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JTT-553, a novel Acyl CoA:diacylglycerol acyltransferase (DGAT) 1 inhibitor, improves glucose metabolism in diet-induced obesity and genetic T2DM mice

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ARTICLE INFO

ABSTRACT

Type 2 diabetes mellitus (T2DM) arises primarily due to lifestyle factors and genetics. A number of lifestyle factors are known to be important in the development of T2DM, including obesity. JTT-553, a novel Acyl CoA:diacylglycerol acyltransferase 1 inhibitor, reduced body weight depending on dietary fat in diet-induced obesity (DIO) rats in our previous study. Here, the effect of JTT-553 on glucose metabolism was evaluated using body weight reduction in T2DM mice.

JTT-553 was repeatedly administered to DIO and KK-A^y mice. JTT-553 reduced body weight gain and fat weight in both mouse models. In DIO mice, JTT-553 decreased insulin, non-esterified fatty acid (NEFA), total cholesterol (TC), and liver triglyceride (TG) plasma concentrations in non-fasting conditions. JTT-553 also improved insulin-dependent glucose uptake in adipose tissues and glucose intolerance in DIO mice. In KK-A^y mice, JTT-553 decreased glucose, NEFA, TC and liver TG plasma concentrations in non-fasting conditions. JTT-553 also decreased glucose, insulin, and TC plasma concentrations in fasting conditions. In addition, JTT-553 decreased TNF-α mRNA levels and increased GLUT4 mRNA levels in adipose tissues in KK-A^y mice.

These results suggest that JTT-553 improves insulin resistance in adipose tissues and systemic glucose metabolism through reductions in body weight.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia via insulin resistance and relative impairment in insulin secretion. T2DM is a common disorder and prevalence rises markedly with increasing degrees of obesity (1). The prevalence of T2DM has risen in the past decade (2) in large part due to trends in obesity and lifestyle changes (3).

Obesity causes peripheral resistance to insulin-mediated glucose uptake (4–6) and may also decrease the sensitivity of beta-cells to glucose (6). These defects are largely reversed by weight loss, leading to a decrease in blood glucose levels toward normal. Obesity is defined as a condition of excessive accumulation of fat tissue. A combination of excessive energy intake and a lack of physical activity are thought to explain most cases of obesity (7). The excessive accumulation of triglycerides (TG) in adipose tissues results in obesity and is associated with organ dysfunction in nonadipose tissues. For example, excessive TG deposition in skeletal muscle and the liver is associated with insulin resistance (8).

The glycerol phosphate (9) and monoacylglycerol pathway (10–12) are two major pathways for TG biosynthesis. In the final reaction for both pathways, a fatty acyl-CoA and diacylglycerol molecule are covalently joined to form TG. This reaction is catalyzed by Acyl CoA:diacylglycerol acyltransferase (DGAT) enzymes. The genes encoding the two DGAT enzymes, DGAT1 and DGAT2, have been identified (13). DGAT enzymes are found in numerous

organs. In particular, DGAT1 is highly expressed in the small intestine and fat tissues (14), while DGAT2 is highly expressed in the liver and fat tissues (15).

Humans with DGAT1 deficiencies have not been identified. However, several common sequence polymorphisms are present in the 5′ noncoding sequences of the DGAT1 gene. One of these polymorphisms, C79T, affects promoter activity and has been associated with alterations in body mass index, diastolic blood pressure, and high-density lipoprotein (HDL) cholesterol levels in Turkish women (16).

In DGAT1-transgenic mice that highly express DGAT1, specifically in fat tissues, the accumulation of TG in fat tissues along with marked increases in body weight were observed when animals were on a high-fat diet (17). Conversely, DGAT1 knockout mice showed resistance to the obesogenic effects of a high-fat diet (18). DGAT1 knockout mice fed a high-fat diet maintained body weights comparable to mice fed a regular diet. TG levels in the liver and skeletal muscles were lower and increased energy expenditure was observed in DGAT1 knockout mice compared with wild type mice. DGAT1 knockout mice demonstrated increased insulin and leptin sensitivity compared with wild-type littermates. Therefore, the phenotypes for DGAT1 gene polymorphisms in humans and DGAT1 deficiencies in mice have generated considerable interest for DGAT1 inhibitors as a potential therapy for obesity and T2DM. We previously reported the discovery of a new DGAT1 inhibitor, JTT-553 ([trans-5′-(4-Amino-7,7-dimethyl-2-trifluoromethyl-7H-pyrimido[4,5-b][1,4]oxazin-6-yl)-2′,3′-dihydrospiro(cyclohexane-1,1′-inden)-4-yl]acetic acid monobenzenesulfonate), using human DGAT1 inhibiting activity (19,20).

In contrast, several DGAT2 variants were identified in a similar population of obese adolescents (21). However, no association between these polymorphisms and obesity phenotypes was found in case−control and family-based association studies involving several hundred subjects.

DGAT2 knockout mice survive for only a few hours after birth (22), but the knockdown of DGAT2 mRNA levels in the liver with specific antisense oligonucleotide (ASO) protected against hepatic steatosis and lipotoxic alterations in insulin sensitivity (23). DGAT2 ASO treatment also protected against hepatic steatosis in mice chronically fed a methionine/choline-deficient diet (21).

Therefore, the anti-obesity effects of DGAT1 deficiency in mice have generated considerable interest in DGAT1 inhibition as a potential therapy for obesity.

In a previous report, we evaluated the pharmacological characterization and anti-obesity effects of JTT-553 (24). Because JTT-553 suppressed fat absorption in the small intestine, fat synthesis in adipose tissues, and food intake, JTT-553 reduced body weight and the weight of adipose tissues in DIO rats. In this study, we evaluated the anti-diabetic effects of JTT-553 using diet-induced obesity (DIO) models and genetic T2DM models because the pathogenesis of T2DM can arise through the influence of genetic or environmental factors (25).

2. Materials and methods

2.1. Chemicals and reagents

JTT-553 was synthesized in Central Pharmaceutical Research Institute, Japan Tobacco Inc. (Osaka, Japan). All other reagents used in this study were obtained commercially.

2.2. Animals and diets

Male B6D2F1 mice were purchased from Charles River Laboratories Japan (Yokohama, Japan). Male KK-A^y mice and male C57BL/

6j mice were purchased from CLEA Japan (Tokyo, Japan). The animals were given free access to water and experimental diets (Table 1). The diets containing 3.1 and 35% (w/w) fat were purchased from Oriental Yeast Co. (Osaka, Japan). The animals were housed under specific pathogen-free conditions in a room controlled for temperature at 23 ± 3 °C and humidity of 55 ± 15% in 12-h light/dark cycles (lights on from 8:00 AM to 8:00 PM). All procedures were conducted according to Japan Tobacco Animal Care Committee's guidelines.

2.3. Anti-diabetic study in the DIO model

B6D2F1 mice were given a 3.1% or 35% fat diet ad libitum for 6 weeks in order to establish conditions of obesity and insulin resistance. The animals were used at the age of 10 weeks. In the previous study, maximal effect of JTT-553 on body weight was observed at 3 mg/kg/day (24). Therefore, JTT-553 was administered as a food admixture at a dose equivalent to 3 mg/kg/day for 31 days. Body weight and food consumption were measured on Day 7, 14, 21 and 27 of administration. Blood samples were collected, and glucose, NEFA, TG, TC and insulin plasma levels were measured on Day 24. Mice were fasted for 24 h from Day 27 and an intraperitoneal glucose tolerance test (1 g/kg) was performed on Day 28. On Day 31, mice were sacrificed by exsanguination under isoflurane anesthesia and the weights of livers and adipose tissues were measured. TG was extracted from the liver using chloroform-methanol (2:1) to determine hepatic TG content.

2.4. Glucose uptake in adipose tissues

C57BL/6j mice were given a 3.1% or 35% fat diet ad libitum for 5 weeks in order to establish conditions of obesity and insulin resistance. In the preliminary study, C57BL/6j mice was superior to B6D2F1 mice in reducing adipose tissues weight using JTT-553, C57BL/6j mice were used to evaluate the glucose uptake and insulin sensitivity in adipose tissues. The animals were used at the age of 10 weeks. In the previous study, maximal effect of JTT-553 on body weight was observed at 3 mg/kg/day, but maximal effect of JTT-553 on TG synthesis in adipose tissues was observed at 10 mg/kg/day (24). And, obvious signs of toxicity in rats treated JTT-553 was not observed in the preliminary toxicology study for 4 weeks. Therefore, JTT-553 was administered as a food admixture at a dose equivalent to 10 mg/kg/day for 39 days. On Day 39, mice were sacrificed by exsanguination under isoflurane anesthesia, and adipose tissues (epididymal and mesenteric fat) were collected. Tissue fragment samples (approximately 50 mg) from the same animal were incubated in Krebs−Ringer phosphate (KRP) buffer containing 1% BSA and insulin (0 or 1 nM) at 37 °C for 30 min. Subsequently, each tissue fragment sample was transferred to KRP buffer containing 1% BSA, insulin (0 or 1 nM) and 6.67 μCi/mL 2-deoxy-D-[1-^{3}H] glucose, and incubated at 37 °C for 5 min. Tissue fragment samples were retrieved and washed with PBS(−) twice. After the wash, tissue fragment samples were

Table 1
Composition of experimental diets.

Macronutrients	Source	3.1% fat diet	35% fat diet
		% (w/w)	
Fat	Lard	3.1	35.0
Carbohydrates	Starch	39.2	7.3
	Sucrose	28.8	28.8
Protein	Casein	14.4	14.4
		(kcal/g)	
Calories		3.291	5.043

shaken with zirconia balls (YTZ® Ball, Nikkato Corporation) in 250 μL of 0.5% SDS aqueous solution to crush the adipose tissue. The tissue solution was then centrifuged at 10,000 ×g for 5 min at room temperature, and 100 μL of the lower water-soluble fraction was collected and radioactivity measured using a liquid scintillation counter.

2.5. Anti-diabetic study in the genetic T2DM model

KK-A^y mice were given a 3.1% or 35% fat diet *ad libitum* for a week. The animals were used at the age of 5 weeks. JTT-553 was administered as a food admixture at a dose equivalent to 3 mg/kg/day for 35 days. Body weight and food consumption were measured on Day 7, 14, 21, 28 and 35 of administration. Blood samples were collected, and glucose, NEFA, TG, TC and insulin plasma levels were measured on Day 14. Mice were fasted for 24 h from Day 31 and blood samples were collected and plasma biochemical parameters were measured on Day 32. On Day 35, mice were sacrificed by exsanguination under isoflurane anesthesia and the weights of livers and adipose tissues were measured. Hepatic TG content was measured as described above. GLUT4 and TNF-α mRNA expression levels in adipose tissues were measured using real-time quantitative polymerase chain reaction (PCR). Total RNA was extracted from the adipose tissues with the Rneasy Lipid Tissue Mini Kit (QIAGEN, Tokyo, Japan). RNA was transcribed into cDNA using M-MLV reverse transcriptase and random primers (Invitrogen, Carlsbad, CA, USA). The reaction mixture was incubated for 10 min at 25 °C, 1 h at 37 °C, and 5 min at 95 °C. Real-time PCR quantification was performed in a 50 μL reaction mixture with an automated sequence detector combined with ABI Prism 7700 Sequence Detection System software (Applied Biosystems, Foster City, CA, USA). The reaction mixture contained 50 ng of synthesized cDNA, 3.5 mmol/L MgCl$_2$, 0.3 μmol/L primers, 0.1 μmol/L probes, and 0.025 units of Ampli Taq Gold®. The cycle parameters included 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The following primers and FAM-conjugated probes were designed using Primer Express software (Applied Biosystems): GLUT4 (forward, TGCCCGAAA-GAGTCTAAAGCG; reverse, CTCTCCAACTTCCGTTTCTCATC; probe, TGATGTGTCTGACGCACTAGCTGAGCTGA), TNF-α (forward, AGACCCTCACACTCAGATCATCTTC; reverse, ACTTGGTGGTTTGCTAC-GACG; probe, CAAAATTCGAGTGACAAGCCTGTAGCCC), and 18s rRNA (purchased from Applied Biosystems).

2.6. Statistical analysis

Statistical analysis was performed using SAS version 8.2 (SAS Institute Inc., CA, USA). Data are expressed as mean values ± s.d. An F test was performed to test homoscedasticity in a single comparison. If homoscedasticity was confirmed, Student's t-test was performed; if not, Welch's t test was used. Differences were considered significant when p < 0.05 (2-sided).

3. Results

3.1. Anti-diabetic effect of JTT-553 in the DIO model

3.1.1. Effect on diet-induced obesity and related parameters

To evaluate the anti-diabetic effect of JTT-553 in the DIO and insulin resistance model, JTT-553 was orally administered as a food admixture to mice fed a 35% fat diet for 31 days. Body weight, body weight gain, and cumulative food consumption in mice fed a 35% fat diet increased compared with mice fed a 3.1% fat diet. JTT-553 significantly decreased body weight gain and cumulative food intake in mice fed a 35% fat diet during the administration period (Fig. 1A–C). In the control group fed a 35% fat diet, the weight of
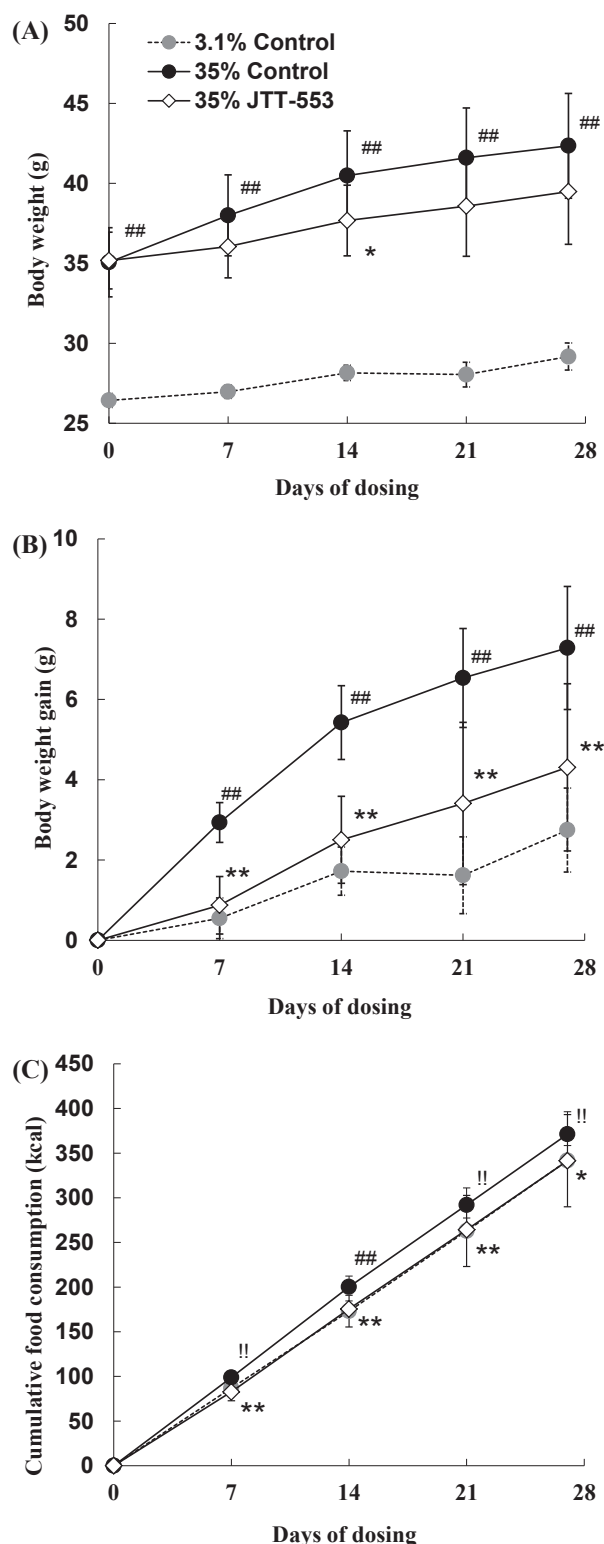

Fig. 1. Effects of repeated administration of JTT-553 in DIO mice. JTT-553 was administered at a dose of 3 mg/kg/day to B6D2F1 mice fed a 35% fat diet for 4 weeks. The changes in body weight (A) and body weight gain (B) and cumulative food consumption (C) are shown. Data represent mean values ± s.d. (n = 8/group). *p < 0.05, **p < 0.01 vs. 35% control (Student's t test), ##p < 0.01 vs. 3.1% control (Student's t-test), !!p < 0.01 vs. 3.1% control (Welch's t test).

adipose tissues increased in association with an elevation in body weight compared with those in the control group fed a 3.1% fat diet. JTT-553 administration also resulted in a decrease in the weight of mesenteric fat as abdominal visceral fat. Liver weight in the control group fed a 35% fat diet did not increase compared with that in the control group fed a 3.1% fat diet, and liver weight in the JTT-553 group was comparable to that in the control group (Fig. 2A). In contrast, TG content in the liver in the 35% fat diet fed control group was significantly elevated compared with that in the 3.1% fat diet fed control group. JTT-553 had a significant lowering effect on TG content in the liver that had been elevated due to a 35% fat diet (Fig. 2B).

3.1.2. Effect on blood chemical parameters

An analysis of blood biochemistry values showed that NEFA, TG, TC and insulin plasma levels in mice fed a 35% fat diet increased compared with those in mice fed a 3.1% fat diet (Table 2). JTT-553 decreased NEFA, TG, TC and insulin plasma levels. Decreases in NEFA, TC and insulin plasma levels were significant. In the glucose tolerance test, the plasma glucose level in the 35% control group was elevated compared with that in the 3.1% control group at fasting after glucose loading (Fig. 3A). Mice fed a 35% fat diet exhibited impaired glucose tolerance. Plasma insulin level in the 35% control group was higher than in the 3.1% control group at

Table 2
Effect of JTT-553 on plasma biochemical parameters in DIO mice.

	3.1% fat diet	35% fat diet	
	Control	Control	JTT-553
Glucose (mg/dL)	225 ± 11	223 ± 22	219 ± 19
NEFA (μEq/L)	416 ± 103	608 ± 120##	410 ± 46$$
Triglycerides (mg/dL)	102 ± 41	234 ± 117!	160 ± 35
Total cholesterol (mg/dL)	109 ± 7	171 ± 23!!	147 ± 12*
Insulin (ng/mL)	0.85 ± 0.28	19.78 ± 15.24##	5.48 ± 4.98$

Data represent mean values ± s.d. (n = 8), *; $p < 0.05$ vs. 35% control (Student's t test), $; $p < 0.05$, $$; $p < 0.01$ vs. 35% control (Welch's t test), ##; $p < 0.01$ vs. 3.1% control (Student's t-test), !; $p < 0.05$, !!; $p < 0.01$ vs. 3.1% control (Welch's t test).

fasting and after glucose loading (Fig. 3B). Mice fed a 35% fat diet exhibited insulin resistance. JTT-553 lowered glucose and insulin plasma levels at fasting and after administration of glucose.

3.1.3. Effect on glucose uptake in adipose tissues

To evaluate the effect of JTT-553 on glucose uptake in adipose tissues in DIO mice, JTT-553 was orally administered as a food admixture to C57BL/6j mice fed a 35% fat diet for 39 days. Regardless of insulin stimulation, administration resulted in
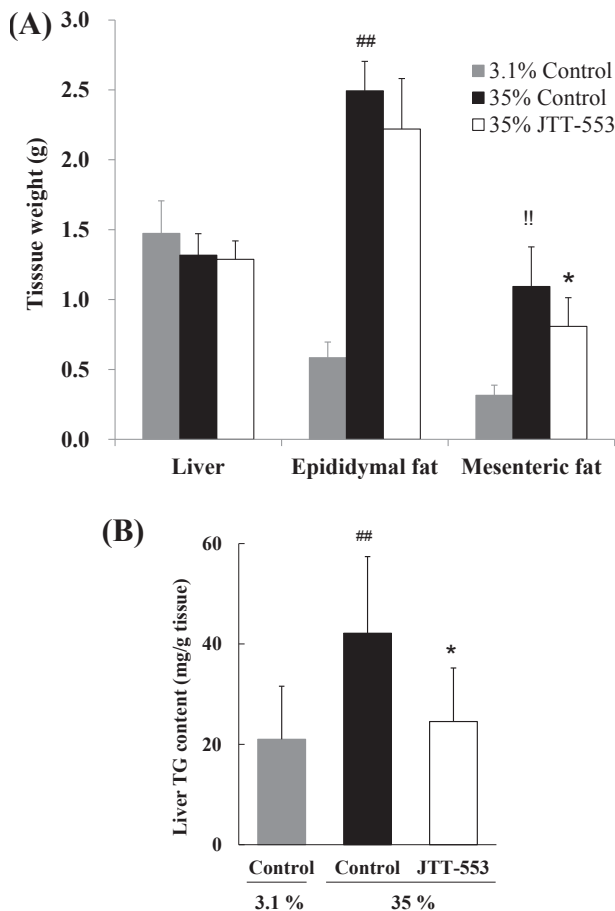

Fig. 2. Effects of repeated administration of JTT-553 in DIO mice. JTT-553 was administered at a dose of 3 mg/kg/day to B6D2F1 mice fed a 35% fat diet for 4 weeks. Tissue weight (A) and liver TG content (B) at 4 weeks of JTT-553 treatment are shown. Data represent mean values ± s.d. (n = 8/group). *$p < 0.05$, **$p < 0.01$ vs. 35% control (Student's t test), ##$p < 0.01$ vs. 3.1% control (Student's t-test), !!$p < 0.01$ vs. 3.1% control (Welch's t test).
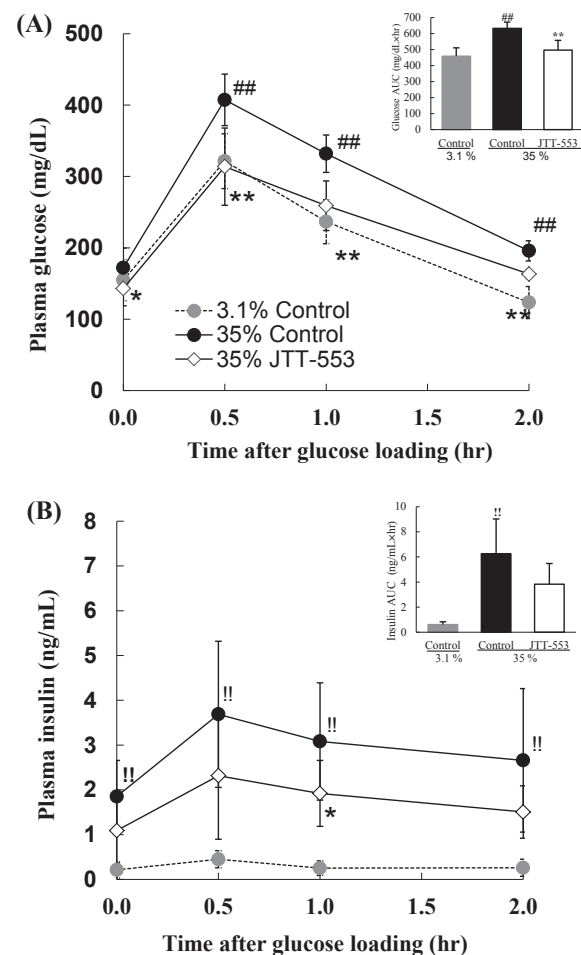

Fig. 3. Effects of repeated administration of JTT-553 in DIO mice. JTT-553 was administered at a dose of 3 mg/kg/day to B6D2F1 mice fed a 35% fat diet for 4 weeks. Changes in glucose (A) and insulin plasma levels (B) in the glucose tolerance test on Day 28 of dosing. Data represent mean values ± s.d. (n = 8/group). The bar graph show the area under the glucose and insulin response curve from 0 to 2 h *$p < 0.05$, **$p < 0.01$ vs. 35% control (Student's t test), ##$p < 0.01$ vs. 3.1% control (Student's t-test), !!$p < 0.01$ vs. 3.1% control (Welch's t test).

significantly decreased glucose uptake in epididymal and mesenteric fat tissues in the 35% fat diet fed group compared with the 3.1% fat diet fed group (Fig. 4A and B). JTT-553 significantly increased glucose uptake in epididymal fat with or without insulin stimulation and JTT-553 significantly increased glucose uptake in mesenteric fat with insulin stimulation.

3.2. Anti-diabetic effect of JTT-553 in the genetic T2DM model

3.2.1. Effect on diet-induced obesity

To evaluate the anti-diabetic effect of JTT-553 in the genetic T2DM model, JTT-553 was orally administered as a food admixture to KK-A^y mice fed a 35% fat diet for 35 days. In mice fed a 35% fat diet, body weight, body weight gain, and cumulative food consumption increased compared with mice fed a 3.1% fat diet (Fig. 5A–C). JTT-553 significantly decreased body weight, and cumulative food consumption.

3.2.2. Effect on blood chemical parameters

For blood biochemical parameters in a non-fasting state, glucose, NEFA, TG, and TC plasma levels in mice fed a 35% fat diet were elevated compared with those in mice fed a 3.1% fat diet (Table 3). JTT-553 significantly decreased glucose, NEFA, and TC
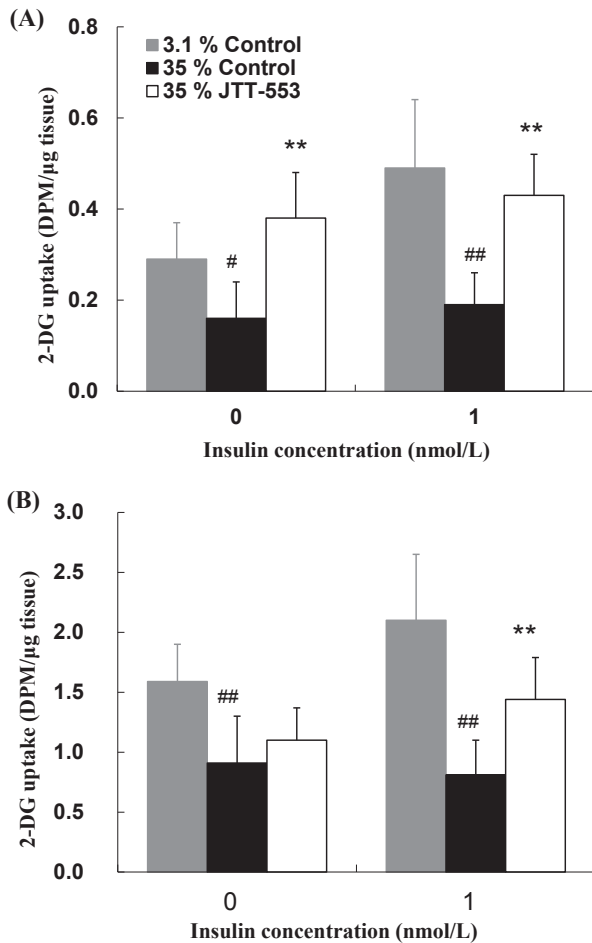
Fig. 4. Effects of JTT-553 on adipose tissue glucose uptake in DIO mice on Day 39 of dosing. JTT-553 was administered at a dose of 10 mg/kg/day to C57BL/6j mice fed a 35% fat diet for 5 weeks. The uptake of 2-deoxyglucose without insulin into epididymal fat (A) and mesenteric fat (B) are shown. Data represent mean values ± s.d. (n = 6/group). **p < 0.01 vs. 35% control (Student's t test), #p < 0.05, ##p < 0.01 vs. 3.1% control (Student's t-test).
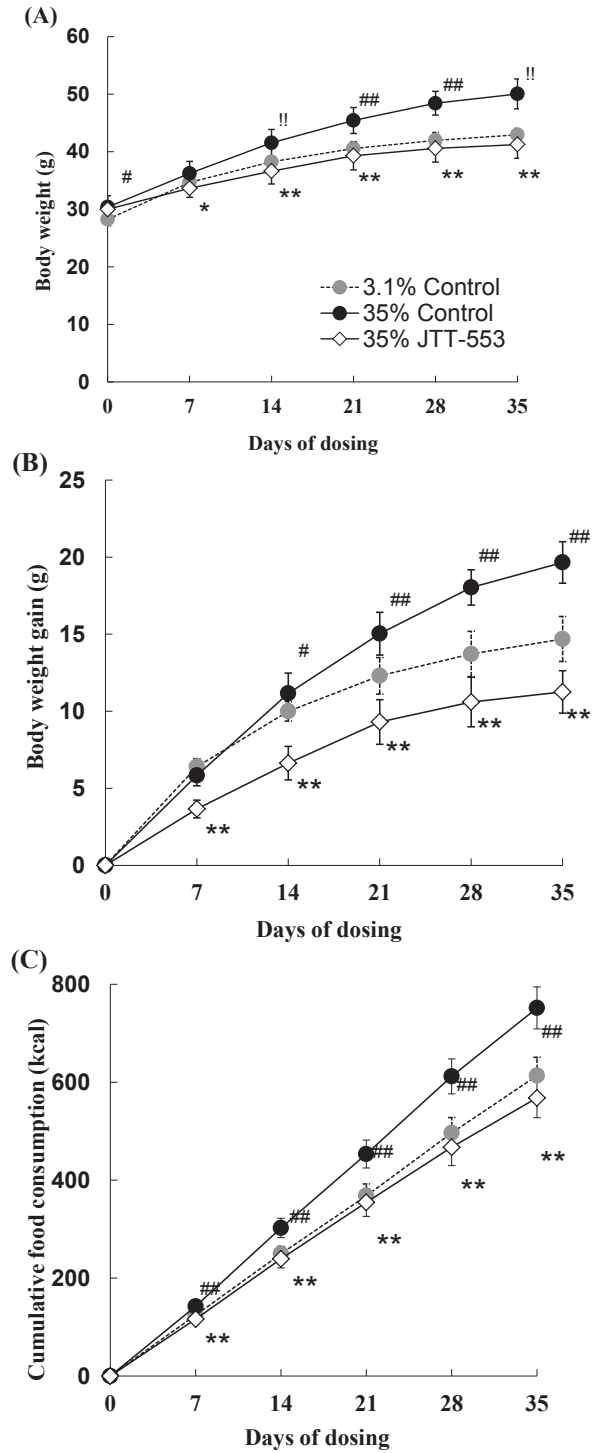
Fig. 5. Effects of repeated administration of JTT-553 in KK-A^y mice. JTT-553 was administered at a dose of 3 mg/kg/day to KK-A^y mice for 5 weeks. The changes in body weight (A) and body weight gain (B) and cumulative food consumption (C) are shown. Data represent mean values ± s.d. (n = 8/3.1% and 35% control groups, n = 7/JTT-553 group). *p < 0.05, **p < 0.01 vs. 35% control (Student's t-test), #p < 0.05, ##p < 0.01 vs. 3.1% control (Student's t-test), !!p < 0.01 vs. 3.1% control (Welch's t test).

plasma levels. For blood biochemical parameters in a fasting state, glucose and insulin plasma levels in mice fed a 35% fat diet were elevated compared with those in mice fed a 3.1% fat diet (Table 3). JTT-553 significantly decreased glucose, TC, and insulin plasma levels.

Table 3
Effect of JTT-553 on plasma biochemical parameters in KK-A^y mice on Day 14 of dosing (non-fasting) and Day 32 of dosing (fasting).

	3.1% fat diet	35% fat diet	
	Control	Control	JTT-553
Non-fasting			
Glucose (mg/dL)	445 ± 82	547 ± 34!!	407 ± 106$$
NEFA (µEq/L)	281 ± 45	684 ± 177!!	501 ± 108*
Triglycerides (mg/dL)	288 ± 92	479 ± 162#	368 ± 149
Total cholesterol (mg/dL)	127 ± 9	167 ± 18##	124 ± 19**
Insulin (ng/mL)	69.62 ± 10.03	42.21 ± 21.87##	36.23 ± 13.66
Fasting			
Glucose (mg/dL)	185 ± 29	287 ± 53##	191 ± 41**
NEFA (µEq/L)	371 ± 65	241 ± 23!!	270 ± 77
Triglycerides (mg/dL)	109 ± 30	120 ± 45	100 ± 44
Total cholesterol (mg/dL)	144 ± 12	149 ± 20	105 ± 9**
Insulin (ng/mL)	1.27 ± 0.54	3.77 ± 1.34!!	1.52 ± 0.86**

Data represent mean values ± s.d. (n = 8), *; $p < 0.05$, **; $p < 0.01$ vs. 35% control (Student's t test), $$; $p < 0.01$ vs. 35% control (Welch's t test), #; $p < 0.05$, ##; $p < 0.01$ vs. 3.1% control (Student's t-test), !!; $p < 0.01$ vs. 3.1% control (Welch's t test).

3.2.3. Effect on fat and the liver

A 35% fat diet increased the weight of mesenteric fat and JTT-553 decreased the weight of epididymal and mesenteric fat (Fig. 6A). A 35% fat diet decreased liver weight compared with a 3.1% fat diet; however, TG content in the liver increased compared with content in animals fed a 3.1% fat diet (Fig. 6B). JTT-553 significantly decreased liver weight and TG content in the liver. In adipose tissue, TNF-α mRNA levels increased after feeding with a 35% fat diet and GLUT-4 mRNA levels decreased after feeding with a 35% fat diet (Fig. 7A and B). JTT-553 significantly decreased TNF-α mRNA levels and increased GLUT-4 mRNA levels.

4. Discussion

The excessive intake of Western diets is known to increase the risk of T2DM through the induction of obesity. We previously reported the pharmacological profile of JTT-553, which reduced body weight gain in DIO rats via suppression of fat absorption in the small intestine, fat synthesis in adipose tissues, and food intake. In the present study, we evaluated the anti-diabetic effect of JTT-553 through the improvement of obesity in DIO and genetic T2DM models, because the pathogenesis of T2DM can arise through the influence of genetic or environmental factors.

In DIO mice, plasma insulin levels were elevated and glucose tolerance was impaired compared with mice fed a 3.1% fat diet. Plasma NEFA, TG and TC levels also increased in DIO mice. DIO mice presented with insulin resistance and dyslipidemia. JTT-553 reduced not only plasma lipid levels, but also plasma insulin levels, and JTT-553 improved glucose tolerance in DIO mice. Because excessive TG accumulation in adipose tissues and the liver impaired insulin sensitivity and glucose metabolism in tissues (8,26), the improvement observed with JTT-553 on glucose tolerance in DIO mice was considered to be due to the reduction in fat weight and liver TG content, and the improvement of insulin resistance in adipose tissues and the liver. In fact, JTT-553 increased insulin-dependent glucose uptake in epididymal and mesenteric fat tissues in DIO mice. It was reported that high fat feeding causes a marked decrease in the expression of GLUT4 in adipose tissues (27). It was inferred that the decrease of glucose uptake in adipose tissues in DIO mice in part due to the decrease of GLUT4 expression and JTT-553 improved the decrease of GLUT4 expression because JTT-553 did not have acute effect on insulin-dependent glucose uptake in 3T3-L1 adipocyte. Moreover, JTT-553 did not affect the adipose cell size evaluated using histological analysis (hematoxylin
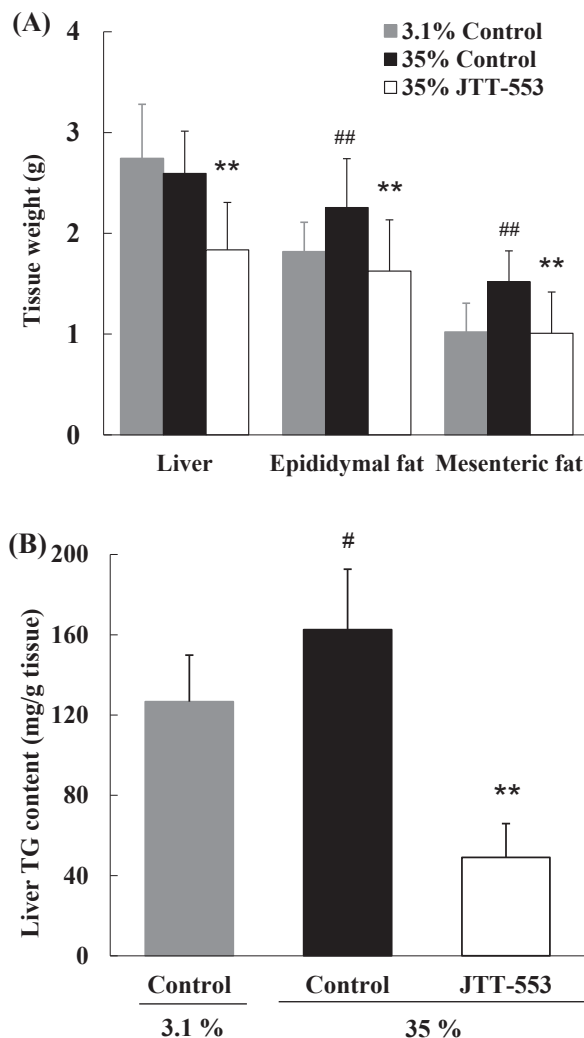

Fig. 6. Effects of repeated administration of JTT-553 in KK-A^y mice. Tissue weight (A) and liver TG content (B) at 5 weeks of JTT-553 treatment at a dose of 3 mg/kg/day are shown. Data represent mean values ± s.d. (n = 8/3.1% and 35% control groups, n = 7/JTT-553 group). *$p < 0.05$, **$p < 0.01$ vs. 35% control (Student's t-test), #$p < 0.05$, ##$p < 0.01$ vs. 3.1% control (Student's t-test).

and eosin staining) in DIO mice (data not shown). Feeding period of 35% fat diet before administration might be long to evaluate the adipose cell size. It could not confirm morphological alterations in the adipose tissue in DIO mice, but JTT-553 improved the adipose tissue functionally. In the previous report, JTT-553 did not affect food intake and body weight in rats fed a 3.1% fat diet (24). In the same study, JTT-553 did not affect plasma TG, glucose, and insulin levels (data not shown). Therefore, it was considered that JTT-553 improved obesity and T2DM derived from high fat feeding in this study.

In KK-A^y mice fed a 35% fat diet, fed and fasting plasma glucose levels were elevated compared with mice fed a 3.1% fat diet. Fed plasma insulin levels in KK-A^y mice fed a 35% fat diet were lower than in mice fed a 3.1% fat diet. However, fasting plasma insulin levels in KK-A^y mice fed a 35% fat diet were elevated compared with mice fed a 3.1% fat diet. This suggested that insulin resistance had worsened and insulin secretion from pancreatic beta-cells may be impaired in KK-A^y mice fed a 35% fat diet. Fed and fasting plasma glucose levels decreased with repeated administration of JTT-553 in KK-A^y mice fed a 35% fat diet. JTT-553 also reduced fasting plasma insulin levels. Because JTT-553 decreased adipose tissue weight and
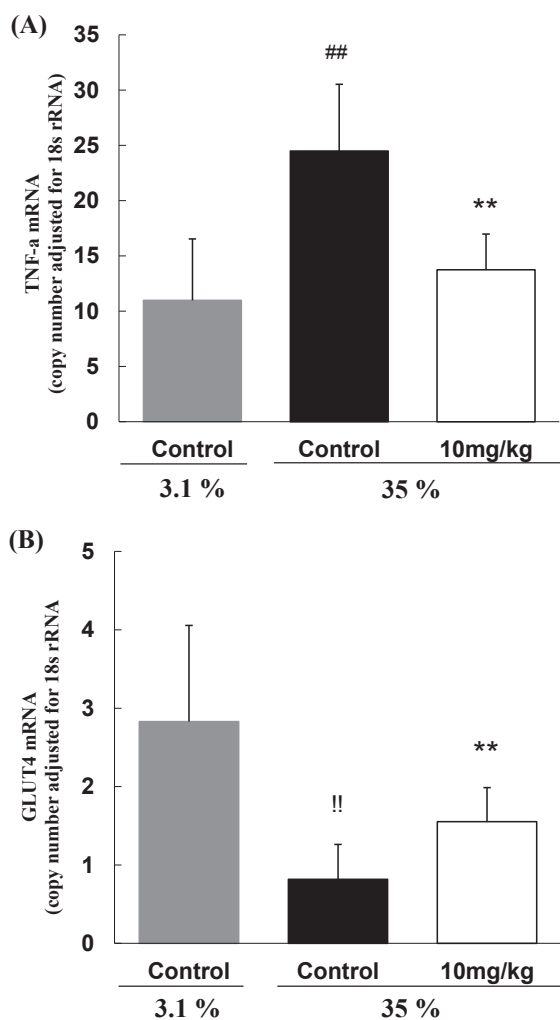
Fig. 7. Effects of repeated administration of JTT-553 in KK-Ay mice. TNF-alpha (A) and GLUT4 (B) mRNA expression levels in adipose tissues at 5 weeks of JTT-553 treatment at a dose of 3 mg/kg/day are shown. Data represent mean values ± s.d. (n = 8/3.1% and 35% control groups, n = 7/JTT-553 group). **$p < 0.01$ vs. 35% control (Student's t-test), ##$p < 0.01$ vs. 3.1% control (Student's t-test), !!$p < 0.01$ vs. 3.1% control (Welch's t test).

liver TG content, the effect of JTT-553 on the improvement of glucose metabolism was considered to be due to the amelioration of insulin resistance in adipose tissues and the liver. In adipose tissues, TNF-α mRNA levels increased after feeding with a 35% fat diet and GLUT-4 mRNA levels decreased after feeding with a 35% fat diet. Studies in genetically obese animals suggest that the increased release of TNF-α from adipose tissues may play a major role in the impairment in insulin activity (28—31) and obese mice genetically lacking TNF-α have more normal insulin sensitivity (28). In another study, weight reduction in obese animals was associated with both improved insulin activity and decreased TNF-α gene expression (31). GLUT4 is the insulin-regulated glucose transporter. Insulin induces a rapid increase in the uptake of glucose by inducing the translocation of GLUT4 from intracellular vesicles to the plasma membrane (32). According to previous reports, it is possible that the incremental change in TNF-α expression in adipose tissues in KK-A^y mice fed a 35% fat diet impaired insulin activity via decreasing GLUT4 expression. Thus, the weight reduction observed with JTT-553 was considered to be associated with both decreased TNF-α mRNA expression and increased GLUT4 mRNA expression, and the effects on adipose tissues considered to ameliorate insulin sensitivity in adipose tissues and the entire body.

In summary, the present findings demonstrate that JTT-553 improves glucose metabolism based on weight reductions in both DIO and genetic T2DM models. These results suggest that JTT-553 may be an effective therapeutic agent for the treatment of T2DM.

Conflict of interest

None declared.

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