

Clinical and experimental studies of
intraperitoneal lipolysis and the
development of clinically relevant
pancreatic fistula after pancreatic surgery

（膵切除後膵液瘻と腹腔内脂肪分解に
ついての臨床および実験的検討）

内田 雄一郎



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Intraperitoneal lipolysis causes clinically relevant pancreatic fistulas

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Complete List of Authors:	Uchida, Yuichiro; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Masui, Toshihiko; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Nakano, Kenzo; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Yogo, Akitada; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Sato, Asahi; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Nagai, Kazuyuki; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Anazawa, Takayuki; Graduate School of medicine, Kyoto University, Division of Hepato-Biliary-Pancreatic and Transplant Surgery, Department of Surgery Takaori, Kyoichi; Kyoto University Graduate School of Medicine, Division of Hepato-Biliary-Pancreatic Surgery and Transplantation Tabata, Yasuhiko; Kyoto university, Department of Biomaterials, Institute for Frontier Medical Sciences Uemoto, Shinji; Kyoto University Graduate School of Medicine, Department of Surgery
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Yuichiro Uchida*, Toshihiko Masui*, Kenzo Nakano*, Akitada Yogo*, Asahi Sato*,
Kazuyuki Nagai*, Takayuki Anazawa*, Kyoichi Takaori*, Yasuhiko Tabata† and Shinji
Uemoto*

*From the division of hepatobiliary pancreatic surgery and transplantation, department
of surgery, Kyoto University, and † from the department of Biomaterials, Institute for
Frontier Medical Sciences, Kyoto University

Corresponding author: Toshihiko Masui
Syogoinkawaharacho 54, Sakyo-ku, Kyoto, Japan
E-mail address: tmasui@kuhp.kyoto-u.ac.jp
TEL: +81-75-751-4323; FAX: +81-75-751-4348

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Summary of surgical relevance

What is already known

Clinically, there are two types of pancreatic fistulas after pancreatic resections; harmless biochemical leakage and harmful clinically relevant pancreatic fistula. Visceral obesity is one of the known risk factors of clinically relevant pancreatic fistula, however, the underlying mechanisms remained to be elucidated.

What is new

The patients with clinically relevant pancreatic fistula had significantly high free fatty acid concentration in drain discharge, suggesting the relationship between intraperitoneal lipolysis and pancreatic fistula. The investigation in rat pancreatic fistula models demonstrated that intraperitoneal lipolysis caused deterioration of pancreatic fistula, indicating that intraperitoneal lipolysis is one of the mechanisms that drives biochemical leakage to clinically relevant pancreatic fistula.

The potential impact on future practice

Intraperitoneal administration of a lipase inhibitor prevented lipolysis as well as pancreatic fistula deterioration in rat models, suggesting future clinical application of lipase inhibitors to prevention of clinically relevant pancreatic fistula.

Background: Visceral obesity is one of the risk factors of clinically relevant pancreatic fistula after pancreatic resections. However, the mechanism that high amount of visceral fat leads to clinically relevant pancreatic fistulas remains unclear. The objective of this study was to evaluate the impact of intraperitoneal lipolysis on postoperative pancreatic fistulas.

Methods: The degree of intraperitoneal lipolysis was investigated by measuring the free fatty acid concentration of drain discharge in 33 patients after pancreatic resection. A rat pancreatic fistula model was prepared by pancreatic transection, and the impact of intraperitoneal lipolysis was evaluated by intraperitoneal administration of triolein (triglyceride) with or without orlistat (lipase inhibitor). A total of 98 rats were used for experiments.

Results: The free fatty acid concentration of drain discharge on postoperative day 1 was significantly associated with the development of a clinically relevant pancreatic fistula ($p=0.004$). A higher free fatty acid concentration of drain discharge was associated with a high amount of visceral adipose tissues ($p=0.009$). In the rat pancreatic fistula models, intraperitoneal lipolysis caused an increased amount of pancreatic juice leakage (4.3 fold when compared with control, $p=0.03$) and multi-organ dysfunction. Intraperitoneal administration of a lipase inhibitor reduced lipolysis and prevented deterioration of the disease state.

Conclusion: Intraperitoneal lipolysis significantly exacerbates pancreatic fistulas after pancreatic resection. Inhibition of lipolysis by intraperitoneal administration of a lipase inhibitor could be a promising therapy that specifically targets the mechanism that drives the biochemical leakage to clinically relevant pancreatic fistula.

Introduction

Postoperative pancreatic fistulas remain among the most frequent and serious complications after pancreatic surgery, although several preventive interventions have been tried.^{1,2} Currently, the ISGPS definition is predominantly used as the grading criteria for pancreatic fistulas worldwide. There are two stages of the development of pancreatic fistulas: one is harmless pancreatic juice leakage (biochemical leak), and the other is harmful (clinically relevant pancreatic fistula (CR-POPF)).³ Because a biochemical leak has negligible clinical impact, the most important issue for improving clinical outcomes is to prevent the deterioration from biochemical leak to CR-POPF. However, the mechanism that drives the biochemical leak toward CR-POPF is unknown.

Obesity is one of the major risk factors related to various surgical procedures.^{4,5} Although it is debatable whether obesity is an independent risk factor for CR-POPF,^{6,7} visceral obesity, which is sometimes present with obesity,⁸ has been reported as an independent risk factor for developing CR-POPF.⁹⁻¹¹ However, the underlying mechanisms remain to be elucidated.

During pancreatic surgery, intraperitoneal saponification around the pancreas is sometimes observed as white spots and considered the result of lipolysis due to pancreatic lipase.¹² Currently, intraperitoneal lipolysis is considered one factor related to deterioration of acute pancreatitis and systemic inflammation.¹²⁻¹⁵ We hypothesized that intraperitoneal lipolysis was associated with the deterioration of pancreatic fistulas.

The objective of this study was to clarify the effects of intraperitoneal lipolysis following pancreatic juice leakage on the deterioration of pancreatic fistulas. Using the peritoneal drainage of patients, the present study showed that intraperitoneal lipolysis is significantly associated with the development of CR-POPF. To determine whether

intraperitoneal lipolysis is the cause of, or only the result of, a pancreatic fistula, a rat pancreatic fistula model was prepared by pancreatic transection. To evaluate the effects of intraperitoneal lipolysis by leaked pancreatic enzymes, we administered triolein (a type of triglyceride) to rats intraperitoneally. The preventive effects of intraperitoneal administration of a lipase inhibitor were also evaluated using this model.

Materials and methods

Patients

A total of 35 patients who had undergone pancreaticoduodenectomy or distal pancreatectomy at Kyoto University Hospital between September 2017 and September 2018 were investigated. Two patients whose drain discharge was less than 5 ml/day were excluded from the analysis. This study was approved by our institutional ethics review board (approval number R0884), and written informed consent was obtained from all participants.

Perioperative management

Operative procedures and perioperative management were performed as previously described.^{16,17}

Pancreatoduodenectomy: All pancreatic anastomoses to the alimentary tract were accomplished using end-to-side pancreatojejunostomy. Two prophylactic drainage tubes were routinely placed intraoperatively. One drainage tube was placed beside the pancreatic anastomosis, and the other was placed behind the biliary anastomosis. The discharge of peripancreatic anastomosis drainage was evaluated in this study.

Distal pancreatectomy: The resection of the pancreas was performed by a linear stapler, and a prophylactic drainage tube was routinely placed beside the pancreatic stump in most cases. Drain free fatty acid concentrations were evaluated on postoperative day

(POD) 1. Drain amylase and lipase values were evaluated on PODs 1 and 3, and drainage tubes were removed on POD 5, except when there was a need for prolonged placement. Pancreatic fistulas were graded according to the 2016 International Study Group of Pancreatic Surgery (ISGPS) definition.³

Visceral/subcutaneous fatty area analysis

Within 4 weeks before surgery, all patients underwent preoperative computed tomography (CT) including an initial non-enhanced acquisition with a section thickness of 5 mm. Patients' fat distribution was analyzed using preoperative CT as previously described¹¹. Briefly, the image at the level of the transverse process of the L3 vertebra was saved as a DICOM image for the analysis. Using Image J freeware (<http://rsb.info.nih.gov/ij/>), the total and visceral compartments were delineated, pixels with densities in the -190 HU to -30 HU range were measured, and the cross-sectional area of each was calculated in cm². The subcutaneous fatty area was obtained by subtracting the visceral fatty area from the total fatty area.

Animal experiments

All animal experiments were approved by the institutional Animal Care and Use Committee of Kyoto University and performed in line with the ARRIVE guidelines. Male, 9-week-old, Sprague-Dawley rats (Shimizu Laboratory Supplies, Kyoto, Japan) were purchased, acclimatized for at least one day prior to use, and used only when their body weight was 280-320 g. Only male animals were used to suppress variations of body weight change or biochemical backgrounds. The animals were housed with a 12-h light/dark cycle, at temperatures ranging from 21 to 25°C, fed standard laboratory chow, and allowed to drink ad libitum. A rat pancreatic fistula model (PT group) was established by pancreatic transection, as previously described.¹⁸⁻²⁰ Briefly, the rats were

anesthetized with sevoflurane and intraperitoneal injection of pentobarbital. Subcutaneous injection of buprenorphine was used for analgesia. After midline laparotomy, the pancreas was encircled at the ventral side of the portal vein without any mobilization. The splenic artery and vein were preserved. The rat pancreatic duct is composed of four branches; the gastric duct, the duodenal duct, the common duct, and the splenic duct.^{18,21} The splenic duct and surrounding pancreatic parenchyma were sharply transected with scissors. The bleeding from the pancreatic stump was easily controlled by compression with gauze for 1 minute. Fatty tissues were kept intact throughout the procedure. To investigate the effect of intraperitoneal lipolysis, some groups (PTTG group and the PTTG+O group) received intraperitoneal administration of either triolein (10 ml/kg body weight) or triolein+orlistat (Tokyo Chemical Industry, Japan, 50 mg/kg body weight, dissolved in the triolein), respectively, after pancreatic transection. The Sham group only underwent laparotomy. The TG group underwent laparotomy and received intraperitoneal triolein (10 ml/kg body weight) without pancreatic transection. The overall experimental setup and abbreviations were summarized in Figure 1 (Fig.1). Animals were euthanized 24 h after operation or observed for 7-28 days for survival analysis. During observation, their body weight was evaluated every two days, and if their body weight reached less than 80% of baseline, they were euthanized to avoid excess distress, which was thought to be a humane end-point. Blood samples were obtained from the inferior vena cava immediately after euthanization. Peritoneal lavage fluid was obtained from the abdominal cavity after irrigation with 4 ml of normal saline, centrifuged to obtain the supernatant and evaluated as ascites. All samples were aliquoted and analyzed immediately or frozen at -80°C for subsequent analyses after a single thaw on ice.

Biochemical Assays

Biochemical assays including AST, ALT, LDH, creatinine, BUN, amylase, and lipase

were performed in a professional clinical laboratory (Japan Clinical Laboratories, Kyoto, Japan).

Measurement of free fatty acid concentrations

The free fatty acid concentration of each sample was determined by high-performance liquid chromatography (Detector Prominence SPD-20A, Shimadzu, Japan; Column YMC pack FA, YMC, Kyoto, Japan) using a labeling drug (XSRFAR01, YMC).²² Margaric acid (C17:0) was used as the internal standard. The total fatty acid (TFA) concentration was calculated by adding individual C12:0, C14:0, C16:0, C18:0, C16:1, C18:1, and C18:2 fatty acids, and unsaturated fatty acid (UFA) was calculated by adding individual C16:1, C18:1, and C18:2 fatty acids, as previously described.¹² Drain discharge was collected from sterile bags 24 h after the operation, centrifuged (300 g, 5 min) to obtain the supernatants, and then subjected to analysis.

Histological analysis

Tissues were extracted 24 h after the operation, fixed with 10% buffered formalin for 48 h, and 4- μ m-thick, paraffin-embedded sections were prepared. Distal pancreas, right median lobe of liver, and right kidney were observed for pancreas, liver, and kidney histology, respectively. Hematoxylin and eosin staining and alizarin red S staining (Sigma-Aldrich, for detection of calcium deposition) were performed according to the standard protocols. TUNEL staining was performed using the DeadEnd™ Colorimetric TUNEL System (Promega, Madison, WI, USA) according to the manufacturer's protocol. The degree of pancreatic inflammation was scored using a previously reported method.²² Briefly, the extents of edema, inflammatory cell infiltration, and acinar necrosis were individually scored on a scale of 0-3, and the scores were summed to obtain the total score. Observations were supervised by a pathologist, and five random sites of each slice were subjected to quantitative analysis and counted using Image J software.

Statistical analysis

Data analyses were carried out using JMP Pro version 12.0 (SAS, Tokyo, Japan). After Levene’s test for variances, groups were compared with the *t*-test, Mann-Whitney U-test, one-way analysis of variance with the post hoc Tukey test, or the Steel-Dwass test, as appropriate. Categorical variables were compared using Fisher’s exact test. Significances were categorized according to levels (N.S. nonsignificant, *P < 0.05, **P < 0.01, ***P < 0.001). Mean±SD values are shown. Five independent animals/samples were analyzed unless otherwise stated. Kaplan-Meier survival analysis and the log-rank test were used to plot and evaluate rat survival.

Results

The relationship among visceral fat, lipolysis and CR-POPF

The patients with CR-POPF had significantly high visceral fatty area (72 vs 115 cm², p=0.015) and significantly high TFA concentrations of drain discharge. (p=0.004) The TFA concentration was found to be a predictor of the development of CR-POPF (area under the curve [AUC]: 0.863, 95% confidence interval [CI]: 0.662–0.953). Based on the receiver operating characteristic curve, the cutoff value of TFA was set to 1 mM (sensitivity: 0.857; 1-specificity: 0.703), and patients were categorized into either a high lipolysis group (TFA ≥1) or a low lipolysis group (TFA <1). Interestingly, high lipolysis group patients had a significantly higher visceral fatty area (70 vs 112 cm², p=0.009) and higher pancreatic enzyme activities in drain discharge. Patients with biochemical leakage were all in the low lipolysis group. (Table 1) Separate analyses based on operative procedures yielded similar results. (Supplemental Table 1 and 2) Based on these findings, we hypothesized that there were specific relationships among intraperitoneal fat, lipolysis and CR-POPF.

Rat PT model demonstrates evident pancreatic juice leakage, but not severe phenotype.

To investigate whether intraoperative lipolysis directly causes CR-POPF, animal experiments were conducted. First, the known rat pancreatic fistula model by pancreatic transection (PT) was validated. In the PT model, transient intraperitoneal pancreatic juice leakage was observed (Fig.2A, B). Macroscopically, there was no obvious intraperitoneal change after PT (Fig.2C). Evaluation of body weight change (Fig.2D) and survival analysis (100% for 28 days, n=5) showed little impact of PT on physiological damage. Although there was slight elevation of serum pancreatic enzymes and AST in the PT group, organ dysfunction, which is an important aspect of CR-POPF, was not observed (Fig.2E).

Intraperitoneal lipolysis dramatically worsens the disease state of PT.

Next, the effect of lipolysis on POPF was investigated using the PT model, triglyceride (triolein), and lipase inhibitor (orlistat). Leakage of triglyceride from fat cells by operative damage was mimicked with triglyceride administration. Intraperitoneal findings of PTTG were dramatically different from those of PT, and many white spots with abnormal dilatation of the intestine were observed (Fig.3A). These white spots were positive for alizarin red staining, suggesting that they are the deposition of free fatty acid calcium salt (Fig.3B). Intraperitoneal lipolysis in the PTTG group was confirmed by a dramatic increase in free fatty acid concentration in ascites, which was largely inhibited by concurrent administration of orlistat (Fig.3C). Survival analysis and body weight change analysis showed a significant deterioration of the disease state in the PTTG group, while the PTTG+O group was equivalent to the PT group (Fig.3D, E).

Multi-organ damage was induced by intraperitoneal lipolysis.

To determine the systemic impact of lipolysis, serum biochemical analysis was performed. Serum AST, ALT, LDH, creatinine, and BUN levels showed severe damage to the liver and kidney in the PTTG group compared with the PT group. Damage was largely prevented by the administration of a lipase inhibitor (Fig.3F). The damage induced apoptosis of the hepatic and renal cells, shown histologically by TUNEL staining (Supplemental Figure. 1A-D). There were also elevations of serum TFA and UFA concentrations in the PTTG group (Supplemental Figure. 1E). These changes were prevented by concurrent administration of a lipase inhibitor.

Intraperitoneal lipolysis increased the amount of pancreatic juice leakage.

When pancreatic enzyme levels were analyzed in the ascites, their levels were significantly elevated in the PTTG group compared with the other groups (Fig.4A). Although there were slightly higher (not significant) serum pancreatic enzyme levels and edematous changes in the pancreas in the PTTG group (Fig.4B, C), the pancreatic inflammation was slight and of the same degree among the PT, PTTG, and PTTG+O groups, suggesting that these changes were not due to pancreatitis (Fig.4D). Histological findings suggesting severe pancreatitis, such as necrosis or intrapancreatic hemorrhage, were not observed in any of the groups. Only in the PTTG group, there was pancreatic surface destruction with saponification spots and invasion of inflammatory cells (Fig.3C, enlarged).

Evaluation of the chemical lipase administration model and free fatty administration model reproduces the changes between the PT and PTTG groups.

To directly clarify the effect of intraperitoneal lipolysis, animal experiments with chemical lipase administration with or without triglyceride and lipase inhibitor were

conducted (Supplemental Figure 2). Concurrent administration of lipase and triglyceride induced similar intraperitoneal changes as found in PTTG and caused significant deterioration of the survival rate and body weight decrease, multi-organ damage including liver, kidney and pancreas. Significant elevations of ascitic pancreatic enzyme activities were also observed. Histological examination showed destruction of the pancreatic surface with deposition of fatty acid calcium salt. All of these changes were ameliorated by the concurrent administration of a lipase inhibitor. Administration of oleic acid (free fatty acid produced by degradation of triolein) to rats induced similar deterioration, including biochemical and histological changes (Supplemental Figure 3).

Discussion

The current study demonstrated, for the first time, that intraperitoneal lipolysis subsequent to pancreatic juice leakage could worsen the disease state of pancreatic fistulas.

Recently, visceral obesity has been considered a risk factor for the development of CR-POPF.⁹⁻¹¹ Triglycerides are stored in fat cells and leak out into the intraperitoneal space with operative damage.²⁴ We hypothesized that, with abundant visceral fatty tissues, the amount of triglycerides that leak into the peritoneum with operative damage could be increased, and leaked triglycerides could have a worsening effect on fistulas. If there were leaked pancreatic juice, triglycerides would be hydrolyzed into free fatty acids by pancreatic lipase. Therefore, the present study focused on free fatty acid concentrations. Indeed, it was found that there was more visceral fatty area in high lipolysis group patients. (Table 1) The TFA concentration of drain discharge was significantly associated with the development of CR-POPF. (Table 1) We thought that these findings may indicate that intraperitoneal lipolysis is related to the pathology of CR-POPF and the mechanisms of fistula deterioration with visceral obesity, but

whether intraperitoneal lipolysis causes severe CR-POPF or is only a result of CR-POPF still needed to be resolved.

To determine the causal relationship between CR-POPF and intraperitoneal lipolysis, animal experiments were conducted. First, the known pancreatic fistula model in which pancreatic juice leakage is induced by simple pancreatic transection¹⁸⁻²⁰ was validated to elucidate the severity and precise biological changes of this model (Fig.2). Because pancreatic juice contains various and vast amounts of digestive enzymes, it is believed that the disease state of POPF could be mainly due to the destruction of surrounding tissues by these enzymes.^{25,26} Interestingly, there was no destruction of surrounding tissues and no severe phenotype in the PT group, even though there was obvious pancreatic juice leakage. This could support the idea that leaked pancreatic juice is not always harmful.

Comparing the PT model to harmless leakage in clinical settings, animal experiments were conducted to show whether intraperitoneal lipolysis could worsen pancreatic fistulas. To mimic intraperitoneal triglyceride leakage with good reproducibility, triglyceride was administered into the peritoneum. We used triolein as a model triglyceride because the most abundant fatty acid chain in triglycerides is that of oleic acid²⁷ (triolein consists of three oleic acid chains) and our clinical data suggest that the most abundant FFA in drain discharge was also oleic acid. It was found that intraperitoneal administration of triglyceride to the PT group (i.e. the PTTG group) dramatically worsened the disease state, even though the triglyceride itself was harmless (Fig.3, Supplemental Figure 4). There was significant body weight loss and decreased survival in the PTTG group. In addition, significant liver and renal damage was found in the PTTG group compared with the PT group. Taken together, the PTTG group might mimic the disease state of CR-POPF Grade C, which is defined as having an adverse effect on survival and shows organ dysfunction.³

The amount of pancreatic juice leakage increased in the PTTG group (Fig.4A). Because the serum pancreatic enzyme levels tended to be higher (not significantly) in the PTTG group (Fig.4B), the degree of pancreatic inflammation was investigated histologically; there was no significant difference among the groups, and it was confirmed that these changes were not the result of pancreatitis (Fig.4C, D). Rather, there was tissue destruction on the pancreatic surface with fatty acid calcium salt deposition only in the PTTG group (Fig.4C), suggesting that the saponification around the pancreas caused the damage to the pancreas itself and induced additional pancreatic juice leakage.

These changes were reproduced in the chemical lipase administration model and the free fatty acid administration model in which amylase leakage would never occur without pancreatic damage (Supplemental Figure 2 and 3), suggesting that saponification around the pancreas caused the damage to the pancreas itself and induced additional pancreatic juice leakage. These results could partly explain the fact that significantly higher pancreatic enzyme activities were found in high lipolysis group patients. (Table 1)

More importantly, these deteriorations, including multi-organ damage and increased pancreatic juice leakage, were effectively prevented by the intraperitoneal administration of lipase inhibitors in both rat models, suggesting that the application of a lipase inhibitor around the pancreas could potentially prevent CR-POPF after pancreatic surgery. Orlistat has not been approved for intraperitoneal usage and it is possible that these effects were caused by the unknown mechanisms of orlistat. Thus, the effect of lipase inhibition was validated using another kind of inhibitor (cetlistat) (Supplemental Figure 5), supporting the notion that the prevention of the severe state was directly due to the lipase inhibition. We are now conducting a trial to develop protective agents for pancreatic surgery using lipase inhibitors.

The findings of this study must be considered in light of several limitations. Although

the intraperitoneal administration of triglyceride is a method that has been used to study the effects of intraperitoneal lipolysis¹⁴ and it was confirmed that the procedure itself did not have a negative physiological impact, the possible differences between these experimental models and physiological condition should be taken into consideration. Although our investigation of clinical cohorts suggests a possible relationship between visceral obesity and CR-POPF, this was not experimentally confirmed. An investigation using obese animals would further clarify the mechanisms for the relationship between POPF and visceral obesity.

Chyle leak is an important complication after pancreatic surgery and this complication could be relevant to our model because chyle contains triglycerides. Recently, chyle leak was found to be significantly associated with CR-POPF.²⁸ We should also note that the major contents of chyle are not free triglycerides but chylomicron, which is a combination of lipoproteins and triglycerides, and pancreatic lipase cannot catalyze lipids within lipoproteins.²⁹ We were unable to investigate this complication because there were few patients suffering from chyle leak. Fatty pancreas could be another important issue regarding our study, but evaluation using CT information lacked reproducibility¹¹ and we were unable to investigate this issue due to a lack of histological data. The underlying mechanisms of multi-organ dysfunction caused by intraperitoneal lipolysis still remain unclear. Others have reported that intraperitoneal lipolysis worsens systemic inflammation and multi-organ disorders via elevation of serum unsaturated fatty acid concentrations and mitochondrial toxicity in the setting of acute pancreatitis.^{13, 14} Of note, unsaturated fatty acids such as oleic acid are known to cause lipotoxicity and induce kidney or liver damage.³⁰⁻³² In the present study, the serum unsaturated fatty acid concentration was increased in the PTTG group, and the acute tubular necrosis found on renal histology in the PTTG group (Supplemental Figure 1) was one of the features of fatty acid-induced renal injury.³¹ The administration

of oleic acid itself also induced multi-organ dysfunction (Supplemental Figure 3). These findings might support the previously reported idea that acute lipotoxicity induces multi-organ dysfunction,¹³ but further investigation is needed to elucidate the mechanism of deterioration. The present patient cohort was relatively small and did not have many obese patients due to the characteristics of our region. Whether our findings could be extrapolated to more obese patients should be investigated in a larger cohort.

In conclusion, intraperitoneal lipolysis is one of the key factors causing deterioration of pancreatic fistulas, which may drive biochemical leak into CR-POPF. This would account for, at least in part, the underlying relationship between visceral obesity and CR-POPF. Intraperitoneal administration of lipase inhibitors effectively prevented the deterioration of pancreatic fistulas in rodent models. We hope that topical administration of a lipase inhibitor could be a breakthrough therapy for preventing the development of CR-POPF.

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Figure legends

Figure 1. Overall experimental setup and group abbreviations

Pancreatic transection was performed under laparotomy. The structural formula of triolein is shown (three oleic acid chains). ip: intraperitoneal administration

Figure 2. Characterization of the rat PT model

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A, Schematic picture of PT, pre (left) and post (right) transection. White line: edge of the pancreas, blue line: portal vein, dashed line: cut line of the pancreas. B, Ascitic amylase and lipase values according to postoperative day (POD) in PT groups. (N=5) The amylase and lipase values of ascites on POD1 in sham groups were shown as controls. (N=5) C, Macroscopic findings of the peritoneum. D, Changes of body weight. (N=5) E, Serum biochemical evaluation. (N=5)

Figure 3. Intraperitoneal lipolysis dramatically worsens the disease state of PT.

A, Macroscopic findings of the peritoneum. Arrow indicates abnormal dilatation of the intestine. B, Alizarin red S staining on the peritoneal surface. Arrow indicates calcium salt deposition. C, Ascitic total free fatty acid concentration. (N=5) D, Kaplan-Meier curves. (N=5 for PT, PTTG+O group, N=7 for PTTG, PTTG (low) group) The PTTG (low) group received a reduced dose of triolein (3 ml/kg body weight). E, Body weight change on POD 3. (N=5) Considering the mortality rate on POD 3, the PTTG low-dose group was evaluated instead of the whole PTTG group. F, Serum biochemical analysis for liver and kidney damage (N=5-7).

The group abbreviations were summarized in figure 1.

Figure 4. Intraperitoneal lipolysis increases the amount of pancreatic juice leakage.

A, Ascitic amylase and lipase values (N=5-7) B, Serum amylase and lipase values (N=5-7) C, Histology of the pancreas, hematoxylin-eosin (HE) staining. Square indicates the area of higher magnification. Left, HE; right, alizarin red S staining. D, Histological scoring for pancreatitis. (N=5)

The group abbreviations were summarized in figure 1.

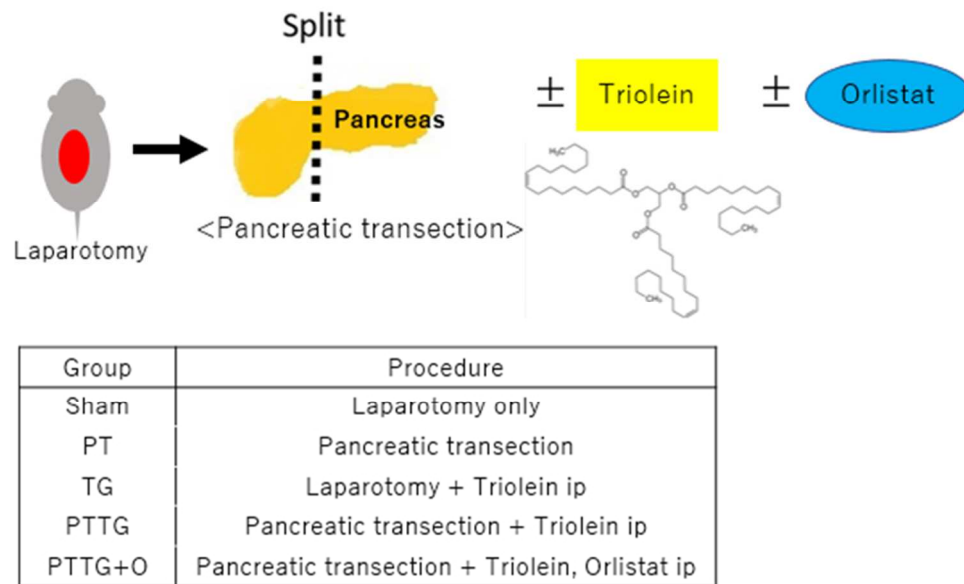
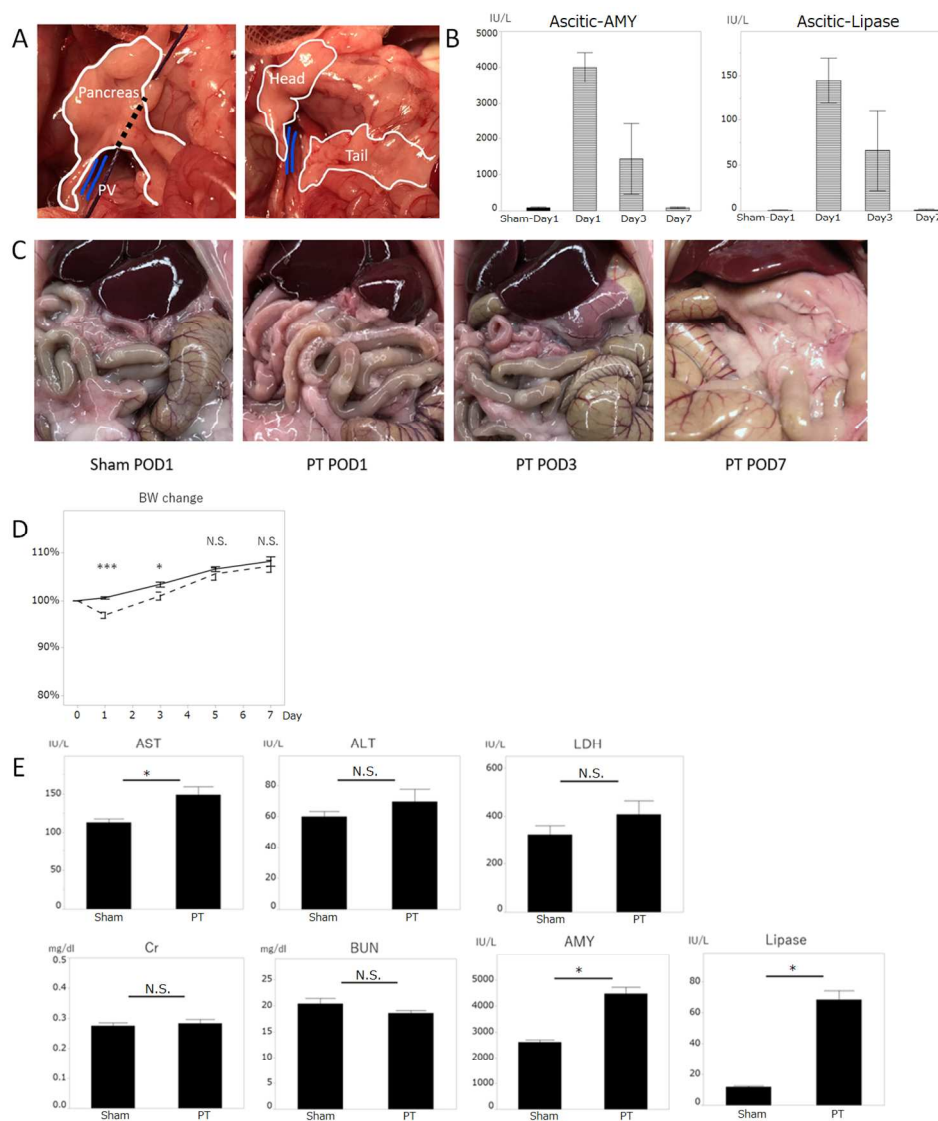


Figure 1. Overall experimental setup and group abbreviations.

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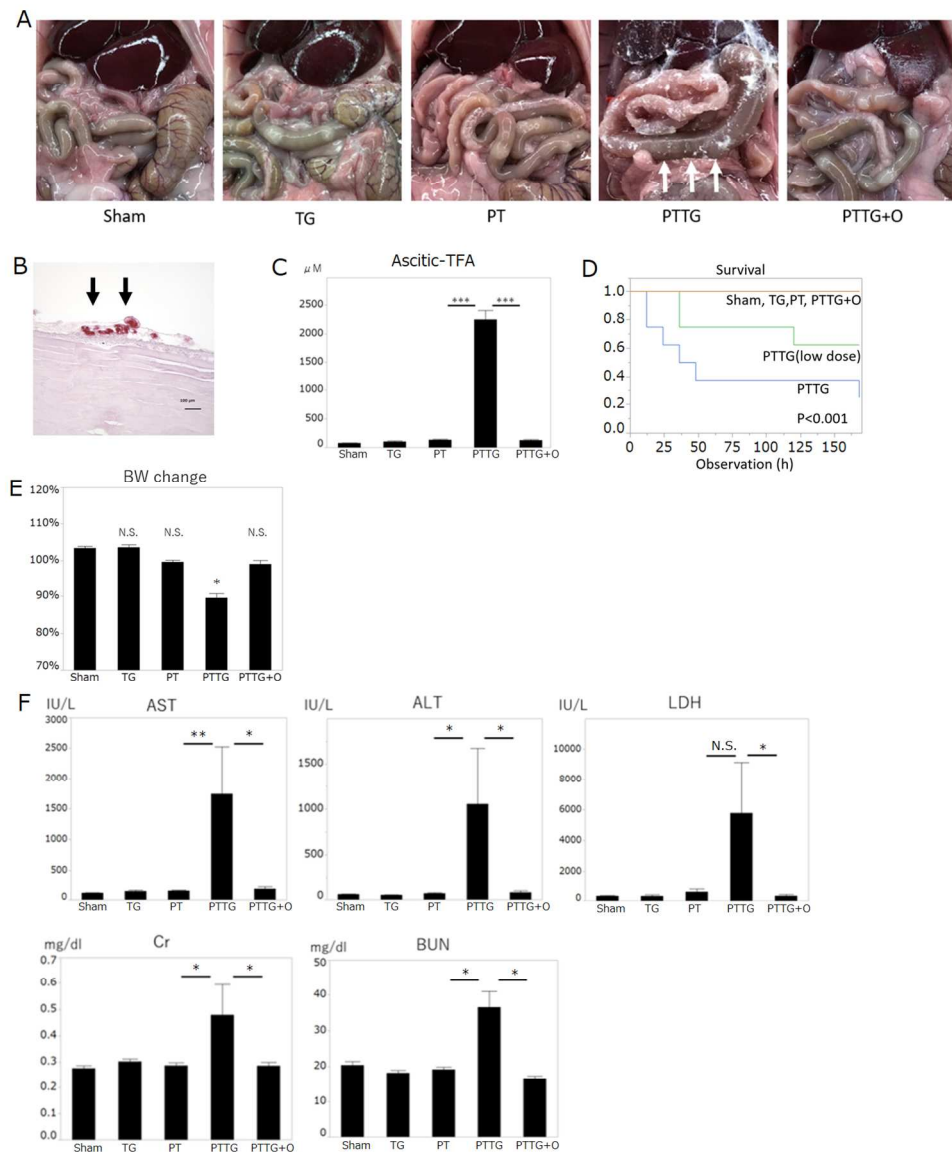


Figure 3. Intraperitoneal lipolysis dramatically worsens the disease state of PT.

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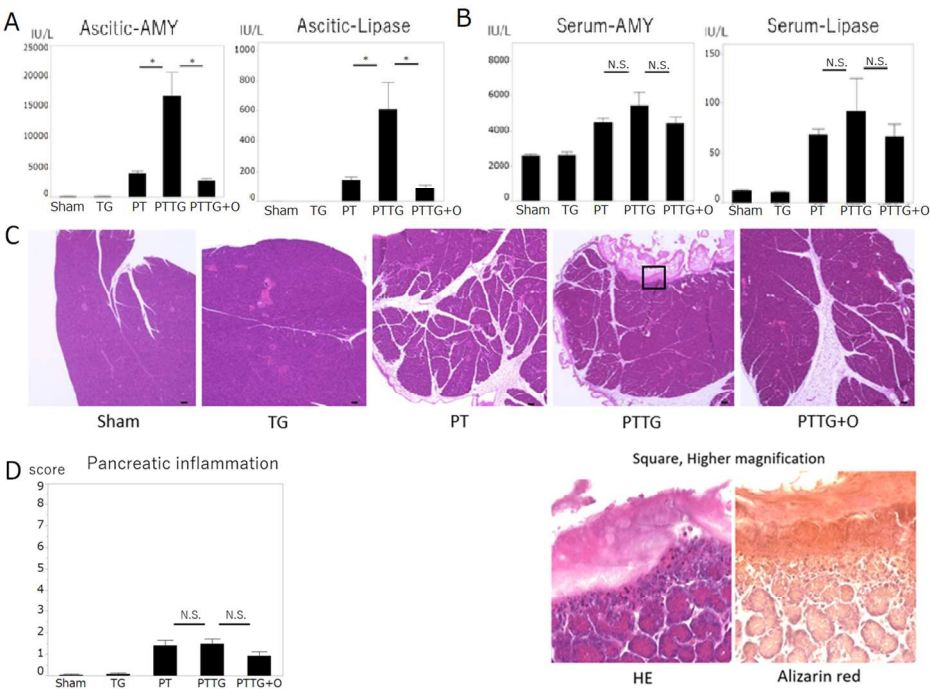


Figure 4. Intraperitoneal lipolysis increases the amount of pancreatic juice leakage.

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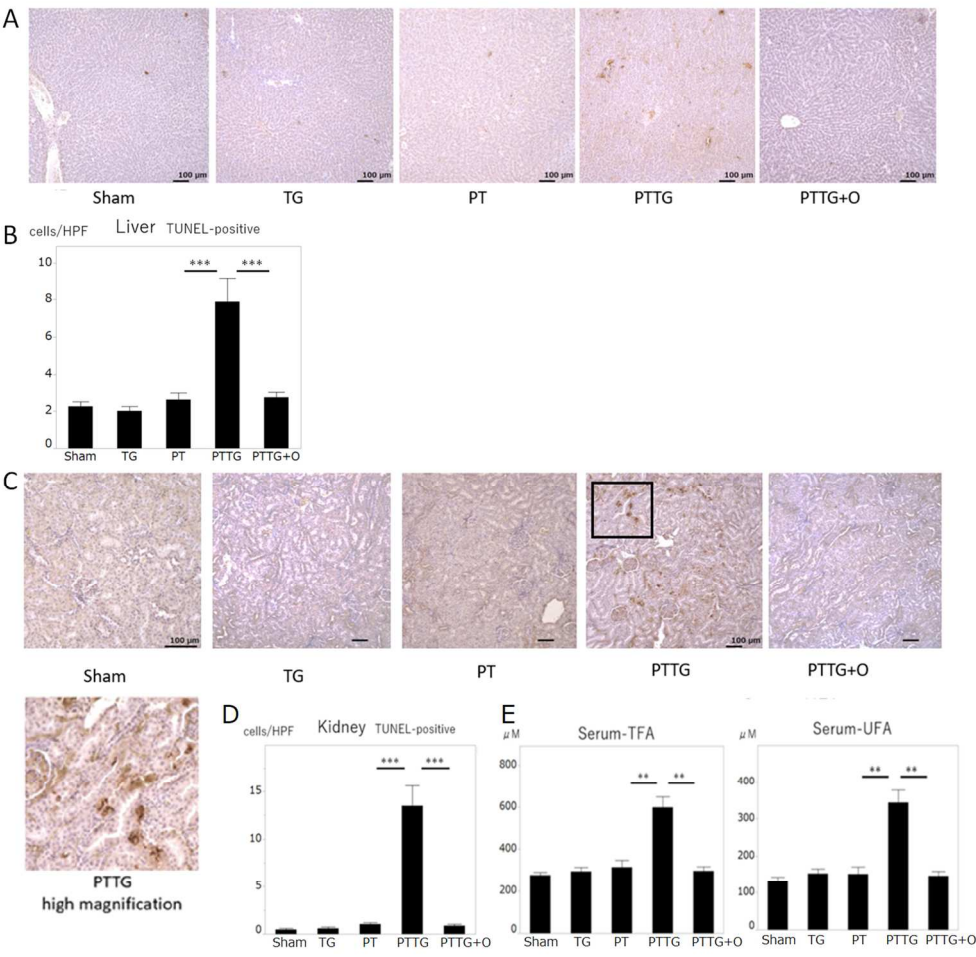
Table 1. Patients' clinical characteristics

	Low lipolysis (n=23)	High lipolysis (n=10)	P
Sex, male	12 (52%)	6 (60%)	0.72
Age (years)	70 (62–76)	71 (65–77)	0.56
BMI (kg/m ²)	20.7 (19.1–23.7)	23.7 (20.9–27.8)	0.11
Total fatty area (cm ²)	150 (68–216)	244 (139–268)	0.04
Visceral fatty area (cm ²)	70 (28–105)	112 (77–131)	0.009
Subcutaneous fatty area (cm ²)	75 (32–110)	97 (68–161)	0.26
Operation type, PD/DP	PD;13 DP;10	PD;6 DP;4	1
Pancreatic texture, soft	7 (30%)	4 (40%)	0.70
POPF non-POPF	15 (68%)	3 (38%)	
Biochemical leakage	6 (27%)	0 (0%)	
CR-POPF	1 (4%)	6 (60%)	0.001
POD1 Drain AMY (IU/L)	437 (99–1975)	1838 (1338–18368)	0.005
POD1 Drain Lipase (IU/L)	635 (48–4555)	3860 (2589–12966)	0.01

Table 1. Patients' clinical characteristics

Patients were categorized into either a high lipolysis group (TFA $\geq 1\text{mM}$) or a low lipolysis group (TFA $< 1\text{mM}$) according to the TFA concentration of drain discharge on POD 1. BMI, body mass index; PD, pancreaticoduodenectomy; DP, distal pancreatectomy.

Each parameter is shown as the median (interquartile range).



409x404mm (96 x 96 DPI)

Supplemental Figure 1

Multi-organ dysfunction is induced by intraperitoneal lipolysis.

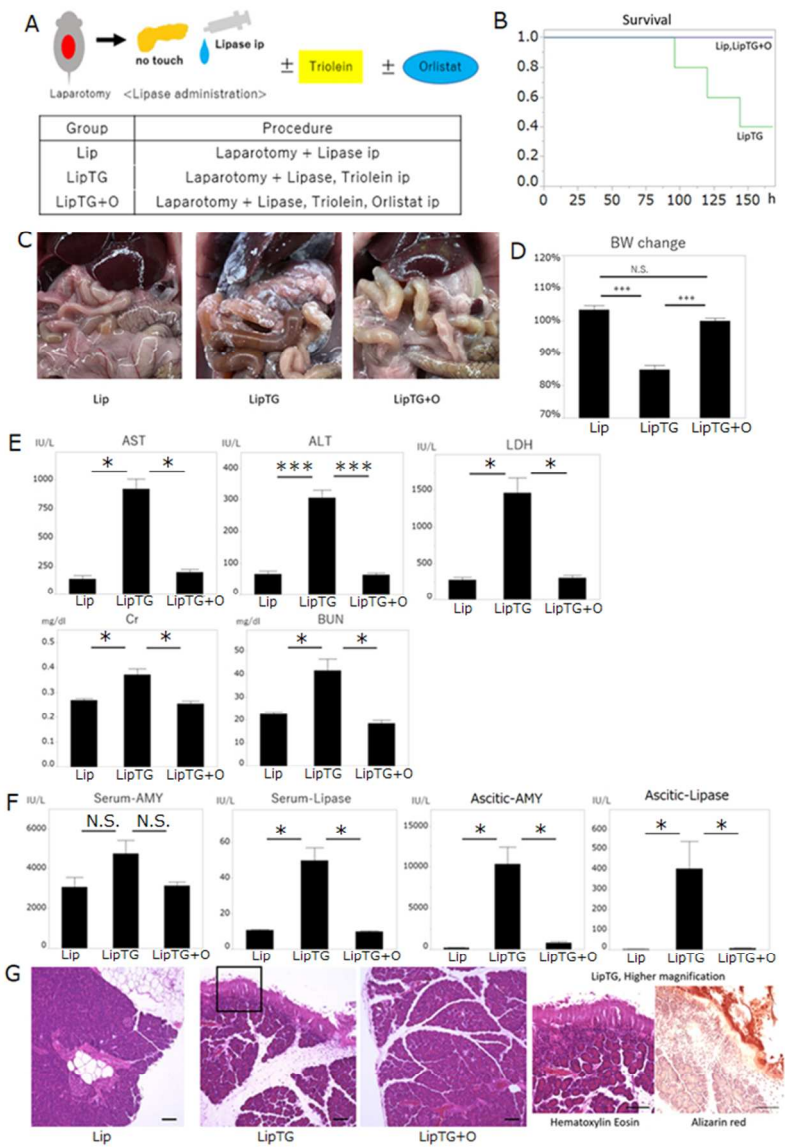
A and B, TUNEL staining for liver histology and its quantification. (N=5) C and D,

TUNEL staining for kidney histology and its quantification. (N=5) Square indicates

the enlarged area. E, serum TFA and UFA concentrations. (N=5)

For histological quantifications, five random sites of each slice were subjected to

quantitative analysis per one animal and counted using Image J software.



202x289mm (96 x 96 DPI)

Supplemental Figure 2

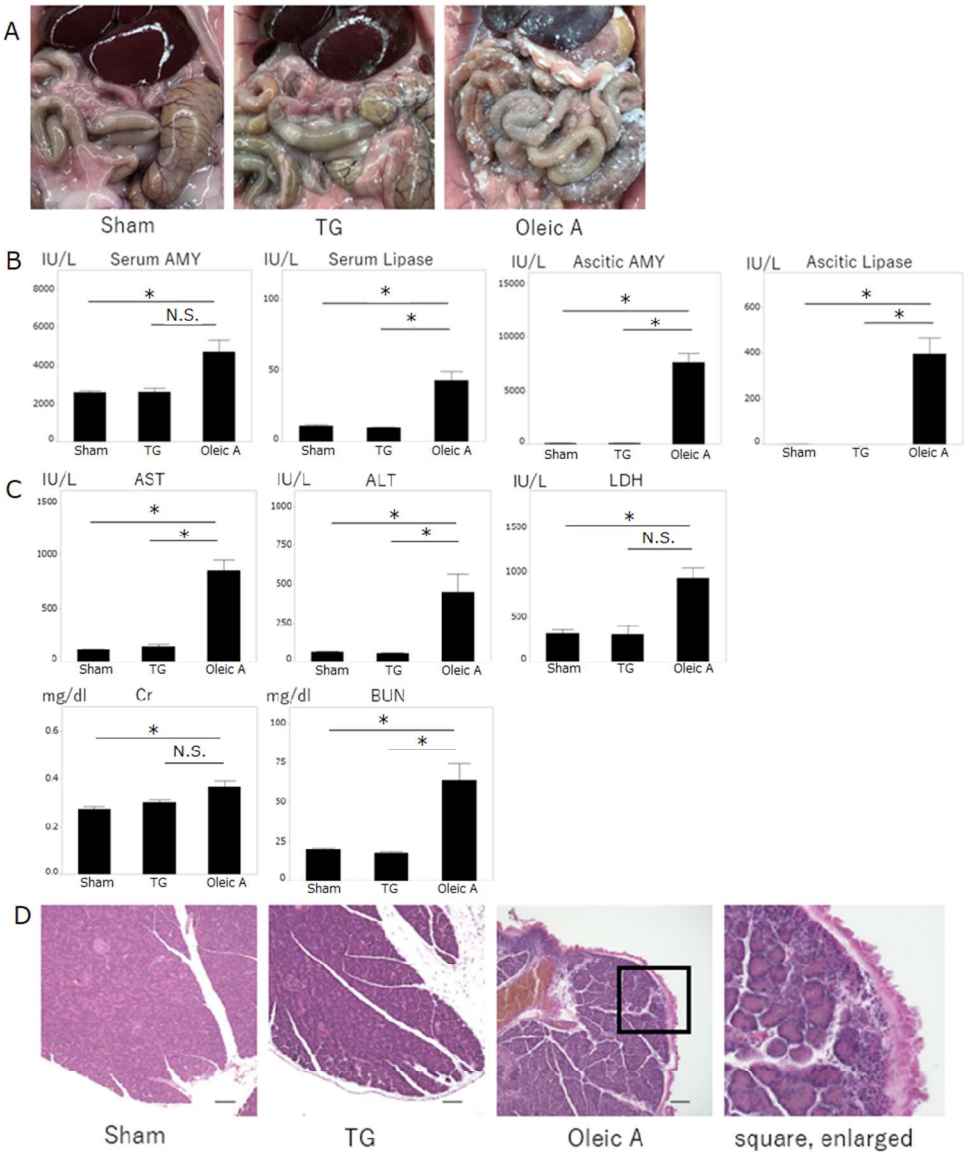
Evaluation of the chemical lipase administration model

The chemical lipase leakage model was established by laparotomy and intraperitoneal administration of lipase (L1754, Sigma Aldrich, St. Louis, MO, USA, 1000 U dissolved in 1 ml of normal saline) (Lip group). The LipTG group and LipTG+0 group received intraperitoneal administration of both lipase and triolein (10 ml/kg body weight) without or with orlistat (50 mg/kg body weight), respectively. Animals were euthanized 24 h after operation or observed for 7 days for survival analysis.

A, Overview of experimental set-up and group abbreviations

B, Kaplan-Meier curves for rat survival. (N=5) C, Macroscopic peritoneal findings.

D, Body weight change on POD 3. (N=5) E, Serum biochemical analysis (N=5) F, Serum and ascitic pancreatic enzyme values. (N=5) G, Histology of the pancreas, hematoxylin-eosin (HE) staining. Square indicates the area of higher magnification (left, HE; right, alizarin red S staining).



266x313mm (96 x 96 DPI)

Supplemental Figure 3

Intraperitoneal administration of oleic acid induced pancreatic juice leakage and damaged liver and kidney.

Oleic acid (Nacalai Tesque, Kyoto, Japan, 10ml/kg body weight) was intraperitoneally administered in Oleic A group after laparotomy. Sham group underwent laparotomy. TG group underwent intraperitoneal administration of triolein after laparotomy.

Experimental methods and drug dose were detailed in main text.

A, Intraperitoneal macroscopic findings

Intraperitoneal white spot was observed in Oleic A group.

B, Serum and ascitic pancreatic enzyme levels.

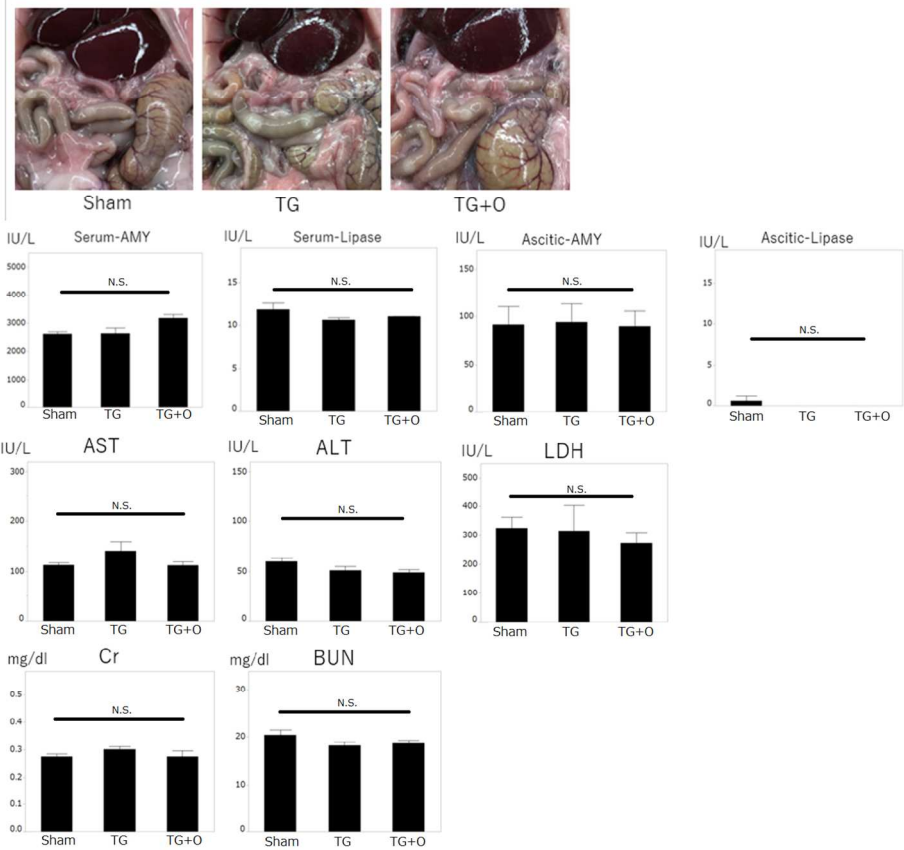
Ascitic pancreatic enzyme levels were significantly elevated in Oleic A group. (N=5)

C, Serum biochemical analysis for liver and kidney damage

Liver and kidney damage were observed in Oleic A group. (N=5)

D, Histological examination on pancreas (Haematoxylin-Eosin staining)

Acinar necrosis with inflammatory cell invasion was observed on the pancreatic surface in Oleic A group. Bar indicates 100 μ m.



409x404mm (96 x 96 DPI)

Supplemental Figure 4

Intraperitoneal administration of triolein or tiolein+orlistat did not have negative physiological impact.

Sham group underwent only laparotomy. TG group underwent intraperitoneal administration of triolein after laparotomy. TG+0 group underwent intraperitoneal administration of triolein+orlistat after laparotomy. Experimental methods and drug dose were detailed in main text.

A, Intraperitoneal macroscopic findings

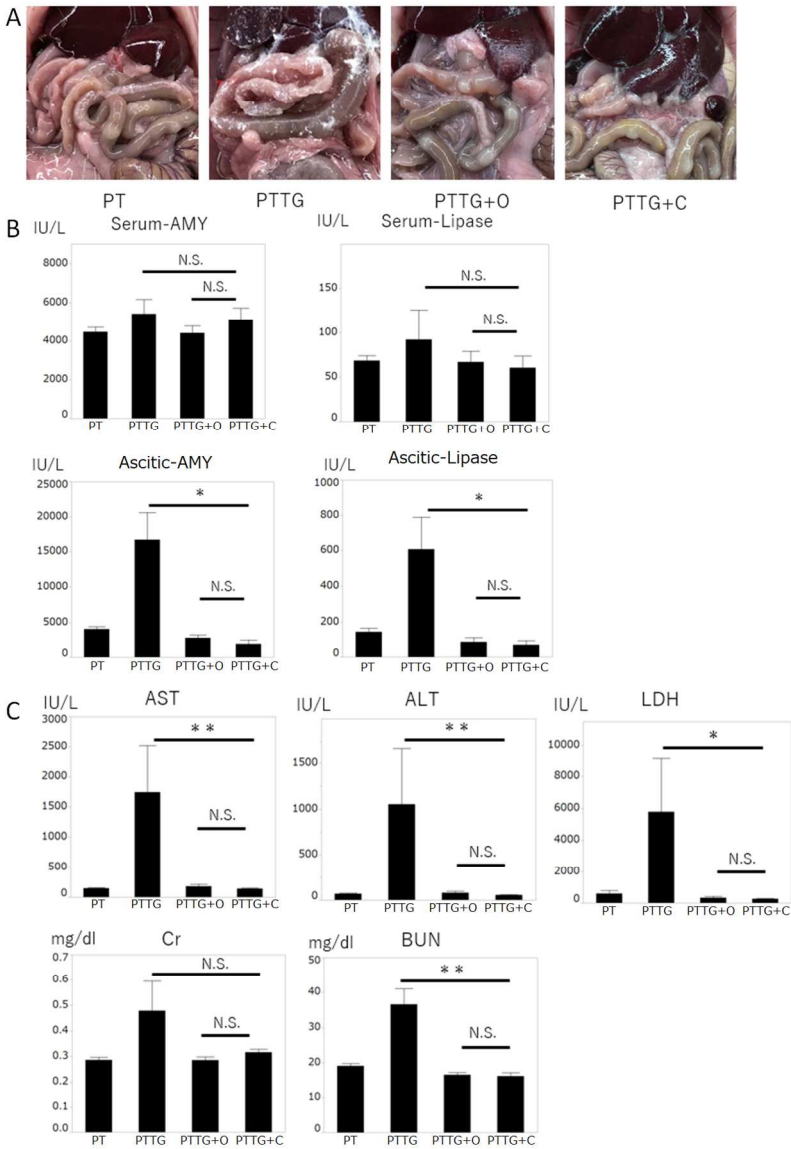
There were no obvious macroscopic change intraperitoneally among groups.

B, Serum and ascitic pancreatic enzyme levels.

Ascitic pancreatic enzyme levels were not significantly different among groups. (N=5)

C, Serum biochemical analysis for liver and kidney damage

Liver and kidney damage were not observed in each group. (N=5)



409x516mm (96 x 96 DPI)

Supplemental Figure 5

Administration of cetilistat (lipase inhibitor) prevent the deterioration of disease state of pancreatic fistula.

Cetilistat (Tokyo Chemical Industry, Tokyo, Japan, 40 mg/kg body weight, dissolved in the triolein) were intraperitoneally administered in PTTG+C group after pancreatic transection. Each animal was euthanized and observed 24 h after operation. Other groups were summarized in figure 1.

A, Intraperitoneal macroscopic findings

Intraperitoneal saponification observed in PTTG group were not existed in PTTG+C group.

B, Serum and ascitic pancreatic enzyme levels.

PLF pancreatic enzyme levels were reduced in PTTG+C group compared with PTTG group.

(N=5)

C, Serum biochemical analysis for liver and kidney damage

Liver and kidney damage observed in PTTG group were not observed in PTTG+C group.

(N=5)

Supplemental Table 1.
Patients' clinical characteristics :Pancreaticoduodenectomy

	Low lipolysis (n=13)	High lipolysis (n=6)	P
Sex, male	9 (69%)	3 (50%)	0.62
Age (years)	69 (62-78)	69 (56-73)	0.56
BMI (kg/m ²)	19.6 (18.1-22.4)	24.8 (22.3-27.5)	0.02
Operation time (minutes)	533 (466-765)	539 (465-628)	0.73
Estimated blood loss (ml)	635 (295-1440)	687 (418-948)	0.48
Total fatty area (cm ²)	116 (41-168)	252 (139-305)	0.01
Visceral fatty area (cm ²)	54 (17-76)	106 (74-116)	0.03
Subcutaneous fatty area (cm ²)	50 (23-95)	139 (74-200)	0.02
Pancreatic texture, soft	3 (23%)	2 (33%)	1
POPF non-POPF	9 (69%)	4 (67%)	
Biochemical leakage	4 (31%)	0 (0%)	
CR-POPF	0 (0%)	2 (33%)	0.045
POD1 drain discharge (ml)	55 (37-121)	55 (30-160)	0.87
POD1 Drain AMY (IU/L)	135 (47-1206)	1563 (1338-4666)	0.04
POD1 Drain Lipase (IU/L)	48 (20-2772)	3860 (2589-17373)	0.03

Patients were categorized into either a high lipolysis group (TFA ≥1mM) or a low lipolysis group (TFA <1mM) according to the TFA concentration of drain discharge on POD 1. BMI, body mass index. Each parameter is shown as the median (interquartile range).

Supplemental Table 2.

Patients' clinical characteristics: Distal pancreatectomy

	Low lipolysis (n=10)	High lipolysis (n=4)	P
Sex, male	3 (30%)	3 (75%)	0.24
Age (years)	70 (62–73)	79 (71–83)	0.09
BMI (kg/m ²)	23.1 (20.2–25.6)	22.4 (18.6–25.9)	0.79
Operation time (minutes)	339 (252–397)	377 (347–483)	0.21
Estimated blood loss (ml)	381 (285–773)	210 (50–1323)	0.99
Total fatty area (cm ²)	197 (154–252)	238 (129–254)	0.88
Visceral fatty area (cm ²)	97 (66–110)	145 (76–180)	0.08
Subcutaneous fatty area (cm ²)	88 (74–173)	73 (42–105)	0.26
Pancreatic texture, soft	4 (40%)	2 (50%)	1
POPF non-POPF	7 (70%)	0 (0%)	
Biochemical leakage	2 (20%)	0 (0%)	
CR-POPF	1 (10%)	4 (100%)	0.007
POD1 drain discharge (ml)	78 (40–134)	39 (18–110)	0.36
POD1 Drain AMY (IU/L)	740 (421–2332)	6771 (1745–16147)	0.09
POD1 Drain Lipase (IU/L)	2142 (602–4640)	5574 (1564–22739)	0.16

Patients were categorized into either a high lipolysis group (TFA $\geq 1\text{mM}$) or a low lipolysis group (TFA $< 1\text{mM}$) according to the TFA concentration of drain discharge on POD 1. BMI, body mass index. Each parameter is shown as the median (interquartile range).