paired *t*-tests comparing the values of each fatty acid as a constituent of triglycerides (TG) between the pre- and post-treatment period. The *x*-axis represents the fold-change values (the medians of ratios between post-treatment value/pre-treatment value), whereas the *y*-axis represents the *q*-values (adjusted *P*-values using the Benjamini–Hochberg method).

FA 14:0 as a constituent of triglycerides. Therefore, the association between weight reduction and a decrease in FA 14:0 as a constituent of triglycerides might not be adiponectin-mediated.

Because blood samples were collected under fasting conditions, plasma triglycerides measured in this study were mainly derived from very low-density lipoprotein particles, and thus were considered to reflect liver fat content. Therefore, it is possible that adipose tissue lipolysis, DNL in the liver, and the effect of diet changed the fatty acid composition of plasma triglycerides, although the mechanism underlying the relatively large decrease in FA 14:0 as a constituent of triglycerides remains unclear.

Lipolysis of adipose tissue is a major source of fatty acids stored in the liver. Insulin suppresses adipose tissue lipolysis. A previous study investigating the changes in individual free fatty acids during an oral glucose tolerance test indicated a remarkable decrease in saturated and monounsaturated fatty acids and a relative increase in polyunsaturated fatty acids[24]. Accordingly, the degree of suppression of adipose tissue lipolysis by insulin may differ among fatty acid species. The increased insulin action in adipose tissue due to anti-diabetic medications or weight reduction can suppress adipose tissue lipolysis, and the difference in the degrees of suppression among fatty acids may have caused the change in the fatty acid composition of triglycerides.

Fatty acid synthesis in the liver can also affect the fatty acid composition of triglycerides. Key enzymes involved in the biosynthesis of fatty acids with relatively lower carbon number (14–18) and those with fewer double bonds (0–1) are fatty acid synthase (FAS), elongation of long-chain fatty acid family member 6 (Elovl6), and stearoyl-CoA desaturase 1 (SCD1), all of which are regulated by sterol regulatory element-binding protein-1c (SREBP-1c)[25–27]. Acetyl-CoA is mainly converted to FA 16:0 (palmitic acid) by FAS, followed by elongation and desaturation. The elongation and desaturation enzymes are Elovl6 and SCD1, respectively. Fatty acid synthase also produces minor amounts of FA 14:0[28], which are converted to FA 16:0 by Elovl6. As mentioned earlier, weight reduction seems to have decreased the circulating insulin, and anti-diabetic medications might also have changed it in the present study. Since insulin stimulates these FA-synthesis-related enzymes *via* SREBP-1c[25], the subsequent change of circulating insulin can affect the fatty acid composition of triglycerides.

Dietary intake affects the fatty acid composition of blood triglycerides and other lipid classes[29]. Although high-fat dairy products contain relatively high amounts of myristic acid, dietary fat from other sources contains only low amounts. In the present study, the influence of dietary fat intake on the change in FA 14:0 in triglyceride was unclear, since we have no information on the intake of high-fat dairy products.

FA 14:0 has both negative and positive effects on mammals. FA 14:0 can predict NASH[30] and potentiate FA 16:0-induced lipotoxicity in hepatocytes[31]. In contrast, it was also shown to

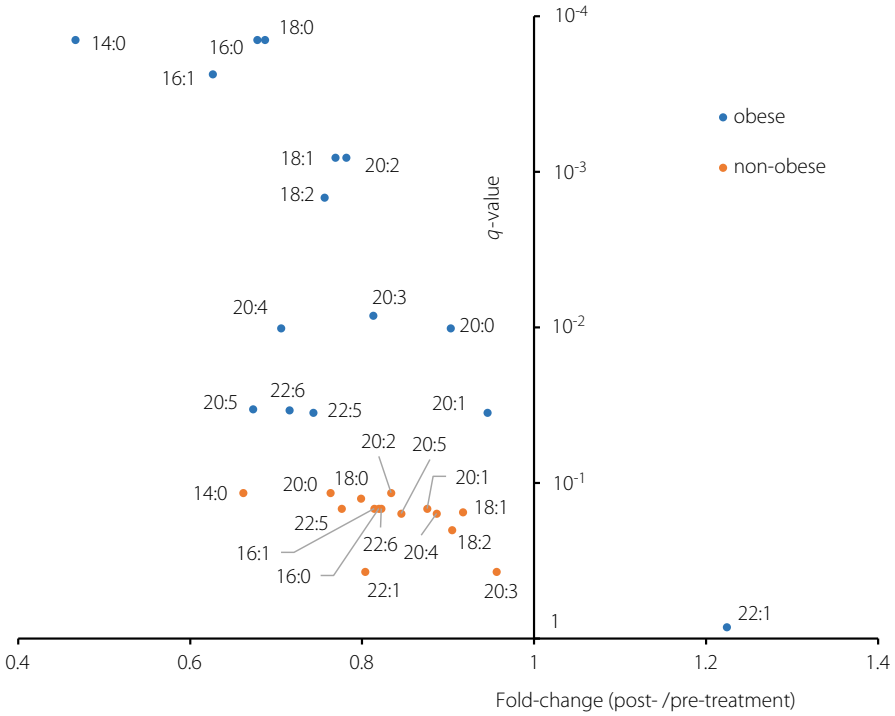

Figure 4 | Comparison of the results of the paired *t*-tests comparing the values of each fatty acid as a constituent of triglyceride (TG) between the pre- and post-treatment period among the obese patients (body mass index [BMI] ≥ 25; *n* = 21) with those among the non-obese patients (BMI < 25; *n* = 10). The *x*-axis represents the fold-change values (the medians of ratios of post-treatment value/pre-treatment value), whereas the *y*-axis represents the *q*-values (adjusted *P*-values using the Benjamini–Hochberg method).

Table 1 | Associations between the changes in fatty acids as constituents of triglycerides and clinical factors

	ΔBMI	ΔAST	ΔALT	Δγ-GTP	ΔFPG	ΔHbA1c	ΔADN
ΔFA 14:0	0.197	0.287	0.174	0.222	0.119	0.122	0.048
ΔFA 16:0	0.138	0.207	0.029	0.252	0.266	0.486*	−0.233
ΔFA 16:1	0.114	0.267	0.106	0.204	0.206	0.358*	−0.071
ΔFA 18:0	0.126	0.250	0.021	0.225	0.241	0.489*	−0.215
ΔFA 18:1	0.028	0.165	0.020	0.255	0.290	0.494*	−0.218
ΔFA 18:2	0.035	0.056	−0.001	0.306	0.325	0.496*	−0.266
Δtotal TG	0.093	0.180	0.032	0.266	0.276	0.471*	−0.213

Pearson's correlation coefficients between the differences in FAs as constituents of TGs before and after treatment and those of clinical factors are presented. The major FAs that constitute triglycerides and total triglyceride are shown in the first column, and major clinical factors are shown in the first row. *$P < 0.05$. ADN, adiponectin; ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; FA, fatty acid; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; TG, triglyceride; γ-GTP, γ-glutamyl transpeptidase.

enhance diacylglycerol kinase δ-dependent glucose uptake in a myotube model[32]. Furthermore, chronic administration of FA 14:0 improved hyperglycemia and insulin resistance in skeletal muscles in an animal model of type 2 diabetes[33]. The fatty acid composition of plasma triglycerides reflects liver fat content, as well as fatty acid transport to peripheral tissue. A relatively large decrease in FA 14:0 as a constituent of triglycerides following comprehensive risk management for diabetes could lead to attenuation of the noxious effect of FA 14:0 on the liver, as well as its beneficial effect on skeletal muscle. However, the beneficial insulin resistance amelioration effect of FA 14:0 on skeletal muscle may not necessarily be required once glycemic control improves. Therefore, a large decrease in FA 14:0 as a constituent of triglycerides after comprehensive risk management for diabetes mainly reflects its beneficial effect on the whole body.

The present study has several limitations. First, there is no detailed patient information about lifestyle-related factors, such

as diet, physical activity, and alcohol consumption. These factors have a significant influence on plasma lipid levels. We also did not have detailed information about the fatty acid composition of the diet given to patients during their hospital stay, although the amount and composition of major nutrients in the food portions given to patients at the hospital were determined by the attending physician according to the patients' respective physical data and diabetes complications. Second, the present study was a pilot study to discover potentially promising hypotheses; the sample size was too small to draw a definite conclusion. Therefore, further studies with larger sample sizes are warranted. Third, we could not assess the change of circulating insulin level, as the majority of the study participants were treated with insulin.

In conclusion, most plasma triglycerides decreased after a 2 week comprehensive risk management for diabetes. Furthermore, FA 14:0 as a constituent of triglycerides showed the largest reduction among the detected fatty acid constituents.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The research protocol was approved by the Ethics Committee of Osaka University Hospital.

Informed consent: Written informed consent was obtained from all the participants after a full explanation of the study.

Registry and the registration no. of the study/trial: 1 May 2017, No. 16374.

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 | Intra- and inter-day variation in the concentrations of 104 triglycerides (TGs) detected in human plasma

Table S1 | Spike-and-recovery test for determination of triglycerides (TGs)

Table S2 | Anti-diabetic medications before and during the comprehensive risk management for diabetes

Table S3 | Changes in all 104 identified triglycerides (TGs) after comprehensive risk management for diabetes