JTT-130, a novel intestine-specific inhibitor of microsomal triglyceride transfer protein, suppresses food intake and gastric emptying with the elevation of plasma peptide YY and glucagon-like peptide-1 in a dietary fat-dependent manner.

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Abbreviations:

JTT-130, diethyl-2-([3-dimethylcarbamoyl-4-[(4’-trifluoromethylbiphenyl-2-carbonyl) amino] phenyl]acetyloxymethyl]-2-phenylmalonate; MTP, microsomal triglyceride transfer protein;

PYY, peptide YY; GLP-1, glucagon-like peptide-1; CCK, cholecystokinin; DPP4, dipeptidyl peptidase-4; ELISA, enzyme-linked immunosorbent assay

Recommended section: Gastrointestinal, Hepatic, Pulmonary, and Renal
Abstract

The microsomal triglyceride transfer protein (MTP) takes part in the mobilization and secretion of triglyceride-rich lipoproteins from enterocytes and hepatocytes. In this study, we investigated the effects of JTT-130 [diethyl-2-([3-dimethylcarbamoyl-4-[(4′-trifluoromethyl)biphenyl-2-carbonyl) amino] phenyl)acetyloxymethyl]-2-phenylmalonate], a novel intestine-specific MTP inhibitor, on food intake, gastric emptying and gut peptides using Sprague-Dawley rats fed 3.1% fat, 13% fat or 35% fat diets. JTT-130 treatment suppressed cumulative food intake and gastric emptying in rats fed a 35% fat diet, but not a 3.1% fat diet. In rats fed a 13% fat diet, JTT-130 treatment decreased cumulative food intake, but not gastric emptying. Also, the treatment with orlistat, a lipase inhibitor, completely abolished the reduction of food intake and gastric emptying by JTT-130 in rats fed a 35% fat diet. On the other hand, JTT-130 treatment increased the plasma concentrations of gut peptides, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) but not cholecystokinin, in the portal vein in rats fed a 35% fat diet. These elevations in PYY and GLP-1 were also abolished by the treatment with orlistat. Furthermore, JTT-130 treatment in
rats fed a 35% fat diet increased the contents of triglycerides and free fatty acids in the intestinal lumen, which might contribute to the elevation of PYY and GLP-1 levels. The present findings indicate that JTT-130 causes satiety responses, decreased food intake and gastric emptying, in a dietary fat-dependent manner, with enhanced production of gut peptides such as PYY and GLP-1 from the intestine.
Introduction

It has been shown that both oral and duodenal administration of fat suppresses hunger and decreases subsequent energy intake (Chapman et al., 1999; Pilichiewicz et al., 2006). Lipase-catalyzed hydrolysis of triglycerides to free fatty acids has been shown to be required for the satiety effects of ingested fat (Feinle et al., 2003; O'Donovan et al., 2003). These studies show that the ingestion of fat triggers suppression of food intake, in which digestion and absorption of fat have been known to play an important role. Free fatty acids have been shown to be involved in the suppression of food intake via gut peptides such as PYY, GLP-1 and cholecystokinin (CCK) synthesized by specific enteroendocrine cells located in the epithelium of the intestine (Chaudhri et al., 2006). PYY and GLP-1 are produced primarily by L cells in the distal small intestine and colon. CCK is secreted from a population of mucosal endocrine I-cells in the proximal intestine. Peripheral injection of exogenous PYY was reported to reduce food intake in rats and human by its action at the Y2 receptor subtype (Batterham et al., 2002). Peripheral administration of GLP-1 has been known to reduce food intake in human and other animals (Donahey et al., 1998; Flint et al., 1998; Verdich et al., 2001). Treatment with
exogenous CCK or CCK-related peptides reduced food intake in rats or humans (Gibbs et al., 1973; Kissileff et al., 1981).

The microsomal triglyceride transfer protein (MTP) has been known to take part in the mobilization and secretion of triglyceride-rich lipoproteins from enterocytes and hepatocytes (Xie et al., 2006). In particular, enteric MTP plays a critical role in the absorption of dietary lipids such as fat and cholesterol (Xie et al., 2006). Recently, we have developed a novel intestine-specific MTP inhibitor, JTT-130, which is designed to be rapidly catabolized to inactive metabolites immediately after its absorption from the intestine, in order to avoid the inhibition of hepatic MTP resulting in hepatic steatosis (Mera et al., in press). In previous reports, we demonstrated that JTT-130 suppresses the absorption of dietary fat and cholesterol in the intestine and lowers plasma triglycerides and cholesterol concentrations without accumulation of hepatic triglycerides (Aggarwal et al., 2005; Mera et al., in press).

It is known that fat absorption in the intestine where MTP plays a pivotal role is a key step in the regulation of food intake. In addition, recent studies have demonstrated that inhibition of intestinal MTP results in suppression of food intake, accompanied by increased circulating
levels of gut peptides such as PYY (Wren et al., 2007). In this study, we examined the effects of JTT-130, an intestine-specific MTP inhibitor, on food intake in Sprague-Dawley rats. In addition, to investigate the characteristics of satiety effects of JTT-130, we evaluated the effects of JTT-130 on gastric emptying and the circulating gut peptides, PYY, GLP-1 and CCK. In order to reveal the effects of dietary fat, we used rats fed diets containing different percentage of fat (3.1%, 13%, and 35%). Furthermore, to examine the contribution of lipase-catalyzed hydrolysis of triglycerides to free fatty acids, we evaluated these effects of JTT-130 in the presence of a lipase inhibitor, orlistat, also known as tetrahydrolipstatin (Hadvary et al., 1988).
Methods

Chemicals

JTT-130, diethyl-2-[(3-dimethylcarbamoyl-4-((4′-trifluoromethylbiphenyl-2-carbonyl) amino) phenyl)acetyloxymethyl]-2-phenylmalonate, was synthesized by Japan Tobacco Inc. (Osaka, Japan). All other reagents used in this study were obtained commercially.

Animals and diets

Male Sprague-Dawley rats (5 weeks) were obtained from Charles River Japan Inc. (Yokohama, Japan) and maintained at a room temperature of 23 ± 3°C and an air humidity of 55 ± 15% in a 12/12-h light/dark cycle (lights on at 10:00 PM; lights off at 10:00 AM). They were given free access to water and the experimental diets (Table 1). The diets were a 3.1% fat diet, a 13% fat diet and a 35% fat diet obtained from Oriental Yeast Co. (Osaka, Japan). All procedures were conducted according to the Japan Tobacco Animal Care Committee’s guidelines.

Evaluation of food consumption

Rats were housed in individual cages and fasted for at least 24 h prior to the experiment. The rats were assigned to vehicle (0.5% methylcellulose solution) or JTT-130 treatment groups.
(n=6). Immediately after oral dosing of either vehicle or JTT-130 (1, 3, 10 and 30 mg/kg), the rats were given free access to the experimental diets. The doses of JTT-130 were decided based on our previous experiment in which we found that oral administration of 10 mg/kg of JTT-130 suppresses the absorption of radio-labeled triglycerides in rats (Mera et al., in press). Food intake was recorded at 0.5, 1, 2, 4, 8, and 24 h after treatment with JTT-130 with correction for spillage. In experiments where the effects of lipase inhibition were assessed, orlistat (100 mg/kg) was administered 30 min before JTT-130 (10 mg/kg) dosing and food intake was recorded at 24 h after treatment with JTT-130. The vehicle for orlistat was a 0.5% methylcellulose solution.

**Evaluation of gastric emptying**

Rats were housed in individual cages and fasted for at least 24 h prior to the experiment. The rats were assigned to vehicle or JTT-130 treatment groups (n=6 or 8). Immediately after oral dosing of either vehicle or JTT-130 (1, 3, 10 and 30 mg/kg), the rats were given free access to the experimental diets. Gastric emptying was measured at 4, 8, or 24 h after treatment with JTT-130. In experiments where the effects of lipase inhibition were assessed, orlistat (100
mg/kg) was administered 30 min before JTT-130 (10 mg/kg) dosing and gastric emptying was measured at 8 h after treatment with JTT-130. Evaluation of gastric emptying was performed as described by Hayes et al (Hayes et al., 2004). Briefly, the remaining contents collected from the stomach were dried overnight at 90°C and weighed. The experimental diets were dried under the same condition as above and weighed, in order to estimate the original moisture content of them and thereby calculate the dry matter weights of the ingested diets. Gastric emptying, expressed as grams of dry matter emptied, was determined by the following equation: total dry matter emptied (g) = dry matter of ingested diet (g) – dry matter of stomach content (g).

**Evaluation of PYY, GLP-1 and CCK levels in the portal vein**

Rats were housed in individual cages and fasted for at least 24 h prior to the experiment. The rats were assigned to vehicle or JTT-130 treatment groups (n=8). Immediately after oral dosing of either vehicle or JTT-130 (10 mg/kg), the rats were given free access to the experimental diets. The rats were anesthetized with diethyl ether and blood samples were collected from the portal vein 4, 8 or 24 h after oral administration of JTT-130. Plasma was isolated by centrifugation. Plasma levels of PYY, GLP-1 and CCK were measured using a PYY ELISA kit.
(Peninsula Laboratories Inc.), a GLP-1 ELISA kit (Linco Research Inc.) and a CCK ELISA kit (Peninsula Laboratories Inc.), respectively. In experiments where the effects of lipase inhibition were assessed, orlistat (100 mg/kg) was administered 30 min before JTT-130 (10 mg/kg) dosing and gut peptides were measured at 8 h after treatment with JTT-130.

**Evaluation of lipid contents in the intestinal tissue and the intestinal lumen**

Rats were housed in individual cages and fasted for at least 24 h prior to the experiment. The rats were assigned to vehicle or JTT-130 treatment groups (n=8). Immediately after oral dosing of either vehicle or JTT-130 (10 mg/kg), the rats were given free access to the experimental diets. The rats were anesthetized with diethyl ether and a segment of jejunum was isolated from each rat 4, 8 or 24 h after oral administration of JTT-130. Approximately 200 mg of jejunum was homogenized in 1.5 ml of methanol. Then, 200 μL of homogenate were vigorously mixed with 400 μL of chloroform and centrifuged at 10,000g for 10 min. Meanwhile, the luminal contents in the whole small intestine were collected, vigorously mixed with 10 mL of a mixture of chloroform-methanol (2:1; v/v), and then centrifuged at 10,000g for 10 min.

The organic phase was collected and evaporated to dryness under a stream of nitrogen gas. The
lipid residue was resuspended in 2-propanol. Triglyceride, cholesterol and free fatty acid contents were determined enzymatically using a triglyceride kit (LiquiTech TGII, Roche Diagnostics K.K.), a cholesterol kit (LiquiTech TCII, Roche Diagnostics K.K.) and a free fatty acid kit (NEFA-C test, Wako), respectively.

Statistical analysis

Data are presented as means ± S.E. Statistical analysis was performed using SAS systems, version 8.2 (SAS Institute, Cary, NC). In this analysis, data were analyzed among three or more groups as follows. When equality of variances was indicated by a Bartlett’s test, statistical analysis was performed using a Dunnett’s multiple comparison test. When equality of variances was not indicated by a Bartlett’s test, statistical analysis was performed using a Kruskal-Wallis test. Comparisons between two groups were made as follows. When equality of variances was indicated by an F-test, statistical analysis was performed using a Student’s t test. When equality of variances was not indicated by an F-test, statistical analysis was performed using a Welch’s t test. P < 0.05 was considered statistically significant.
Results

Effects of JTT-130 on Food Intake

The effects of JTT-130, an intestine-specific MTP inhibitor, on food intake were evaluated in Sprague-Dawley rats on 3.1% fat, 13% fat, and 35% fat diets (Table 1). JTT-130 treatment decreased cumulative food intake for 24 h after dosing in a dose-dependent manner in rats on a 35% fat diet (Fig. 1). Significant reductions in the cumulative food intake were observed at 8 h or later after JTT-130 treatment at the dosages of 10 and 30 mg/kg. However, the reduction of food intake by JTT-130 was attenuated on lower fat diets (Fig. 2). Little effect of JTT-130 on cumulative food intake was shown with a 3.1% fat diet (Fig. 2C). On the other hand, treatment with orlistat, a lipase inhibitor, significantly reversed the suppression of food intake by JTT-130 after 24 h of JTT-130 treatment in rats fed a 35% fat diet (Fig. 3). In addition, orlistat-treated rats had more food intake than vehicle-treated rats (Fig. 3).

Effect of JTT-130 on Gastric Emptying

The effects of JTT-130 on gastric emptying were evaluated in rats on a 35% fat diet. JTT-130-treated rats showed decreased gastric emptying for 4, 8 and 24 h after dosing JTT-130.
as compared with vehicle-treated rats (Fig. 4A). JTT-130 treatment decreased gastric emptying in a dose-dependent manner (Fig. 4B). Significant reductions in gastric emptying were observed at the dosages of 10 and 30 mg/kg. Like the suppression of food intake, the reduction of gastric emptying by JTT-130 was attenuated on lower fat diets (Fig. 4C).

Furthermore, we assessed the effects of orlistat on the reduction of gastric emptying by JTT-130 in rats on a 35% fat diet. Orlistat treatment significantly reversed the reduction of food intake by JTT-130 on a 35% diet (Fig. 4D).

**Effects of JTT-130 on Gut Hormones**

In order to assess the effects of JTT-130 on gut peptides known to be involved in the regulation of food intake, we measured the plasma levels of PYY, GLP-1 and CCK in the portal vein. JTT-130 treatment significantly increased plasma levels of PYY in the portal vein at 4 h or later after dosing JTT-130 on a 35% fat diet (Fig. 5A). Also, JTT-130-treated rats showed higher levels of plasma GLP-1 at 8 h or later after dosing than vehicle-treated rats on the mean-value basis (Fig. 5B). There was a near-significant elevation (P=0.06) of plasma GLP-1 level 24 h after dosing JTT-130 (Fig. 5B). On the other hand, JTT-130 treatment did not show any
elevation of CCK levels, but rather decreased CCK levels in the portal vein compared with vehicle treatment on a 35% fat diet (Fig. 5C). Meanwhile, on a 3.1% fat diet, treatment with JTT-130 produced no significant effects on plasma PYY, GLP-1 and CCK levels (data not shown).

Additionally, we evaluated whether orlistat can reverse the effects of JTT-130 on gut peptides as well as on food intake and gastric emptying in rats fed a 35% fat diet. As a result, orlistat treatment completely abolished the elevation of plasma PYY and GLP-1 levels by JTT-130 on a 35% fat diet (Fig. 6A and 6B). We also found orlistat-treated rats showed lower levels of plasma GLP-1 than vehicle-treated rats (Fig. 6B). On the other hand, no significant change was observed on CCK levels after treatment with orlistat (Fig. 6C).

**Effects of JTT-130 on lipid levels in the intestinal tissue and luminal content**

The effects of JTT-130 on lipid levels in the intestinal tissue were evaluated in rats on a 35% fat diet. JTT-130 treatment significantly increased the triglyceride content in the intestinal tissue at 4, 8 and 24h after JTT-130 dosing in rats on a 35% fat diet (Table 2). Meanwhile, JTT-130 treatment showed no significant effects on the levels of free fatty acid and cholesterol in the
intestinal tissue (Table 2). We also measured lipid levels in luminal contents in the intestine of rats on a 35% fat diet. The respective levels of triglyceride, free fatty acid and cholesterol in the luminal contents were significantly elevated in the JTT-130-treatment group (Table 3).
DISCUSSION

JTT-130 is an intestine-specific MTP inhibitor designed to be rapidly catabolized to inactive metabolites immediately after its absorption in the intestine, in order to avoid inhibition of hepatic MTP resulting in hepatic steatosis (Mera et al., in press). In our previous report, we demonstrated that JTT-130 suppressed the absorption of dietary fat by inhibiting MTP in the intestine (Mera et al., in press). Some reports have shown that ingested fat in the intestine triggers the suppression of food intake (MacIntosh et al., 2001; Pilichiewicz et al., 2006). Additionally, treatment with orlistat, a lipase inhibitor, revealed that the hydrolysis of triglyceride to free fatty acids in the intestinal lumen is required for the satiety effects of ingested fat (Feinle et al., 2003; O'Donovan et al., 2003). The digestion and absorption of fat in the intestine are thought to play an important role in the regulation of the food intake, but so far, little is known about the effects of intestinal MTP inhibition on food intake.

In this study, we revealed that the inhibition of intestinal MTP by JTT-130 leads to the suppression of food intake in Sprague-Dawley rats fed a 35% fat diet. On the other hand, JTT-130 did not suppress food intake in lower fat diets, particularly in a 3.1% fat diet, where no
significant suppression was observed. Furthermore, orlistat treatment completely abolished the

reduction of food intake by JTT-130 on a 35% fat diet. Taken together, these results suggest that
dietary fat and its degradation seem to be essential for the satiety effect of JTT-130.

Several recent reviews describe that the gastrointestinal tract is the largest endocrine organ in
the body and many gut peptides have been shown to influence energy intake (Chaudhri et al.,
2006; Murphy et al., 2006; Cummings and Overduin, 2007). The hydrolysis of ingested fat into
free fatty acids is involved in the suppression of food intake via several gut peptides. For
instance, PYY and GLP-1 are well-known gut peptides secreted in response to free fatty acids
from L cells primarily localized in the distal intestine. The administration of exogenous GLP-1
is known to reduce food intake in human and rat (Donahey et al., 1998; Flint et al., 1998;
Verdich et al., 2001). Also, the peripheral administration of exogenous PYY3-36, a bioactive
product cleaved off from PYY1-36 by dipeptidyl peptidase-4 (DPP4), is shown to reduce food
intake in human and rat (Batterham et al., 2002). In this study, we found that JTT-130 increased
plasma concentrations of PYY and GLP-1 in the portal vein in rats fed a 35% fat diet and, like
the suppression of food intake, orlistat treatment completely reversed this elevation of plasma
PYY and GLP-1 levels. Also, JTT-130 had no significant effects on plasma PYY and GLP-1 levels in rats fed a 3.1% fat diet, where its satiety effects were not observed. Thus, considering that the elevation of PYY and GLP-1 synchronized with the suppression of food intake, increased concentrations of PYY and GLP-1 are thought to be involved in the satiety effects of JTT-130 as a part of its mechanism of action. However, further studies are needed to elucidate the mechanisms underlying satiety effects of JTT-130.

In the present study, we also found JTT-130 increased the levels of triglycerides in the intestinal tissue and the luminal contents of triglycerides, free fatty acids and cholesterol in rats fed a 35% fat diet. The accumulation of triglycerides in the intestinal tissue is also found in the intestine-specific MTP deficient mice (Xie et al., 2006). In a previous study using radioactive tracers for triglycerides, we confirmed that JTT-130 treatment increased unabsorbed lipids in the intestinal lumen (Mera et al., in press). Thus, these elevations of lipids in the intestinal tissue and lumen are thought to be caused by MTP inhibition in the enterocytes. On the other hand, taking into consideration that free fatty acids increase the secretion of PYY and GLP-1 from L cells and also that treatment with orlistat which inhibits hydrolysis of triglycerides to free fatty
Acids reversed the elevation of PYY and GLP-1 by treatment with JTT-130, increased free fatty acids in the intestinal lumen might cause the elevation of PYY and GLP-1 in the portal vein. However, as the time course of the elevation of luminal free fatty acids was not consistent with that of the elevation of PYY and GLP-1, further investigations are required to clarify the relationship between the elevation of free fatty acids and the elevation of PYY and GLP-1 by treatment with JTT-130.

PYY and CCK are known to decrease gastric emptying as well as food intake (Chaudhri et al., 2006; Murphy et al., 2006; Cummings and Overduin, 2007). In this study, we also found that JTT-130 decreased gastric emptying in a dietary fat-dependent manner and this suppression of gastric emptying was completely reversed by orlistat treatment like food intake. The reduction of gastric emptying is observed in conditions where JTT-130 decreases food intake. This suggests that decreased gastric emptying could be involved in the suppression of food intake by JTT-130.

Interestingly, in contrast with PYY and GLP-1, JTT-130 treatment decreased rather than increased the plasma concentration of CCK, which is also known to be secreted in response to
free fatty acids from I cells. In the previous study, we showed JTT-130 treatment accumulated lipids mainly in the jejunum, in which the MTP is predominantly expressed (Mera et al., in press). Thus, the elevation of free fatty acid contents resulting from MTP inhibition is predicted to be milder in the proximal intestine where I cells are localized than in the distal intestine where L cell are localized. In addition to this, decreased gastric emptying by JTT-130 is supposed to result in decreased inflow of dietary nutrients such as proteins into the intestine, which are also known to stimulate the release of CCK from I cells (Liddle et al., 1985). Consequently, the different localization of endocrine cells for these gut peptides may result in the different responses to JTT-130 treatment.

Orlistat is known to reduce fat absorption by lipase inhibition in the intestine and is used for the treatment of obesity. However, the weight loss due to orlistat treatment is less than expected from the level of suppressed fat absorption, in part due to the attenuation of the acute inhibitory effects of oral fat on food intake (O'Donovan et al., 2003). In this study, we found orlistat treatment elevated cumulative food intake. Furthermore, our data revealed that GLP-1 levels in the orlistat treatment group were lower than in the vehicle treatment group. It is consistent with
a previous report where GLP-1 is reduced by orlistat treatment in humans (Ellrichmann et al., 2008). The reduction of GLP-1 is supposed to contribute to the elevated food intake by orlistat.

Since JTT-130 is able to suppress food intake as well as fat absorption (Mera et al., in press), it could be a promising new drug for the treatment of obesity and obesity-related diseases.

In summary, the present findings demonstrate that JTT-130, an intestine-specific MTP inhibitor, suppresses food intake and gastric emptying with the elevation of plasma PYY and GLP-1 in a dietary fat-dependent manner. These results suggest that JTT-130 could be useful as a drug for the treatment of obesity and obesity-related metabolic disorders.
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Authorship Contributions

Participated in research design: Hata, Mera, Ishii, Tadaki, Tomimoto, Kuroki, Kawai, and Kakutani

Conducted experiments: Hata, Mera, Ishii, Tadaki, Tomimoto, Kuroki, and Kawai

Performed data analysis: Hata, Mera, Ishii, Tadaki, Tomimoto, Kuroki, and Kawai

Wrote or contributed to the writing of the manuscript: Hata, Mera, Ota, and Kakutani
References


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Legends for figures

Fig. 1. Effects of JTT-130 on cumulative food intake

JTT-130 was administered orally to rats at the indicated dosages after 24 h food deprivation.

Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130.

Cumulative food intake was monitored until 24h after JTT-130 dosing. Data are presented as means ± S.E. from six animals. *, p < 0.05; **, p < 0.01 versus vehicle group.

Fig. 2. Effects of dietary fat on the suppression of food intake by JTT-130

JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation.

Rats were allowed to have free access to 35% fat (A), 13% fat (B) or 3.1% fat (C) diets immediately after JTT-130 dosing. Cumulative food intake was monitored until 24h after dosing JTT-130. Data are presented as means ± S.E. from six animals. *, p < 0.05; **, p < 0.01 versus vehicle group.

Fig. 3. Effects of JTT-130 on cumulative food intake in the presence of orlistat
JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation. Orlistat, a lipase inhibitor, was orally administered 30 min before JTT-130 dosing at a dosage of 100 mg/kg. Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130. Cumulative food intake was measured for 24h after JTT-130 dosing. Data are presented as means ± S.E. from six animals. **, p < 0.01 versus vehicle group. ##, p < 0.01 versus JTT-130 group.

Fig. 4. Effects of JTT-130 on gastric emptying

A. JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation. Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130. Gastric emptying was evaluated for 4, 8 and 24h after dosing JTT-130. B. Gastric emptying for 8h after JTT-130 treatment at indicated dosages was evaluated on a 35% fat diet. C. Gastric emptying for 8h after JTT-130 treatment at a dosage of 10 mg/kg was evaluated for 35% fat, 13% fat and 3.1% fat diets. D. Effects of JTT-130 at a dosage of 10 mg/kg on gastric emptying for 8h were evaluated in the presence or absence of orlistat (100 mg/kg), a lipase inhibitor, on a
35% fat diet. Orlistat was administered 30 min before JTT-130 dosing. Data are presented as means ± S.E. from six or eight animals. *, p < 0.05; **, p < 0.01 versus vehicle group. ##, p < 0.01 versus JTT-130 group.

**Fig. 5. Effects of JTT-130 on gut peptides**

JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation. Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130. Plasma levels of PYY (A), GLP-1 (B) and CCK (C) in the portal vein were measured 4, 8 and 24h after dosing JTT-130. Data are presented as means ± S.E. from eight animals. *, p < 0.05; **, p < 0.01 versus vehicle group.

**Fig. 6. Effects of JTT-130 on gut peptides in the presence of orlistat**

JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation. Orlistat, a lipase inhibitor, was administered orally 30 min before dosing JTT-130 at a dosage of 100 mg/kg. Rats were allowed to have free access to a 35% fat diet immediately after JTT-130
dosing. Plasma levels of PYY (A), GLP-1 (B) and CCK (C) in the portal vein were measured 8
h after dosing JTT-130. Data are presented as means ± S.E. from eight animals. *, p < 0.05; **, p < 0.01 versus vehicle group. ##, p < 0.01 versus JTT-130 group.
Table 1 Composition of experimental diets

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<th>13% fat diet</th>
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Table 2 Effects of JTT-130 on lipid levels in the intestinal tissue

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<th>Cholesterol (mg/g tissue)</th>
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<td></td>
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<tr>
<td>4 hr</td>
<td>114.37±10.46</td>
<td>153.27±7.24**</td>
<td>30.78±2.84</td>
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<td>115.39±8.15</td>
<td>143.65±6.14*</td>
<td>32.23±2.15</td>
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JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation.

Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130. The rats were anesthetized with diethyl ether 8 h after JTT-130 dosing. A segment of jejunum was isolated from each rat. Jejunal lipid levels were measured as described in the Methods section.

Data are presented as means ± S.E. from eight animals. *, p < 0.05; **, p < 0.01 versus vehicle group.
Table 3 Effects of JTT-130 on lipid levels in the luminal content in the intestine

<table>
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<tr>
<th></th>
<th>Triglyceride (mg/g tissue)</th>
<th>Free Fatty Acid (µEq/g tissue)</th>
<th>Cholesterol (mg/g tissue)</th>
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<td>10.03±1.73</td>
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<td>7.30±0.48</td>
</tr>
<tr>
<td>8 hr</td>
<td>5.38±1.28</td>
<td>11.13±1.38**</td>
<td>5.73±0.47</td>
</tr>
<tr>
<td>24 hr</td>
<td>0.90±0.18</td>
<td>3.85±0.73**</td>
<td>3.68±0.36</td>
</tr>
</tbody>
</table>

JTT-130 was administered orally to rats at a dosage of 10 mg/kg after 24 h food deprivation. Rats were allowed to have free access to a 35% fat diet immediately after dosing JTT-130. The rats were anesthetized with diethyl ether 8 h after dosing JTT-130. Luminal contents in the whole small intestine were collected. Their lipid levels were measured as described in the Methods section. Data are presented as means ± S.E. from eight animals. *, p < 0.05; **, p < 0.01 versus vehicle group.
Figure 1
Figure 2

Graph A: Cumulative food intake (g) vs. Time after feeding (h) for Vehicle (35% fat) and JTT-130 (35% fat).

Graph B: Cumulative food intake (g) vs. Time after feeding (h) for Vehicle (33% fat) and JTT-130 (33% fat).

Graph C: Cumulative food intake (g) vs. Time after feeding (h) for Vehicle (3.1% fat) and JTT-130 (3.1% fat).
Figure 3
Figure 4
**Figure 6**

(A) Plasma PYY (ng/mL)

(B) Plasma GLP-1 (pmol/mL)

(C) Plasma CCK (ng/mL)

<table>
<thead>
<tr>
<th>JTT-130</th>
<th>Orlistat</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
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</table>

**Notes:**
- **:** Indicates statistical significance.
- **##** Indicates strong statistical significance.
- **:** Indicates moderate statistical significance.

**Legend:**
- White bars represent control conditions.
- Gray bars represent JTT-130 treatment.
- Hatched bars represent Orlistat treatment.

**Sample Data:**
- JTT-130: - + + -
- Orlistat: - - + + +