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Original Article

Laparoscopic Roux-en-Y gastric bypass in super obese Göttingen minipigs

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Abstract: Background: The specific mechanisms behind weight loss and comorbidity improvements in obese patients after Roux-en-Y gastric bypass (RYGBP) are still poorly understood. The aim of this study was to establish and evaluate the feasibility of a long-term survival RYGBP model in super obese Göttingen minipigs in order to improve the translational potential relative to current animal models. Methods: Eleven Göttingen minipigs with diet-induced obesity underwent laparoscopic RYGBP and were followed up to 9 months after surgery. Intra- and post-operative complications, body weight (BW), food intake and necropsy data were recorded. Results: Five minipigs survived without complications to the end of the study. Four minipigs developed surgical related complications and were euthanized while two minipigs died due to central venous catheter related complications. BW and food intake is reported for the six minipigs surviving longer than 4.5 months post-surgery. Weight loss and reduced food intake was seen in all minipigs. After 2-3 months of weight loss, weight regain was evident in all but two minipigs which seemed to continue losing weight. Necropsy revealed some variation in the length of the alimentary, biliary and common limb between minipigs. Conclusion: The use of obese Göttingen minipigs as a translational RYGBP model is feasible and has potential for the study of RYGBP-related changes in gut function, type-2 diabetes and appetite regulation. Still, the surgical procedure is technically highly demanding in obese Göttingen minipigs and the peri-operative animal care and follow up requires close monitoring.

Keywords: Gastric bypass, obesity, diabetes, weight loss, animal model, pig

Introduction

Obesity is a rapidly growing problem worldwide and morbidly obese patients are at significantly increased risk of developing related metabolic disorders such as type 2-diabetes and cardiovascular disease [1-3]. Laparoscopic Roux-en-Y gastric bypass (RYGBP) is the most commonly used surgical procedure to treat morbidly obese patients [4]. Besides weight loss, patients undergoing RYGBP achieve extensive improvements in comorbidities, such as remission of type 2-diabetes, superior to those achieved by diet, exercise and behavioral intervention possibly due to mechanisms associat-

ed with the surgical alteration of the gastrointestinal tract [1, 5]. The specific mechanisms involved in the amelioration of metabolic abnormalities remain poorly understood, but may be due to changes in energy metabolism, gastrointestinal peptides and food preferences [6, 7]. Initially weight loss after RYGBP was believed to result from restriction of food intake due to reduced gastric volume and because of altered absorption as the first part of the small intestine is bypassed [8]. However, this cannot fully explain the observed acute postsurgical improvements, which more likely can be attributed to neuroendocrine mechanisms. From this notion, it is clear that the specific neuroendo-

crine mechanisms following surgery are highly complex involving both physiological and behavioral changes [6, 9].

A better understanding of the neuroendocrine mechanisms behind the unexpected immediate response to RYGBP could pave the way for design of new anti-obesity drugs and a noninvasive treatment of obesity [6]. Animal models, particularly rats, have been used to study the physiological changes after gastric bypass [10-12]. However, the pig may be a more appropriate animal model because of its genetic, physiologic and phenotypic similarities to humans including gut function, metabolism, lipoprotein profile and omnivorous habits [12-17]. Gut peptide responses to fasting and feeding are also similar to humans [18, 19]. Another advantage with pigs is the ability to collect larger blood volumes and tissue samples compared to small animal models.

Pig models of RYGBP have been established but few have been long-term survival models and none have used obese pigs [12, 20-22]. A review of literature published reveals seven laparoscopically performed RYGBP porcine survival studies with postoperative follow-up ranging from 1 week to 6 months [22-28]. In these studies, domestic pigs such as Yorkshire and Large White x Landrace have been used [22, 23, 28]. Due to genetic selection these breeds have a lean phenotype and are not prone to develop obesity and associated comorbidities. This is in contrast to the Göttingen minipig which is prone to diet-induced obesity and metabolic syndrome and may thus be considered a better translational model to study the effect of RYGBP [29-32].

Göttingen minipigs and especially the ovariectomized females will develop massive obesity already by the age of 18 months when given *ad libitum* access to food [29, 33]. Adult Göttingen minipigs normally have a body weight (BW) range of 35 to 40 kg and food restriction is necessary to maintain a lean phenotype. Even though obese Göttingen minipigs do not become diabetic they develop insulin resistance and cardiovascular complications [13, 32]. The obese Göttingen minipig has also proven to be superior for the study of severe obesity compared to rodents. In contrast to rodents, the obese minipig body composition is very similar to that reported for severely obese people. They have the ability to obtain a BW more

than two times normal weight equivalent to humans and they eat in a meal pattern during daylight [29]. Also, the effect of liraglutide, a human glucagon-like peptide-1 analog, has been shown to have similar effects in humans and obese Göttingen minipigs by reducing food take and causing weight loss [29, 34]. Altogether, this makes the obese Göttingen minipig a very attractive model to study the effects of RYGBP on appetite regulation, insulin sensitivity, organ adaptation and neuroendocrine mechanisms. The aim of this study was to establish and evaluate the feasibility of a long-term survival model of RYGBP in super obese Göttingen minipigs.

Materials and methods

Experimental animals and housing

Animals were treated in accordance with the Animal Experimentation Act of Denmark, which is in accordance with the Council of Europe Convention ETS 123. The study was approved by the National Animal Experimentation Board.

Eleven ovariectomized obese Göttingen minipigs (age 4-6 years) (Ellegaard Göttingen minipig A/S, Denmark) were used in this study. Ovariectomy was done at the breeder facility when minipigs were one year old. Thereafter diet-induced obesity was initiated by providing *ad libitum* access to minipig chow diet (Altromin 9023 (Metabolizable energy (ME) 10.5 MJ/kg), Brogaarden, Denmark). At the age of 2-3 years and an average BW of 90 kg they were transferred to the lab animal facility (Laboratory Animal Unit, Frederiksberg Campus, University of Copenhagen, Denmark) and continued on restrictive feeding (Altromin 9023, Brogaarden, Denmark) in order to keep the BW at this level. Before this study, the minipigs had been used for pharmacological testing of anorexic drug candidates. Consequently, a sufficient wash out period of 4-6 months was included prior to this study. The minipigs were single housed in pens with straw bedding and had free access to water. All minipigs were exercised daily (15-30 min of walk outside their pen) and BW was measured weekly.

Jugular catheter placement

Approximately one month before RYGBP surgery, two central venous catheters (CVC)

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(Silastic silicone tubes, 0.040 in. ID x 0.085 in. OD, Dow Corning, Belgium) were implanted in the external jugular vein after surgical cut down in anesthetized minipigs [35]. Catheters were tunneled under the skin and exteriorized from the dorsum. The exteriorized catheters were protected by a little bag loosely sutured to the skin. Catheters were locked with concentrated heparin solution (Heparin LEO 5000 IE/ml, LEO Pharma, Denmark) to prevent thrombosis. Every 3 to 4 days, the catheter lock solution was withdrawn, flushed with saline, and refilled with the heparin lock.

Anesthesia and pre-operative preparation

The minipigs were accustomed to fluid nutrition (Nutridrink, Nutricia Advanced Medical Nutrition, Denmark) for 2-3 weeks before RYGBP surgery by daily giving them small amounts of Nutridrink (10-40 ml) orally using a large syringe. Two days before surgery, straw bedding was removed from their pens and only 200 ml of Nutridrink (1260 KJ/300 kcal, 20 g protein, Nutridrink Protein, Nutricia Advanced Medical Nutrition, Denmark) was given twice daily. Twelve hours prior to surgery they were restricted from all food. Water was provided *ad libitum*.

On the day of surgery, the minipigs were sedated with a mixture of 0.5 mg/kg midazolam (Midazolam Hameln 5 mg/ml, Hameln Pharmaceuticals gmbh, Germany) + 0.4 mg/kg metadon (Metadon 10 mg/ml, Nycomed, Denmark) + 5 mg/kg ketamine (Ketaminol Vet 100 mg/ml, Intervet, Denmark) intravenously (i.v.) to effect. Anesthesia was induced with 1-4 mg/kg propofol i.v. (Rapinovel Vet 10 mg/ml, Schering-Plough Animal Health, Denmark) and the minipig was intubated and mechanically ventilated with 60-100% oxygen using the pressure control ventilation - volume guaranteed (PCV-VG) mode with a tidal volume of 350-400 ml and a positive end-expiratory pressure (PEEP) level of 8 cm H₂O. Perioperative antibiotic prophylaxis (Metronidazol 10 mg/ml, Veterinary Pharmacy, University of Copenhagen, Denmark; 20 mg/kg + Cephazolin Fresenius 1 g, Fresenius Kabi, Germany; 20 mg/kg) were administrated i.v. at the time of induction. Cephazolin was repeatedly administrated every second hour until the end of surgery. Anesthesia was maintained with propofol (Rapinovel Vet 10 mg/ml; 2-6 mg/kg/h) and fentanyl (Haldid 50 µg/ml,

Janssen-Cilag, Belgium; 10-35 µg/kg/h). In four minipigs repeated i.v. boli of ketamine 0.5 mg/kg (Ketaminol Vet 100 mg/ml, Intervet, Denmark) were also given. A continuous i.v. infusion of Ringers-acetate solution (Fresenius Kabi, Denmark) was administrated at 5-15 ml/kg/h. Corneal reflexes, heart rate, ECG, temperature, non-invasive blood pressure, oxygen saturation and end tidal CO₂ was monitored throughout the procedure. A Bair Hugger and rescue-blankets were used to avoid hypothermia during the surgical procedure.

Atropine was given as a bolus if the heart rate was lower than 50 min⁻¹ (Atropin DAK 1 mg/ml, Nycomed, Denmark; 0.01 mg/kg). Pigs with low urine production during surgery were given a bolus of furosemide (Furix 10 mg/ml, Nycomed, Denmark; 1 mg/kg). Hypotension was treated with dopamine infusion (Dopmin 40 mg/ml, Orion Pharma A/S, Denmark; 0.1-0.3 mg/kg/h) or plasma expanders (Voluven 60 mg/ml, Fresenius Kabi, Denmark; 200-300 mg/kg/h).

Before surgery, a urinary catheter (Rüsch GmbH, Germany, Size Ch. 10) was placed in the bladder and urine was collected in a closed system. In addition, after insertion of an oro-gastric tube, gastric lavage was performed with lukewarm water in order to remove any residual stomach contents. **Figure 1** shows representative images from the pre-operative preparation, the surgical procedure and the post-surgical management of an obese Göttingen minipig.

Surgical procedure

Animals were placed in dorsal recumbency with the cranial end of the table elevated approximately 20 degrees. After standard aseptic preparation, each trocar site was infiltrated with a mixture of xylocain and bupivacaine (Xylocain 20 mg/ml, AstraZeneca A/S, Denmark and Marcain 5 mg/ml, AstraZeneca A/S, Denmark) before skin incision. Five trocars (Endopath EXCEL, Ethicon Endo-Surgery, Denmark) were placed in the upper abdominal region under visual guidance (**Figure 1C** and **1D**) and CO₂ was inflated until an intraperitoneal pressure of 10-12 mm Hg was reached. The peritoneal cavity was inspected and the liver retracted with a retractor (Endo Paddle Retract, Covidien, Denmark) in order to oversee the visceral aspect of the stomach. With a harmonic scalpel (Harmonic Ace, Ethicon Endo-

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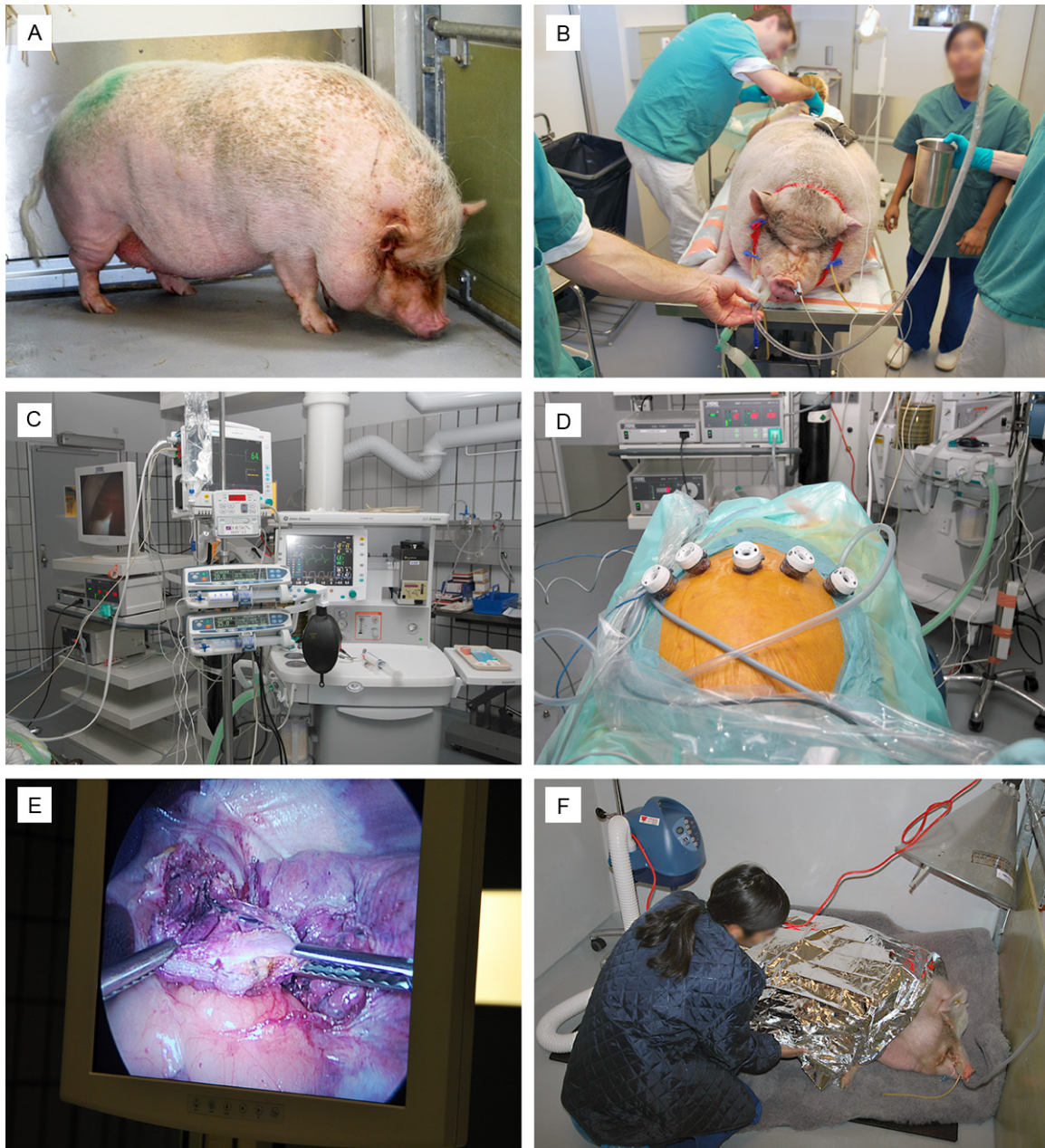


Figure 1. Images showing the pre-operative preparation, the surgery procedure and the post-surgical management of an obese Göttingen minipig. A: An obese Göttingen minipig. B: Urinary catheter placement and gastric lavage done in an anesthetized pig. Note little bag placed on the back of the pig containing central venous catheters. C: Anesthesia and laparoscopic equipment. D: Placing of ports. E: Creation of the new small gastric pouch. F: Recovery of minipig after surgery. Supporting oxygen is provided and the pig is covered with a Bair Hugger and a blanket.

Surgery, Denmark), beginning at the oral part of the lesser curvature, a retrogastric tunnel was created by incising the gastrohepatic ligament and dissecting dorsally towards the angle of His (incisura cardiaca), sparing the vagal nerve. After creation of the retrogastric tunnel a gastric pouch of estimated 20-30 ml was created with the use of a linear 60 mm GIA stapler

(Echelon Endopath Stapler, Ethicon Endo-Surgery, Denmark). Two interconnected staple lines in an inverted L-shape were used; one running horizontally from the lesser curvature towards the greater curvature and one from the end of this first staple line vertically toward the angle of His avoiding incorporation of the ventricular diverticulum in the gastric pouch. Three

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to five staple applications in total were used for creating the gastric pouch (**Figure 1E**). The gastric pouch was opened over an oro-gastric tube with a harmonic scalpel (Harmonic Ace, Ethicon Endo-Surgery, Denmark). From the point of emergence from its retroperitoneal course, a 120 cm section of the jejunum was measured. At this point, the jejunum was incised with harmonic scalpel and anastomosed to the gastric pouch with the 60 mm linear stapler and the stapler hole was closed with running sutures in two layers (Vicryl 4-0 and Monocryl 4-0). From the gastro-jejunosomy, another 240 cm of jejunum was measured. At this point the jejunum was incised with harmonic scalpel and anastomosed to the jejunum immediately oral to the gastro-jejunosomy with a 45 mm linear stapler (Endopath ETS, Ethicon Endosurgery, Denmark) and the stapler hole was closed with a running suture in one layer (Vicryl 4-0). The jejunum was then transected between the gastro-jejunosomy and the jejuno-jejunosomy with the 45 mm linear stapler creating a 240 cm alimentary (Roux) limb; a 120 cm biliary limb and a common limb of approximately 5-600 cm. Considering that the total length of the small intestine is longer in pigs than in humans, the lengths of the alimentary and biliary limbs relative to the common limb was set to be approximately the same as in human gastric bypass. The abdomen was flushed with saline, deflated and trocar sites closed with skin staplers.

Post-surgical management

Buprenorphine 0.02-0.05 mg/kg i.m. (Temgesic 0.3 mg/ml, Reckitt Benckiser, United Kingdom) was administrated every 8 hours for 2 days and utilized as necessary for breakthrough pain in the first 4 postoperative days. Fluid therapy (Ringers acetate, 5% glucose infusion) was given if assessed necessary. The clinical status of the animals was monitored intensively on the days following surgery and thereafter twice daily. Body temperature, pulse and respiratory rate were recorded until the minipig was assessed to be in a stable condition. Additional antibiotics (metronidazol 20 mg/kg + enrofloxacin, Baytril Vet 50 mg/ml; 5 mg/kg i.v.) were given if fever occurred. Symptoms such as changes in behaviour, reduced or loss of appetite and gastroesophageal reflux were treated individually with additional non-steroidal analgesia (meloxicam 0.4 mg/kg i.m., Metacam 20 mg/ml, Boehringer Ingelheim, Denmark), appe-

tite stimulants (dexamethasone 0.06 mg/ml i.m., Voren Vet 1 mg/ml, Boehringer Ingelheim, Denmark), antiemetic and gastrointestinal motility stimulants (metoclopramide 0.2-0.25 mg/kg i.m., Primperan 5 mg/ml, Sanofi-Aventis, Denmark), and gastric protectiva (sucralfat 50 mg/ml orally, Antepsin 200 mg/ml, Orion Pharma, Denmark).

To avoid vitamin deficiencies dietary supplements were provided after surgery (2 tablets daily (Matas Multivitamin tablets, Matas, Denmark) and a vitamin B12 substitute (1 ml i.m. every 3. month, Betolvex 1 mg/ml, Actavis, Denmark)) [36]. In addition, minipigs developing low haematocrit levels in the months after surgery were treated with iron paste (Hyofer P, Salfarm Denmark A/S, Denmark).

Food intake measurements after surgery

Upon full recovery from anesthesia feeding was restarted. Initially, small amounts of Nutridrink (e.g. 10 ml) were offered as frequent small meals. During the first 1-2 weeks after surgery the daily amount of Nutridrink was slowly increased from 300 to 600 ml per day. Subsequently dry diet was added in small amounts (Altromin 9023, Brogaard, Denmark) and the fluid nutrition was gradually altered to dry diet again. Approximately 3-4 weeks after surgery the minipigs were ready to be moved to pens with *ad libitum* access to dry diet [29]. Individual food consumption was recorded daily.

Post mortem examination

After 8-9 months, minipigs were euthanized with i.v. injection of pentobarbital (Pentobarbital 200 mg/ml, Glostrup Apotek, Denmark; 150 mg/kg) and necropsy was performed. Intestinal length (alimentary limb, biliary limb, common limb and colon) was recorded. Tissue was harvested from selected organs for later assessment.

Statistical analysis

Where relevant, results are presented with mean \pm standard deviation (SD) and ranges.

Results

The study flow and post-operative complications are presented in **Figure 2**. The laparoscopic RYGBP was completed in all animals

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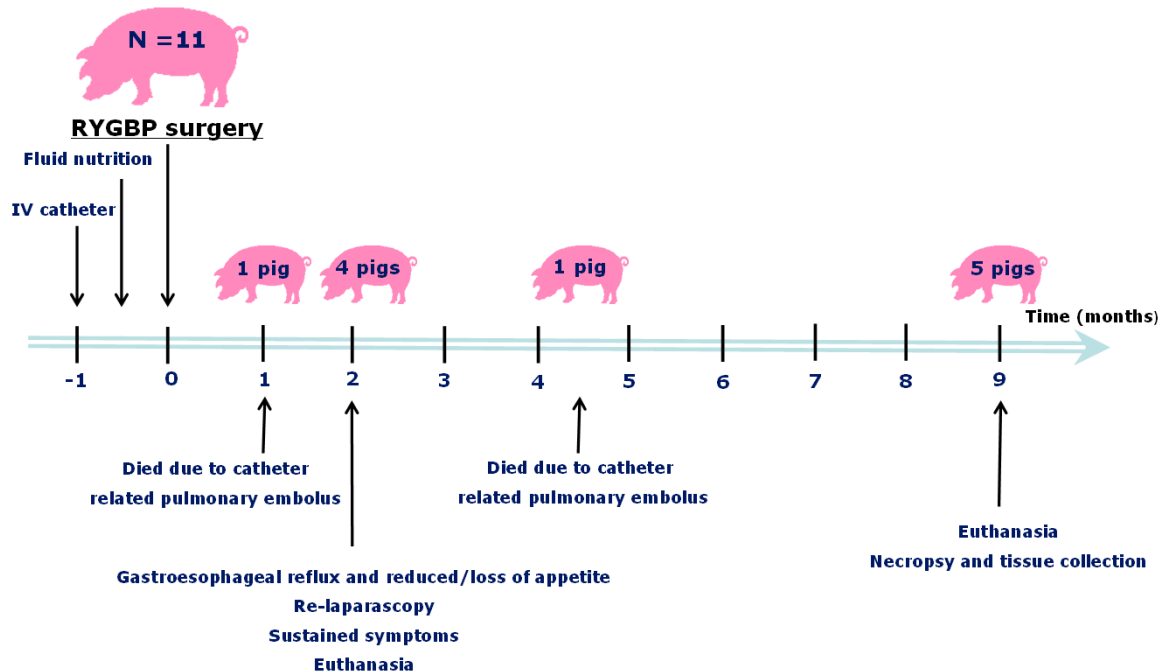


Figure 2. Study flow diagram.

and no intraoperative surgery related deaths occurred. The mean operative time was 216 ± 78 min (range 120-360 min). Of the 11 minipigs, five minipigs survived without complications to the end of the study period. Four minipigs developed signs of gastroesophageal reflux and reduced or lost appetite within 2 months after surgery. Following re-laparoscopy, internal herniation and anastomosis-related strictures were identified and corrected in these minipigs. However, due to sustained symptoms and surgical complications these minipigs were euthanized. In addition, two minipigs died suddenly (1 and 4.5 months post-surgery respectively) most likely due to CVC related pulmonary emboli as clots around the catheter tip were noted at necropsy.

Intra-operative complications

Due to anatomical differences between pigs and humans, some adjustments to the technique used in humans were required to achieve satisfactory results. Several animals developed slight to moderate bleeding from lacerations of the liver or spleen mostly after retraction. In one case the bleeding delayed the procedure. Two animals experienced small colon lesions caused by endoscopic instruments. Both of these were repaired by suturing or stapling and did not give rise to further complications.

A declining blood pressure was observed in all minipigs during surgery but medical intervention was only necessary in four of the minipigs. The position of the animal on the surgery table was found to have marked adverse effects on the circulation. In a few cases, hypotension could be minimized by changing the angle of the table slightly. A slow increase in pulse was seen in all minipigs during the surgery procedure even after adequate pain management. Lowering the abdominal pressure to 9-10 mm Hg was found to have beneficial effects by lowering the high pulse rate and increasing the blood pressure. Consequently, longer periods with high intraperitoneal pressure (12 mm Hg) was avoided.

Post-operative complications

Gastroesophageal reflux and subsequent reduced or lost appetite was observed in four minipigs starting 1-3 weeks after surgery. Initially meal sizes were reduced and analgetics were given to rule out loss of appetite due to pain. Subsequently two minipigs were treated with metoclopramide, which only seemed to worsen the symptoms as the minipigs developed transient signs of increased abdominal pain following injection. Also, this treatment had no effect on either reflux or the appetite. The remaining two minipigs were treated once with dexameth-

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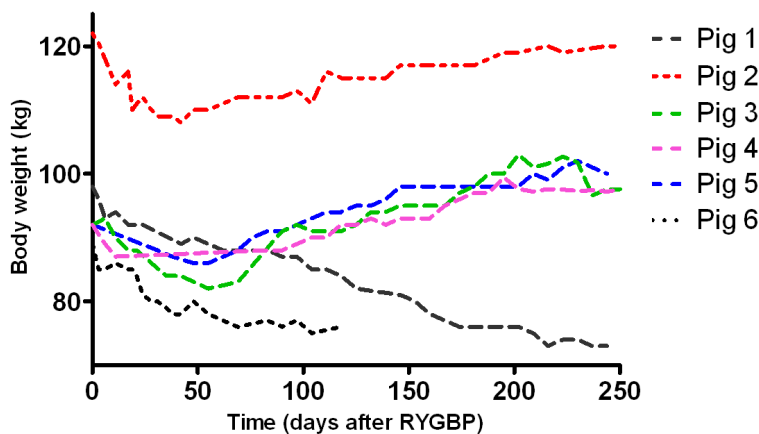


Figure 3. Body weight evolution following Roux-en-Y gastric bypass (RYGBP) in pigs surviving more than 4 months after surgery.

asone also without any effect. The minipig with most severe reflux was also given sucralfat orally to protect against development of esophagitis. As clinical signs continued in all four minipigs, re-laparoscopy was performed. Internal herniation and anastomosis-related strictures were identified and corrected. However, re-operation and correction of strictures or herniations was insufficient to prevent persistence of clinical signs and these minipigs were euthanized based on a poor prognosis for recovery.

In addition, one minipig had a rise in body temperature a few days after surgery. This returned to normal following treatment with antibiotics. Approximately seven months after surgery, two minipigs developed bacterial skin infections at the exit site of the jugular vein catheters. Even though only one of these minipigs had slight fever they were both treated successfully with antibiotics.

Body weight and food intake

The mean initial BW was 98.5 ± 13.3 kg (range 88-122 kg). BW and food intake is only reported for the minipigs which survived to the end of the study including the minipig which survived until 4.5 months after surgery. Weight loss was most pronounced during the first 2-3 months after surgery (**Figure 3**). Thereafter, four of the minipigs gained weight again and even gained more weight than their initial weight. Only one minipig kept losing weight throughout the study. The minipig which died suddenly probably due to a pulmonary thrombus also seemed to lose weight up to the time of death

Average daily food intake was $1.61 \text{ kg} \pm 0.17 \text{ kg}$ (range 1.3-1.8 kg, $\sim 13.7\text{-}18.9$ MJ) before surgery and $0.63 \text{ kg} \pm 0.3 \text{ kg}$ (range 0.17-0.95 kg, $\sim 1.8\text{-}10.0$ MJ) after surgery. The average difference in daily food intake before and after surgery was $0.98 \pm 0.27 \text{ kg}$ (range 0.65-1.43 kg, $\sim 6.8\text{-}15.0$ MJ).

Necropsy findings

In 7/11 minipigs the RYGBP had been performed as planned and without any visible surgery related complications (alimentary limb 164 ± 47 cm, biliary limb 150 ± 39 cm and common limb 715 ± 149 cm). Small bowel lengths for individual minipigs are given in **Table 1**. In four minipigs necropsy revealed surgery related complications.

In two minipigs which were euthanized within two months after surgery, the biliary limb was much longer than planned (approximately 450 cm). In addition, both of these minipigs had pronounced anastomosis related strictures, one at the site of the gastro-jejunostomy and the other just distal to the jejuno-jejunostomy (Y-anastomosis). Marked bowel distention was evident proximal to the stricture. In the third minipig a pronounced proximal stricture at the gastro-jejunostomy was also found. This minipig was re-operated and shortly afterwards euthanized due to sustained symptoms. These necropsy findings corresponded well with the clinical signs observed in these three minipigs.

In the fourth minipig which was re-operated, an internal herniation of the small intestine was identified and corrected. Immediately post-operatively this minipig was euthanized because of cardiovascular collapse due to hepatic lacerations causing severe intraabdominal bleeding. No anastomosis related strictures were identified and the internal herniation had successfully been corrected.

Two minipigs died suddenly and necropsy findings revealed catheter related fibrinopurulent thrombus formation, and multiple lung abscesses. Based on these findings pulmonary embolism may have been the cause of sudden death.

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Table 1. Length of small intestinal limbs following Roux-en-Y gastric bypass

Pig ID	Alimentary limb (cm)	Biliary limb (cm)	Common limb (cm)	Comments
Pig 1	240	165	445	
Pig 2	212	190	750	
Pig 3	177	212	800	
Pig 4	165	114	656	
Pig 5	142	130	752	
Pig 6	150	150	700	Catheter thrombus and pulmonary emboli
Pig 7	140	140	970	Catheter thrombus and pulmonary emboli
Pig 8	88	96	648	Gastrojejunostomi stricture
Pig 9	-	-	-	Liver laceration after re-laparoscopy
Pig 10	-	450	-	Gastrojejunostomi stricture
Pig 11	75	450	60	Y-anastomosis stricture

No anastomotic leaks were identified at necropsy in any minipigs and no signs of acute inflammation in the abdominal cavity were present. In six minipigs, mild to moderate fibrous adhesions were found between small intestines and the visceral surface of the liver, and from the liver to the diaphragm. No gastro-gastric fistulas were present but fibrous adhesions between the small gastric pouch and the bypassed stomach was seen in one case. In two minipigs large gallstone-like bezoars were found in the bypassed stomach the largest being approximately 10 x 5 cm.

Discussion

To the best of our knowledge, this is the first description of laparoscopic RYGBP in an obese minipig model and the first reports of an up to 9 months follow up after RYGBP in a porcine model. The model differs from other animal models as continued post-surgical weight loss was observed in some minipigs, which makes the model a much closer reflection of obese human patients.

Anesthesia and surgery were well tolerated in all minipigs. The balanced total intravenous anesthesia protocol provided haemodynamic stability during surgery and ensured few cardiovascular side effects as well as a fast recovery. In seven minipigs, the surgical procedure was performed successfully without any surgery related complications. Five of these minipigs survived to the end of study while two died before time most likely due to pulmonary embolism. Our data show that the use of obese Göttingen minipigs as a RYGBP model is feasible but challenging. Even for very experienced

and skilled surgeons the surgical procedure is technically very demanding. Pilot studies were done in lean farm pigs as only a limited number of obese minipigs were available. The surgical procedure was found to be far more challenging in obese minipigs compared to lean farm pigs. Peri-operative animal care requires intensive monitoring with particular focus on intra- and post-operative analgesia. Also the post-operative care requires close monitoring with regard to food intake and CVC maintenance in order to avoid dumping symptoms and catheter related complications.

When using the obese Göttingen minipig as a survival model of RYGBP the surgical technique in particular needs special attention. Standardized RYGBP technique as described in human bariatric surgery is not directly applicable in obese Göttingen minipigs [12, 20, 22]. Pigs have a dense peritoneum, a thick stomach wall, a short lesser omentum and a large ventricular diverticulum making creation of a small gastric pouch with a defined size very difficult [20, 26]. The course of the bile duct in pigs also makes it more prone to damage when creating the gastric pouch. Also due to very fragile intestines, manipulation of porcine small bowel in particular requires caution in order to avoid anastomotic leakage and stricture formation as well as intraoperative bleeding and intestinal laceration [12, 21]. In the present study, three minipigs were found to have strictures while leakage was not observed. Moreover, impacted bile was found in the bypassed stomach in two minipigs. This could be due to retrograde flow of bile into the stomach and precipitation of bile components forming gallstone-like bezoars. Gallstone-related complications after RYGBP is

also often reported in humans further stressing the pig as an appropriate translational animal model [37].

The optimal length of the alimentary limb in order to obtain weight loss and comorbidity improvements have been subjected to a number of studies. Pigs have approximately twice as long intestines as humans and the length also varies between pigs [12]. In order to standardize and to make the porcine RYGBP model more comparable to humans it has been suggested that the length of the alimentary and biliary limb in pigs should be calculated as a fraction (1/3 or 1/4) of the total small bowel length [12, 20] or just twice the length normally used in humans [22]. In a porcine study by Gandarillas and co-workers, there were no significant differences in BW following RYGBP among experimental groups with 300, 600 and 900 cm alimentary limbs [23]. However, as the exact length of the alimentary limb was not measured after euthanasia the degree of variation within each group was not known. In the present study, two minipigs were found having biliary limbs that were too long. This situation can be explained by the greatly impaired visualization and identification of specific anatomic structures because of extensive visceral fat in the super obese Göttingen minipigs. Consequently, even though we aimed at creating uniform lengths of alimentary and biliary limbs some variation was found at necropsy. This may partly explain differences in effect on weight loss as also observed in humans [38, 39].

In the present study, weight loss was most pronounced in the first months after surgery. To some extent however, this was a reflection of loss of appetite. Subsequently some minipigs started to regain weight and even exceeded their initial weight. Weight regain after a period of weight loss is also frequently seen in humans following RYGBP, however not as soon after surgery as observed in this study [38-40]. Still, the exact mechanisms underlying weight regain following an initial weight loss regardless of reduced calorie intake is yet unknown, but could possibly be due to changes in compensatory mechanisms controlling BW homeostasis [7, 39, 40]. Even though all minipigs significantly reduced their daily food intake after RYGBP the majority still gained weight again. However, the two minipigs with the lowest feed intake were also the minipigs that showed ongoing

weight loss. In contrast to our findings, it was shown in another study that pigs with *ad libitum* access to feed following open RYGBP did not reduce their daily caloric intake and consequently this study concluded that restricted feeding is necessary to reduce daily calorie intake [20]. In this study, non-obese mature miniature swine (Yucatan and Hanford) were used to evaluate the hormonal changes (e.g. ghrelin, peptide YY and glucagon-like peptide-1) after RYGBP. However, due to the limited number of animals included, variation in surgical technique and different feeding patterns, adequate and meaningful assessment of the effect of RYGBP was not achieved [20].

In the present study, two minipigs died due to catheter related thrombosis. Long-term venous catheter implantation is necessary in order to easily obtain blood samples at frequent intervals (e.g. meal tolerance testing and intravenous glucose tolerance testing) without stressing the animal. Catheter related infections with risk of occlusions, thromboembolisms and focal infections are known complications in pigs and underscore the importance of meticulous peri-operative animal care with aseptic handling and maintenance of the catheters to avoid catheter related morbidity and mortality [41, 42]. Still, it must be emphasized that the duration of the present study is considerable longer compared to previous porcine studies with chronic implanted CVC and consequently increased risk of such complications.

In conclusion, the use of super obese Göttingen minipigs in RYGBP surgery is feasible and holds promise as a phenotypically appropriate RYGBP model. However, the use of super obese Göttingen minipigs is associated with pitfalls that need to be taken into consideration. The surgical procedure is technically highly demanding and the peri-operative animal care and follow up requires close monitoring. Still we believe that this model has future potential in the study of RYGBP-related changes in gut function, type-2 diabetes and appetite regulation. Hopefully, this new animal model can lead to further development of better pharmacological strategies for the management of obesity and type-2 diabetes.

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Disclosure of conflict of interest

The authors have no conflicts of interest. All authors have read and agreed the content within the final manuscript.

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References

- [1] Nguyen N, Champion JK, Ponce J, Quebbemann B, Patterson E, Pham B, Raum W, Buchwald JN, Segato G and Favretti F. A review of unmet needs in obesity management. *Obes Surg* 2012; 22: 956-966.
- [2] Kral JG, Kava RA, Catalano PM and Moore BJ. Severe obesity: the neglected epidemic. *Obesity Facts* 2012; 5: 254-269.
- [3] Must A, Spadano J, Coakley EH, Field AE, Colditz G and Dietz WH. The disease burden associated with overweight and obesity. *JAMA* 1999; 282: 1523-1529.
- [4] Smith BR, Schauer P and Nguyen NT. Surgical approaches to the treatment of obesity: bariatric surgery. *Endocrinol Metab Clin North Am* 2008; 37: 943-964.
- [5] Vetter ML, Ritter S, Wadden TA and Sarwer DB. Comparison of bariatric surgical procedures for diabetes remission: efficacy and mechanisms. *Diabetes Spectr* 2012; 25: 200-210.
- [6] Tam CS, Berthoud HR, Bueter M, Chakravarthy MV, Geliebter A, Hajnal A, Holst J, Kaplan L, Pories W, Raybould H, Seeley R, Strader A and Ravussin E. Could the mechanisms of bariatric surgery hold the key for novel therapies? *Obes Rev* 2011; 12: 984-994.
- [7] Rao RS. Bariatric surgery and the central nervous system. *Obes Surg* 2012; 22: 967-978.
- [8] Mason EE, Printen KJ, Lewis JW, Scott DH and Blommers TJ. Gastric bypass criteria for effectiveness. *Int J Obes* 1981; 5: 405-411.
- [9] Akkary E. Bariatric surgery evolution from the malabsorptive to the hormonal era. *Obes Surg* 2012; 22: 827-831.
- [10] Seyfried F, le Roux CW and Bueter M. Lessons learned from gastric bypass operations in rats. *Obesity Facts* 2011; 4: 3-12.
- [11] Bueter M, Ashrafian H, Frankel A, Tam F, Unwin R and Bloom S. Energy expenditure after Roux-en-Y gastric bypass in rats. *Obes Surg* 2009; 19: 988-989.
- [12] Rao RS, Rao V and Kini S. Animal models in bariatric surgery—a review of the surgical techniques and postsurgical physiology. *Obes Surg* 2010; 20: 1293-1305.
- [13] Bellinger DA, Merricks EP and Nichols TC. Swine models of type 2 diabetes mellitus: Insulin resistance, glucose tolerance, and cardiovascular complications. *ILAR J* 2006; 47: 243-258.
- [14] Nilsson C, Raun K, Yan FF, Larsen MO and Tang-Christensen M. Laboratory animals as surrogate models of human obesity. *Acta Pharmacol Sin* 2012; 33: 173-181.
- [15] Spurlock ME, Gabler NK. The development of porcine models of obesity and the metabolic syndrome. *J Nutr* 2008; 138: 397-402.
- [16] Gandarillas M, Bas F. The domestic pig (*Sus scrofa domestica*) as a model for evaluating nutritional and metabolic consequences of bariatric Surgery practiced on morbid obese humans. *Cien Inv Agr* 2009; 36: 163-176.
- [17] Wernersson R, Schierup MH, Jorgensen FG, Gorodkin J, Panitz F, Staerfeldt HH, Christensen OF, Mailund T, Hornshøj H, Klein A, Wang J, Liu B, Hu S, Dong W, Li W, Wong GK, Yu J, Wang J, Bendixen C, Fredholm M, Brunak S, Yang H and Bolund L. Pigs in sequence space: a 0.66X coverage pig genome survey based on shotgun sequencing. *BMC Genomics* 2005; 6: 70.
- [18] Sheikh SP, Holst JJ, Orskov C, Ekman R and Schwartz TW. Release of PYY from pig intestinal mucosa; luminal and neural regulation. *Regul Pept* 1989; 26: 253-266.
- [19] Salfen BE, Carroll JA and Keisler DH. Endocrine responses to short-term feed deprivation in weanling pigs. *J Endocrinol* 2003; 178: 541-551.
- [20] Flum DR, Devlin A, Wright AS, Figueredo E, Alyea E, Hanley PW, Lucas MK and Cummings DE. Development of a porcine Roux-en-Y gastric

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- bypass survival model for the study of post-surgical physiology. *Obes Surg* 2007; 17: 1332-1339.
- [21] Scott DJ, Provost DA, Tesfay ST and Jones DB. Laparoscopic Roux-en-Y gastric bypass using the porcine model. *Obes Surg* 2001; 11: 46-53.
- [22] Escareno CE, Azagury DE, Flint RS, Nedder A, Thompson CC and Lautz DB. Establishing a reproducible large animal survival model of laparoscopic Roux-en-Y gastric bypass. *Surg Obes Relat Dis* 2012; 8: 764-769.
- [23] Gandarillas M, Boza C, Barrales L, Hodgkinson SM and Bas F. Nutrient absorption, feed intake and liveweight change as a consequence of Roux-en-Y gastric bypass with increasing alimentary limb lengths in the pig model. *Cien Inv Agr* 2011; 38: 31-39.
- [24] Gentileschi P, Gagner M, Milone L, Kini S and Fukuyama S. Histologic studies of the bypassed stomach after Roux-en-Y gastric bypass in a porcine model. *Obes Surg* 2006; 16: 886-890.
- [25] Potvin M, Gagner M and Pomp A. Laparoscopic Roux-en-Y gastric bypass for morbid obesity: A feasibility study in pigs. *Surg Laparosc Endosc* 1997; 7: 294-297.
- [26] Nocca D, Gagner M, Abente FC, Del Genio GM, Ueda K, Assalia A, Rogula T and Bertani D. Laparoscopic gastric bypass with silicone band in a pig model: Prevention of anastomotic dilatation feasibility study. *Obes Surg* 2005; 15: 523-527.
- [27] Waage A, Gagner M, Biertho L, Jacob BP, Kim WW, Faife B, Sekhar N and del GG. Comparison between open hand-sewn, laparoscopic stapled and laparoscopic computer-mediated, circular stapled gastro-jejunostomies in Roux-en-Y gastric bypass in the porcine model. *Obes Surg* 2005; 15: 782-787.
- [28] Kim WW, Gagner M, Biertho L, Waage A and Jacob B. Taking posterior rectus sheath laparoscopically to reinforce the gastrojejunostomy in laparoscopic Roux-en-Y gastric bypass. *Obes Surg* 2003; 13: 258-262.
- [29] Raun K, von Voss P and Knudsen LB. Liraglutide, a once-daily human glucagon-like peptide-1 analog, minimizes food intake in severely obese minipigs. *Obesity* 2007; 15: 1710-1716.
- [30] Daniele A, Cammarata R, Masullo M, Nerone G, Finamore F, D'Andrea M, Pilla F and Oriani G. Analysis of adiponectin gene and comparison of its expression in two different pig breeds. *Obesity* 2008; 16: 1869-1874.
- [31] Larsen MO, Rolin B, Sturis J, Wilken M, Carr RD, Porksen N and Gotfredsen CF. Measurements of insulin responses as predictive markers of pancreatic beta-cell mass in normal and beta-cell-reduced lean and obese Göttingen minipigs in vivo. *Am J Physiol Endocrinol Metab* 2006; 290: E670-E677.
- [32] Johansen T, Hansen HS, Richelsen B and Malmlof R. The obese Göttingen minipig as a model of the metabolic syndrome: dietary effects on obesity, insulin sensitivity, and growth hormone profile. *Comp Med* 2001; 51: 150-155.
- [33] Bollen PJ, Madsen LW, Meyer O and Ritskes-Hoitinga J. Growth differences of male and female Göttingen minipigs during ad libitum feeding: a pilot study. *Lab Anim* 2005; 39: 80-93.
- [34] Astrup A, Rossner S, Van GL, Rissanen A, Niskanen L, Al HM, Madsen J, Rasmussen MF and Lean ME. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet* 2009; 374: 1606-1616.
- [35] Christoffersen BO, Gade LP, Golozubova V, Svendsen O and Raun K. Influence of castration-induced testosterone and estradiol deficiency on obesity and glucose metabolism in male Göttingen minipigs. *Steroids* 2010; 75: 676-684.
- [36] Aasheim ET, Bjorkman S, Sovik TT, Engstrom M, Hanvold SE, Mala T, Olbers T and Bohmer T. Vitamin status after bariatric surgery: a randomized study of gastric bypass and duodenal switch. *Am J Clin Nutr* 2009; 90: 15-22.
- [37] Nagem RG, Lazaro-da-Silva A, de Oliveira RM and Morato VG. Gallstone-related complications after Roux-en-Y gastric bypass: a prospective study. *Hepatobiliary Pancreat Dis Int* 2012; 11: 630-635.
- [38] Himpens J, Verbrugghe A, Cadiere GB, Everaerts W and Greve JW. Long-term results of laparoscopic Roux-en-Y Gastric bypass: evaluation after 9 years. *Obes Surg* 2012; 22: 1586-1593.
- [39] White S, Brooks E, Jurikova L and Stubbs RS. Long-term outcomes after gastric bypass. *Obes Surg* 2005; 15: 155-163.
- [40] Magro DO, Geloneze B, Delfini R, Pareja BC, Callejas F and Pareja JC. Long-term weight regain after gastric bypass: a 5-year prospective study. *Obes Surg* 2008; 18: 648-651.
- [41] Swindle MM, Smith AC and Goodrich JA. Chronic cannulation and fistulization procedures in swine: a review and recommendations. *J Invest Surg* 1998; 11: 7-20.
- [42] Moritz MW, Dawe EJ, Holliday JF, Elliott S, Mattei JA and Thomas AL. Chronic central vein catheterization for intraoperative and long-term venous access in swine. *Lab Anim Sci* 1989; 39: 153-155.