Regular Article

Pharmacological Characterization of [*trans*-5'-(4-Amino-7,7-dimethyl-2-trifluoromethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)-2',3'-dihydrospiro(cyclohexane-1,1'-inden)-4-yl]acetic Acid Monobenzenesulfonate (JTT-553), a Novel Acyl CoA:Diacylglycerol Transferase (DGAT) 1 Inhibitor

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Acyl CoA:diacylglycerol acyltransferase (DGAT) 1 is an enzyme that catalyzes the final step in triglyceride (TG) synthesis. This enzyme is considered to be a potential therapeutic target for obesity and diabetes. Here, results of an investigation of the pharmacological effects of JTT-553 [*trans*-5'-(4-amino-7,7-dimethyl-2-trifluoromethyl-7*H*-pyrimido[4,5-*b*][1,4]oxazin-6-yl)-2',3'-dihydrospiro(cyclohexane-1,1'-inden)-4-yl]acetic acid monobenzenesulfonate, a novel DGAT1 inhibitor, are reported. To measure the inhibitory activity of JTT-553 against DGAT1, TG synthesis using [¹⁴C]-labeled oleoyl-CoA was evaluated. Similarly, the inhibitory activity of JTT-553 against DGAT2, an isozyme of DGAT1, and acyl-CoA cholesterol acyltransferase (ACAT) 1, which is highly homologous to DGAT1, were evaluated. JTT-553 selectively inhibited human DGAT1 and showed comparable inhibitory effects on the activity of human, rat, and mouse DGAT. *In vivo*, JTT-553 suppressed plasma TG and chylomicron TG levels after olive oil loading in Sprague-Dawley (SD) rats. JTT-553 also inhibited TG synthesis in epididymal fat after [¹⁴C] oleic acid injection in C57BL/6J mice. Food intake was evaluated in SD rats fed 3.1%, 13%, or 35% (w/w) fat diets. In rats fed the 35% fat diet, JTT-553 reduced food intake. This reduction of food intake was observed 2 h after feeding, lasted for 24 h, and correlated with dietary fat content. Furthermore, JTT-553 reduced daily food intake and body weight gain in diet-induced obese rats after 4-week repeated administration. JTT-553 exerted multiple effects on intestinal fat absorption, adipose fat synthesis, and food intake, and consequently induced body weight reduction. Therefore, JTT-553 is expected to be an effective novel therapeutic agent for the treatment of obesity.

Key words obesity; triacylglycerol; acyl CoA:diacylglycerol acyltransferase (DGAT) 1

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.¹⁾ Obesity is also a risk factor for the development of various diseases, particularly cardiovascular diseases, type 2 diabetes, dyslipidemia and hypertension.²⁾ The excessive accumulation of triglycerides (TG) in adipose tissue results in obesity and is associated with organ dysfunction in non-adipose tissues. For example, excessive TG deposition in skeletal muscle and the liver is associated with insulin resistance, deposition in the liver is associated with nonalcoholic steatohepatitis, and deposition in the heart is associated with cardiomyopathy.^{3,4)} As a result of increases worldwide in the prevalence of obesity and other diseases of excessive TG accumulation, decreasing excessive TG accumulation is considered of biomedical importance.

The glycerol phosphate⁵⁾ and the monoacylglycerol pathway^{6–8)} 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 two DGAT enzymes, DGAT1 and DGAT2, have been identified.^{9,10)} DGAT enzymes are found in numerous organs. In particular,

DGAT1 is highly expressed in the small intestine and fat tissues,⁴⁾ while DGAT2 is highly expressed in the liver and fat tissues.¹¹⁾

Humans with DGAT1 deficiencies have not been identified. However, several common sequence polymorphisms are present in the 5' noncoding sequences of the DGAT1 gene.¹²⁾ 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.

In DGAT1-transgenic mice that highly express DGAT1 specifically in fat tissues, accumulation of TG in fat tissues accompanied by marked increases in body weight were observed when the animals were on a high-fat diet.¹³⁾ Conversely, DGAT1 knockout mice showed resistance to the obesogenic effects of a high fat diet.¹⁴⁾ 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 had 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 consider-

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able interest for DGAT1 inhibitors as a potential therapy for obesity. 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.^{15,16)}

In this study, we examined the anti-obesity effects of a novel DGAT1 inhibitor, JTT-553, using diet induced obese (DIO) models. Given information about DGAT1 distribution and the phenotype of DGAT1 deficient mice, DGAT1 is suggested to be involved in the absorption of TG *via* the small intestine and in the synthetic pathway of TG in fat tissues. The presence of fatty acids and glycerides in the gastrointestinal tract also reportedly leads to the suppression of appetite, although the mechanism remains unclear.^{17–19)} Therefore, inhibition of DGAT1 may possibly exert a suppressive effect on appetite through the accumulation of free fatty acids and glycerides *via* DGAT1 inhibition in the small intestine. Subsequently, we evaluated the pharmacological effects of JTT-553 on fat absorption in the small intestine, on fat synthesis in adipose tissue, and on food intake. From these evaluations, the anti-obese effects of JTT-553 were determined.

MATERIALS AND METHODS

Chemicals and Reagents JTT-553 was synthesized in the Central Pharmaceutical Research Institute within Japan Tobacco Inc. (Osaka, Japan). [1^{14}C] oleoyl-coenzyme A (oleoyl-CoA) and [1^{14}C] oleic acid were purchased from Amersham biosciences. All other chemicals were standard reagent grade.

Animals and Diets Male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories (Yokohama, Japan). Male C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan). The animals were maintained on CRF-1 (Charles River Japan), as standard laboratory chow diets, and water *ad libitum*. For the evaluation of food consumption and for repeated administration studies, animals were given free access to water and experimental diets (Table 1). The diets contained 3.1%, 13% and 35% (w/w) fat and were purchased from Oriental Yeast Co. (Osaka, Japan). The animals were housed under specific pathogen-free conditions in a room controlled for temperature at $23 \pm 3^\circ\text{C}$ and humidity of $55 \pm 15\%$ in 12-h light/dark cycles (lights on from 8:00 a.m. to 8:00 p.m.). All procedures were conducted according to guidelines from Japan Tobacco's Animal Care Committee.

Enzyme Assays Human DGAT1 (hDGAT1), human DGAT2 (hDGAT2) and human ACAT1 (hACAT1) were cloned into pFASTBAC1 vectors and expressed in Sf9 in-

sect cells using a baculovirus expression system. Sf9 cells were infected and membrane fractions isolated as enzyme sources as described by Cases *et al.*³⁾ The reaction mixtures for hDGAT1 and hDGAT2 enzyme assays contained 100mM Tris-HCl (pH 7.5), 250mM sucrose, 150mM MgCl_2 , 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.1% bovine serum albumin (BSA), 0.2mM 1,2-di-oleoyl-*sn*-glycerol and 5 μg of protein/mL of recombinant hDGAT1 or 60 μg of protein/mL of recombinant hDGAT2. The reaction mixtures of the hACAT1 enzyme assay contained 100mM Tris-HCl (pH 7.5), 250mM sucrose, 150mM MgCl_2 , 0.1% CHAPS, 0.1% BSA, 0.5mM cholesterol and 40 μg of protein/mL of recombinant hACAT1. For measurement of inhibitory activity of JTT-553, serial dilutions of JTT-553- $\text{C}_6\text{H}_5\text{SO}_3\text{H}$ with dimethyl sulfoxide (DMSO) were added to the reaction mixture at a final concentration of 4% DMSO. The reaction was initiated by adding 15 μM [1^{14}C] oleoyl-CoA for a final volume of 125 μL and the mixture was incubated for 10 min at 25°C . The reaction was terminated by the addition of 500 μL chloroform-methanol (2:1) solvent. After mixing, the reaction mixture was centrifuged ($2000 \times g$, 10 min) and lipids extracted in the organic phase were separated by thin layer chromatography (TLC) using a hexane-diethylether-acetic acid (80:20:1) solvent system. The radioactivity of synthesized [1^{14}C] triacylglycerol was measured using the BAS-2500 imaging system (FUJIFILM, Tokyo, Japan). Human, rat and mouse intestinal microsomal DGAT enzyme assays were performed in the same manner as the DGAT1 enzyme assay, except for using 5 $\mu\text{g}/\text{mL}$ of human intestinal microsomes (Tissue Transformation Technologies, MD, U.S.A.), 40 $\mu\text{g}/\text{mL}$ rat intestinal microsomes or 10 $\mu\text{g}/\text{mL}$ mouse intestinal microsomes instead of recombinant DGAT1.

Olive Oil Loading Test Olive oil (5 mL/kg) was orally loaded into 8-week old male SD rats, and plasma TG concentrations were measured at 1, 3, 5, 8, and 24 h after oil loading. The effect of JTT-553 (orally dosed as a 0.5% methylcellulose suspension 30 min prior to olive oil loading) on increases in plasma TG concentrations was investigated. In addition, the effect on increases in chylomicron TG concentrations at 3 h after olive oil loading and the time at which plasma TG concentrations peaked in the control group, were investigated. Plasma samples were used at the time of group assignment and at the time of peak plasma TG concentrations in the control group. Samples were applied onto REP LIPO-30 agarose gel plates, and subjected to electrophoresis at 400 V for 15 min using an REP system (Helena Laboratories, Saitama, Japan). Triglycerides were stained using Chol/Trig Combo TG, which used the method of generating formazan pigments by glycerol-3-phosphate dehydrogenase. After desiccation, pigmentation level was determined using a densitometer at a wavelength 570 nm. The ratio of peak areas in the densitogram was analyzed using included software (Ed-Bank2, K.K. Helena Laboratories) to calculate chylomicron TG ratio.

Evaluation of TG Synthesis in Adipose Tissue Eleven-week old male C57BL/6j mice were fasted before lights were turned off on the day prior to administration. Immediately after the lights were turned off, JTT-553 was administered orally, and feeding was resumed. Sixty minutes after administration, [1^{14}C] oleic acid solution was administered intraperitoneally. Ninety minutes after administration of [1^{14}C] oleic acid solution, epididymal adipose tissue was collected from

Table 1. Composition of Experimental Diets

Macronutrients	Source	3.1% Fat diet	13% Fat diet	35% Fat diet
		% (w/w)		
Fat	Lard	3.1	13.0	35.0
Carbohydrate	Starch	39.2	29.3	7.3
	Sucrose	28.8	28.8	28.8
Protein	Casein	14.4	14.4	14.4
		(kcal/g)		
Calories		3.291	3.834	5.043

animals. Lipids were extracted from tissues, and developed and separated *via* TLC. The signal intensity of the TG fraction and the total lipid fraction on a TLC plate were measured using a BAS-2500 imaging system. The ratio of the TG fraction to the total lipid fraction from the same sample was calculated as the TG synthesis ratio.

Food Intake Study in Rats Seven-week old male SD rats were acclimatized to a 3.1%, 13%, or 35% (w/w) fat diet for 28 d. Rats were fasted for 24 h before lights were turned off on the day prior to administration. Immediately after the lights were turned off, JTT-553 was administered orally. Feeding was resumed immediately after administration and food was weighed at 0.5, 1, 2, 4, 8, and 24 h. Cumulative food consumption was calculated from the differences in food weight before and after feeding.

Anti-obesity Study in Diet-Induced Obesity (DIO) Model Male SD rats were provided 3.1% or 35% (w/w) fat diet *ad libitum* for 10 weeks in order to establish a condition of obesity, after which animals were used. JTT-553 was administered orally once daily for 29 d from the day of group assignment (day 0) until day 28. Rats and food were weighed every seven days to assess the effects on body weight gain, food consumption and food efficiency. Food efficiency was calculated by dividing body weight gain by cumulative food consumption. On day 29, mesenteric fat weights were measured.

Statistical Analysis Statistical analyses were performed using SAS version 8.2 (SAS Institute Inc., CA, U.S.A.). Data are expressed as the mean $\pm$ S.D. Bartlett's homoscedasticity test was performed in the dose dependency study. If homoscedasticity was confirmed, a Dunnett's multiple comparison test was to be performed, while a Steel's multiple comparison test was to be used if homoscedasticity was not confirmed. An *F* test was performed to test homoscedasticity in a single comparison. If homoscedasticity was confirmed, a Student's *t*-test was to be performed; and a Welch's test was used if homoscedasticity was not confirmed. Differences were considered significant if *p* was <0.05 (2-sided).

RESULTS

Enzyme Assay We identified a novel DGAT1 inhibitor, JTT-553, as shown in Fig. 1. Fifty percent maximal inhibitory concentration (IC_{50}) of JTT-553 for human DGAT1 was 2.38 ± 0.14 nM (Table 2). JTT-553 showed comparable inhibitory effects on DGAT activity derived from human, rats and mouse intestinal microsomes (Table 3). IC_{50} values for human DGAT2, an isozyme of DGAT1, and ACAT1, which is highly homologous to human DGAT1, were greater than 10μ M (Table

2).

Effect of JTT-553 on Fat Absorption The effect of JTT-553 on fat absorption was evaluated by measuring increments of plasma TG and chylomicron TG after olive oil loading in SD rats. In the control group, Δ TG elevated until 3 h after olive oil loading and gradually decreased thereafter. JTT-553 suppressed Δ TG in a dose-dependent manner (Fig. 2A), and statistical significant differences were observed at doses of 0.3 and 1 mg/kg. At 3 h after olive oil loading, Δ TG value in the control group was 198.1 ± 115.8 mg/dL and those in the 0.03, 0.1, 0.3, and 1 mg/kg JTT-553 dosing groups were 184.1 ± 169.7 , 80.1 ± 32.2 , -13.5 ± 64.2 and -0.3 ± 42.1 mg/dL, respectively. Δ Chylomicron TG values were measured using plasma samples collected 3 h after olive oil loading. JTT-553 also decreased Δ chylomicron TG in a dose-dependent manner (Fig. 2B). In the control group, Δ chylomicron TG was 39.8 ± 46.4 mg/dL, 39.4 ± 48.9 , 4.1 ± 7.5 , -11.6 ± 5.6 and -16.4 ± 12.2 mg/dL in the 0.03, 0.1, 0.3, and 1 mg/kg JTT-553 dosing groups, respectively. Significant effects were observed at doses of 0.3 and 1 mg/kg.

Effect of JTT-553 on TG Synthesis The systemic effect of JTT-553 on TG synthesis after [$1-^{14}$ C] oleic acid administration in C57BL/6J mouse epididymal adipose tissues was evaluated. The effect of JTT-553 on TG synthesis in the liver was also examined. JTT-553 dose dependently decreased TG synthesis in mouse epididymal adipose tissues. TG synthesis rates were 0.62 ± 0.05 , 0.58 ± 0.06 , 0.41 ± 0.02 , 0.34 ± 0.09 , and 0.26 ± 0.04 in the 0.1, 0.3, 1, 3, and 10 mg/kg JTT-553 dosing groups, respectively, compared with 0.64 ± 0.09 in the control group. Significant effects on TG synthesis rate were observed at doses of 1 mg/kg and higher (Fig. 3).

Comparatively, in the liver, TG synthesis rate in the JTT-553 group was similar to that in the control group (data not shown).

Effect of JTT-553 on Food Intake The effect of JTT-553 on food consumption in SD rats fed a high fat (35% [w/w] fat) diet was evaluated. The time course of changes in cumulative food consumption is shown in Fig. 4A. In rats fed a high-fat diet, JTT-553 dose-dependently decreased food consumption. In the control group, cumulative food consumption was 10.47 g for 2 h after feeding, and those in the 0.1, 0.3, 1, and

Table 2. Inhibitory Effects of JTT-553 on Human DGAT1, Human DGAT2 and Human ACAT1

	hDGAT1	hDGAT2	hACAT1
IC_{50} (nM)	2.38 ± 0.14	>10000	>10000

Enzyme activities were determined by using [$1-^{14}$ C] oleoyl-CoA and membrane fractions containing recombinant enzymes from Sf9 cells. IC_{50} values were calculated in a semilogarithmic proportional manner from the two points flanking 50% inhibition. Each value represents the mean $\pm$ S.E. from three independent experiments.

Table 3. Inhibitory Effects of JTT-553 on Activity of Human, Rat and Mouse Small Intestinal DGAT

	Human	Rat	Mouse
IC_{50} (nM)	1.55 ± 0.02	1.50 ± 0.07	0.59 ± 0.06

Enzyme activities were determined by using [$1-^{14}$ C] oleoyl-CoA and human, rat and mouse small intestinal microsomes. IC_{50} values were calculated in a semilogarithmic proportional manner from the two points flanking 50% inhibition. Each value represents the mean $\pm$ S.E. from three independent experiments.

Fig. 1. Chemical Structure of JTT-553

Fig. 2. Effects of JTT-553 on Fat Absorption in the Small Intestine

The compound was administered orally to male SD rats 30 min prior to olive oil loading. Plasma TG levels were measured prior to and 1, 3, 6, 8, and 24 h after oil loading. Plasma chylomicron-TG levels were measured 3 h after olive oil loading. Changes in plasma TG levels (A) and changes in plasma chylomicron-TG levels (B) were calculated. Data represent the mean $\pm$ S.D. ($n=6$ /group). * $p<0.05$ vs. control (Steel test); # $p<0.05$, ## $p<0.01$ vs. non-loaded (Student's *t*-test); ! $p<0.05$, !! $p<0.01$ vs. non-loaded (Welch's *t* test).

3 mg/kg JTT-553 dosing groups were 7.37 ± 1.38 g, 6.43 ± 1.80 g, 6.27 ± 1.12 g, and 5.63 ± 1.30 g, respectively. Significant effects were observed at 2 and 4 h after feeding in the 0.1 mg/kg dosing group, and at 2, 4, and 8 h after feeding in the 0.3 mg/kg dosing group. In the 1 and 3 mg/kg dosing groups, food consumption significantly decreased at 2, 4, 8, and 24 h after feeding.

In addition, the dietary fat dependent effect of JTT-553 on food consumption using SD rats fed three types of diets containing fat (3.1%, 13%, and 35% [w/w] fat diets) was evaluated. In the 3 mg/kg JTT-553 dosing groups, 24 h cumulative food consumption was comparable to those observed in rats fed 3.1% (w/w) fat diet in the control group (Fig. 4B). However, 24 h cumulative food consumption in the JTT-553 dosing groups was significantly reduced compared with rats fed 13% (w/w) fat diet in the control group (Fig. 4B). In rats fed 35% (w/w) fat diet, cumulative food consumption was significantly reduced compared with the control group (Fig. 4B).

Anti-obesity Effect of JTT-553 in a DIO Model To evaluate the anti-obesity effect of JTT-553, JTT-553 was ad-

Fig. 3. Effects of JTT-553 on TG Synthesis in Mouse Epididymal Adipose Tissue

The compound was administered orally to C57BL/6j mice after 24 h of food deprivation. Mice were given standard diet immediately after compound administration. One hour after administration, [14 C] oleic acid was administered intraperitoneally. Another 1.5 h later, epididymal adipose tissue was collected from animals. The ratio of radioactivity in the TG fraction to radioactivity in the total lipid fraction was calculated. Data represent the mean $\pm$ S.D. ($n=5$ /control and 0.1 mg/kg groups, $n=6$ /0.3–10 mg/kg groups) ** $p<0.01$ vs. control (Dunnett's test).

Fig. 4. Effects of JTT-553 on Food Consumption in Rats

The compound was administered orally to male SD rats after 24 h of food deprivation. Rats were given diet immediately after administration of the compound. (A) The cumulative food consumption in rats fed 35% fat diet was monitored until 24 h after administration. (B) Twenty-four hours cumulative food consumption in rats fed 3.1%, 13%, or 35% fat diets after administration. Data represent the mean $\pm$ S.D. ($n=6$ /group). * $p<0.05$; ** $p<0.01$ vs. control (Dunnett's test).

Fig. 5. Effects of Repeated Administrations of JTT-553 in Diet-Induced Obese Rats

The compound was administered orally once-a-day to diet-induced obese SD rats for 4 weeks. Changes in body weight gain (A), daily food consumption (B), and food efficiency (C) are shown, respectively. Mesenteric fat weight (D) at 4 weeks of JTT-553 treatment is shown. Data represent the mean $\pm$ S.D. ($n=8$ /group). * $p<0.05$, ** $p<0.01$ vs. 35% control (Dunnett's test), ## $p<0.01$ vs. 3.1% control (Student's *t*-test), ! $p<0.01$ vs. 3.1% control (Welch's *t*-test).

ministered for 4 weeks to DIO rats and body weight, weight gain, food consumption, food efficiency, and mesenteric fat weight were measured. In obese rats fed a high-fat diet, JTT-553 dose-dependently decreased body weight gain, daily food consumption, and food efficiency. Significant effects were observed for weight gain, daily food consumption and food efficiency at doses of 1 mg/kg and higher. Comparatively, in rats fed a low-fat diet, body weight gain, daily food consumption and food efficiency in the JTT-553 group were comparable to those in the control group (Figs. 5A–C).

In addition, JTT-553 caused decreases in mesenteric fat in obese rats fed a high-fat diet. Significant effects were observed at doses of 0.3 mg/kg and higher. However, in rats fed a low-fat diet, visceral fat weight was comparable in the JTT-553 and control groups (Fig. 5D).

DISCUSSION

JTT-553 is a novel selective DGAT1 inhibitor that inhibits hDGAT1 enzyme activity. JTT-553 almost completely suppressed the elevation of plasma TG and plasma chylomicron TG levels in SD rats after olive oil loading. In adipose tissues, JTT-553 inhibited TG synthesis from [1- 14 C] labeled oleic acid. JTT-553 decreased food consumption depending on dietary

fat content, and repeated administrations of JTT-553 reduced body weight gain, food consumption and food efficiency in DIO rats.

DGAT1 is an enzyme that catalyzes the final step of TG synthesis, and DGAT2 is a known isozyme of DGAT1. DGAT2-deficient mice are lipopenic and die soon after birth, reportedly due to profound reductions in substrates for energy metabolism and due to impaired permeability barrier function of the skin.²⁰ DGAT1 is a member of the same family as ACAT1. Absence of ACAT-1 causes dry eye and cutaneous xanthomatosis in mice.²¹ Toxicological effects induced by various classes of ACAT inhibitors have consistently been observed in the adrenal glands of certain species.^{22,23} Thus, it is important that DGAT1 inhibitors have no inhibitory effects on DGAT2 and ACAT1 activity due to the safety risk. Based on these principles, we developed a selective DGAT1 inhibitor, JTT-553.

In single dose *in vivo* studies, JTT-553 dose-dependently inhibited increases in plasma TG concentrations as well as chylomicron secretion in rats after olive oil loading (Figs. 2A, B). JTT-553 also completely inhibited chylomicron secretion. The effects of JTT-553 on postprandial lipemia are consistent with the importance of TG synthesis for the assembly and secretion of chylomicrons. JTT-553 is expected to ameliorate extra fat

absorption in obese subjects.

JTT-553 inhibited TG synthesis from radiolabeled oleic acid in epididymal adipose tissue in mice in a dose-dependent manner (Fig. 3). JTT-553 is considered to inhibit TG accumulation in adipose tissue. This effect likely results in prevention of hypertrophy of adipose tissue by inhibiting TG accumulation. This finding indicates that the systemic effect of JTT-553 may improve increases in fat mass in obesity. Alternatively, JTT-553 failed to inhibit hepatic TG synthesis from radiolabeled oleic acid in mice (data not shown). DGAT2 was therefore considered the dominant enzyme in liver TG synthesis.

JTT-553 dose-dependently decreased food consumption in rats fed a high fat diet (Fig. 4A), and this anti-feeding effect was dependent on dietary fat content (Fig. 4B). In this study, plasma concentrations of JTT-553 at 2 h after administration were $0.17 \pm 0.07 \mu\text{M}$ in the 0.1 mg/kg dosing group, $0.62 \pm 0.17 \mu\text{M}$ in the 0.3 mg/kg dosing group, and $1.69 \pm 0.44 \mu\text{M}$ in the 1 mg/kg dosing group. Anti-feeding effect of JTT-553 was appeared at the plasma concentrations of $0.17 \mu\text{M}$ and above, and the maximum effect was appeared at the plasma concentrations of $1.69 \mu\text{M}$. Given the plasma concentrations of JTT-553 in this study, it was considered that JTT-553 was reached a concentration sufficient to inhibit DGAT1 activity in the small intestine and the adipose tissue. The presence of fatty acids and glycerides in the gastrointestinal tract reportedly leads to induction of satiety, or suppression of appetite.^{22,23} Intraduodenal infusion of TG suppressed appetite and food intake, and elevated plasma cholecystokinin (CCK) and glucagon-like peptide (GLP)-1 levels in humans. Gut hormones such as CCK, GLP-1, and peptide YY (PYY) are known to decrease food intake.²⁴ Based on findings in these reports, JTT-553 inhibition of fat absorption *via* the small intestine may be hypothesized as causing the accumulation of fatty acids and glycerides in the intestinal lumen, thus suppressing food consumption *via* secretion of gut hormones. Further investigations are required to elucidate the mechanisms underlying satiety effect of JTT-553.

In a repeated dose administration study, JTT-553 decreased body weight gain and visceral fat weight in obese rats fed a high-fat diet (Figs. 5A, D). The suppressive effect of JTT-553 on food consumption was not observed in rats fed a low-fat diet; however, was observed in rats fed a high-fat diet (Fig. 5B). This finding is consistent with results from the anti-feeding study. In addition, JTT-553 decreased food efficiency (Fig. 5C), suggesting that the reducing effect on body weight and visceral fat weight were attributed to inhibitory effects of JTT-553 on small-intestinal fat absorption and TG synthesis in adipose tissues, as well as suppressive effects on food consumption. Although it was considered that plasma free fatty acid (FFA) levels were elevated in the JTT-553 groups than in the control group due to the inhibitory effect of JTT-553 on TG synthesis in adipose tissue, JTT-553 did not change plasma FFA levels after four weeks administration. In the control group, plasma FFA level was $570 \pm 208 \mu\text{Eq/L}$, and those in the 0.1, 0.3, 1, and 3 mg/kg JTT-553 dosing groups were 528 ± 153 , 493 ± 127 , 428 ± 114 , and $481 \pm 139 \mu\text{Eq/L}$, respectively. JTT-553 increased FFA contents in feces in DIO rats may be due to the inhibitory effect on small-intestinal fat absorption (data not shown). And, JTT-553 suppressed food consumption in rats fed a high fat diet. Because JTT-553 reduced dietary fat intake, JTT-553 did not elevate plasma FFA levels. Plasma

FFA levels in DGAT1 deficient mice did not change compared with wild type mice, either.¹⁴ Reduction of body weight and visceral fat weight ameliorates insulin sensitivity in adipose tissues and the entire body.²⁵ Thus, JTT-553 is expected to show anti-diabetic effects, as well as anti-obesity effects.

In summary, the present findings demonstrate that JTT-553 improves diet-induced obesity based on effects on fat absorption *via* the small intestine, TG synthesis in fat tissues, and food intake. These results suggest that JTT-553 may be an effective therapeutic agent for the treatment of obesity.

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Conflict of Interest The authors declare no conflict of interest.

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