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Sleeve Gastrectomy in Mice using Surgical clips

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TITLE:

Sleeve Gastrectomy in Mice using Surgical Clips

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KEYWORDS:

Bariatric surgery, sleeve gastrectomy, diabetes, obesity, high fat diet, mice

SUMMARY:

The prevalence of diabetes and obesity is continuously increasing worldwide. The mechanisms between diabetes, obesity and their associated mortality and co-morbidities

need to be further investigated. Here, we present a protocol for sleeve gastrectomy (SG) in animals as an uncomplicated preclinical model of bariatric surgery.

ABSTRACT:

The number of people who are overweight and obese is continually increasing both in the adult and adolescent populations. This coincides with the increased universal phenomenon of type 2 diabetes (T2D) and other metabolic problems. Bariatric surgery, such as SG, is currently one of the most effective and commonly used long-term treatment for obesity and T2D, but the association between them is not completely explored yet. The mechanisms underlying the outcomes seen after bariatric surgery in humans can be investigated based on preclinical animal studies. The SG reduces body weight, glucose levels and many metabolic parameters, and is easy to perform with a low incidence of complications. The goal of this work is to provide a simple method and an uncomplicated preclinical model of bariatric surgery in animals for researchers.

INTRODUCTION:

The prevalence of obesity has increased nearly threefold worldwide since 1975. In 2016, more than 1.9 billion adults older than 18 years were overweight and over 650 million adults were obese. The prevalence of T2D in the adult population has also doubled from 4.7% to 8.5% with the number rising from 108 million to 422 million adults between 1980 and 2014 globally[1]. Most bariatric surgeries result in significant weight loss, a reduction of body fat, and improvement in glucose tolerance, insulin resistance and diabetes control[2]. In addition to weight loss and remission of T2D, bariatric surgery further produces additional health advantages such as hypertension control and a lower incidence of certain types of obesity related cancer development and progression[3]. Bariatric surgery also induces durable complete, partial remission of T2D, and decreases the risk of ten-year major coronary heart disease (CHD) and some degree of cerebrovascular risk. However, the underlying mechanisms are not completely understood[4].

There are several procedures for bariatric/metabolic surgery. Both restrictive and gastrointestinal bypass surgeries provide positive effects on metabolism[5]. Of the bariatric surgical models, SG and modified Roux-en-Y gastric bypass have higher success and lower mortality rates and demonstrate reliable restrictive and gastrointestinal bypass surgery models in mice[6]. Although the gastrointestinal bypass surgery provides more weight loss and a significant improvement of glucose tolerance and liver steatosis than the restrictive procedure in the long term, the SG still produces good control of body weight and glucose levels and is easy to perform with a low incidence of complications[6]. The proportion of SG increased from 30% to 54% and Roux-en-Y gastric bypass surgery decreased from 52% to 32%

from 2008 to 2014[7]. Currently, laparoscopic SG is the most commonly performed bariatric procedure at the national level within academic centers in the United States[8]. Although there have been many published reports regarding the pathophysiological processes between bariatric surgery, diabetes and obesity, we need more animal experiments to further explore unknown mechanisms.

This protocol aims to produce an animal method to investigate the mechanisms underlying the outcomes seen after bariatric surgery in humans. The current translational study may provide insights on the mechanism of obesity and T2D treatment from the SG.

PROTOCOL

All procedures for animal use were approved by the National Yang-Ming University Institutional Animal Care and Use Committee and complied with the "Guide for the Care and Use of Laboratory Animals. 8th edition, 2011". The anesthetic procedure was performed according to the guideline for the pain control, anesthesia, pre-operative, and postoperative care for the experimental animals from National Yang Ming University, ICAUC-016, 2015, 1st edition.

1. Animal preparation

1.1. Obtain 20 8-week-old wild type C57BL/6 male mice from the National Laboratory Animal Center (NLAC) that weigh around 16-18 grams. Randomly assign these mice into the SG or the sham operation group with 10 mice each.

1.2. Start the mice on chow or a high-fat diet (see **Supplemental File**) prior to the bariatric or sham surgeries[9]. Produce diet-induced obesity with at least 2 preoperative weeks of the HFD.

1.3. House the mice in the National Yang-Ming University Laboratory Animal Center under a light-dark cycle of 12:12 with light onset at 07:00 A.M. and allow free feeding to standard rodent chow and water.

1.4. Restrict all animals from food but allow free water access on the night before surgery.

2. General preoperative preparation

2.1. Fast the mice for at least 6 hours before the surgery. Induce anesthesia in an induction chamber with 5% isoflurane and oxygen (both 3-4 L/min).

2.1.1. Check the depth of anesthesia ensuring that pinch stimulation (blunt curved serrated smooth micro-forceps) of the hind paw, the forepaw, and the ear does not evoke any motor reflex. Administer antibiotics (25 mg/kg cefazolin) via subcutaneous injections before the operation.

2.2. Assign a particular work zone for surgical procedures. Clean the surgical area and spray the surgical table with 75% alcohol solution before an operation.

2.3. Place the mouse in the nose cone and maintain anesthesia with 2% isoflurane (2 L/min) and oxygen (4 L/min).

2.4. For sleeve gastrectomy and sham operation, after confirming the appropriate depth of anesthesia by toe stimulation, apply the 0.2% Carbomer eye gel on the mouse's eyes to prevent dry eyes.

2.5. Use an electric razor to shave hair from the abdomen to the sternum. Remove and clean the remaining hair with a depilatory cream. Place and fix the mouse on the surgical table in a supine position.

2.6. Disinfect the abdominal wall of the mouse with povidone-iodine solutions alternating with 75% alcohol for 3 applications.

3. Sleeve gastrectomy and sham procedures

3.1. Median laparotomy

3.1.1. Use a micro-scissor to make a 1-1.5 cm length midline incision at the upper abdomen.

3.2. Stomach and intestine externalization

3.2.1. Perform the SG procedure with the aid of a magnifying dissecting microscope or magnifier as necessary to prevent the unpredictable bleeding and hypovolemic shock.

3.2.2. Use two curved smooth serrated micro-forceps to gently move the stomach and complete externalize it.

3.2.3. Carefully divide the gastrosplenic ligament connecting the left stomach to the spleen using a cotton-tipped probe and electrocautery as needed, thereby dissecting the gastric fundus from the surrounding spleen and other internal organs.

4. Stomach isolation and clip

NOTE: The SG for mice was performed using the new clip applier technique as previously published[10].

4.1. Stretch the fundus and pylorus of the stomach gently and laterally with forceps, identify the midline (**Figure 1** and **Figure 2**), and carefully apply surgical clips to half of the medial side of the stomach midline from the gastroesophageal junction inferiorly and the lower pole superiorly. Clamp and exclude around 75-80% of stomach, thereby creating the entire lateral sleeve of the stomach (**Figure 1** and **Figure 2**).

4.2. Move the intestine to the bare skin on the side and cover it with a warm-saline moistened gauze and perform intraperitoneal hydration as needed to prevent dehydration and hypothermia.

4.3. Resect the lateral clipped stomach, remove the excluded sleeve of the stomach with microscissors and then sterilize the cut edge of the stomach with povidone-iodine solution.

4.4. Oversew the clip line with a 5-0 monofilament nonabsorbable suture to ensure no leakage. Knot the ends of the suture and anchor to the clips on either end.

4.5. Return the stomach and intestine to the proper position in the abdominal cavity and close the abdomen with a running 5-0 monofilament nonabsorbable continuous and discontinuous suture to the fascia and abdominal wall.

4.6. Administer analgesics with ketoprofen (2-5 mg/kg) and antibiotics with cefazolin (25 mg/kg) intraperitoneally after the whole procedure.

5. Sham procedure of SG

5.1. Perform a similar procedure as described previously with a midline laparotomy, and externalize the intestine and stomach using 37 °C wet warm saline gauze coverage for 5 min.

5.2. Return the stomach and intestine to the proper sites of these internal organs.

5.3. Close the abdominal wall carefully as previously described with 2 layers of continuous and discontinuous closure to the fascia and skin using slow or non-absorbable monofilament sutures. Administer analgesics with ketoprofen (2-5 mg/kg) and antibiotics with cefazolin (25 mg/kg) intraperitoneally after the whole procedure.

6. General postoperative care

6.1. Stop isoflurane and continue with a room air flow of 3-5 L/min until the mouse is fully awake.

6.2. Keep watching the mouse while it regains mobility and begins to walk around the cage.

6.3. Place the mouse in a 30 °C independent incubator for 5 days. Make sure there is only one mouse per cage to prevent the mice from injuring each other.

6.4. Return free access to a gel diet food (high-fat gel diet: 10% lard, 10% liquid sugar, 57% water) for 3 days after surgery and reintroduce the previous assigned diet 3 days after surgery.

6.5. Subcutaneously or intraperitoneally inject ketoprofen (2-5 mg/kg), and cefazolin (25 mg/kg) for 1 day after the operation.

6.6. Assess the mouse body weight weekly through the whole study period. Provide ketoprofen (2-5 mg/kg) by intraperitoneal injection for pain control once daily as needed if the animal is in distress after the operation.

NOTE: Here, the survival rate of SG was 90% after the learning period.

7. Metabolic parameter assessment

7.1. Fast mice for 6 h, and take baseline blood samples (0 min). Obtain all blood samples from the tip of the tail vein of freely moving mice[11,12].

7.2. Deliver 1 mg/g of 50% dextrose by intraperitoneal injection for the glucose tolerance test.

7.3. Measure the blood glucose from the tips of the tail veins of freely moving mice at 0, 5, 15, 30, 60, and 120 min after glucose administration on duplicate samples using glucometers

and test strips.

7.4. Keep the whole blood sample (n = 10 for each group) at room temperature with clotting for 30 minutes.

7.5. Centrifuge the blood sample at 3,000 x *g* for 10 minutes at 4 °C.

7.6. Transfer the plasma into separate tubes without disturbing blood clots and store at -80 °C.

7.7. Study the plasma samples at the end of the study using commercial mice ELISA kits for hemoglobin A1c (HBA1c), glucose, cholesterol, and insulin levels according to the manufacturer's guidelines.

REPRESENTATIVE RESULTS:

Results of the operation are shown in **Figure 3** and **Figure 4**. The survival rate of the study was 90%. One mouse died in the sham group because of weakness and another mouse died in the SG group on the third day after operation for unknown reasons. Results obtained on weight loss (**Figure 3A**) after operation (at week 3) on HFD fed mice were quite similar to those observed in humans, with a final body weight loss of about 15-20%[13]. The study also demonstrated that the cholesterol level (**Figure 3B**) decreased significantly after SG surgery in the HFD fed mice.

Better insulin sensitivity and glucose tolerance were observed soon after the surgery by an intraperitoneal glucose tolerance test (IPGTT). In the current study, we demonstrated that the HFD-induced obesity evoked insulin resistance and glucose intolerance in wild type C57BL/6 mice and can be corrected by SG. After SG, the insulin resistance, glucose, and lipid levels all improved in this study.

Figure 1: Surgical procedures of SG. (**A**) The 5 mm surgical clip used in this experimental model. (**B**) Check the depth of anesthesia with pinch stimulation. (**C,D**) Apply the clip to the half of medial side of the stomach midline from gastroesophageal junction inferiorly and lower pole superiorly. (**E**) Sterilize and suture the cut edge of the stomach to ensure no leakage. (**F-H**) Hydrate the mice with warm saline, return the stomach and intestine back to the proper site in the abdominal cavity. Close the abdominal cavity carefully. Recover from the anesthetizing status in the induction chamber and return to the cage when the condition was fully recovered and stabilized.

Figure 2: Sites of surgical clip usage to the stomach

Figure 3: The effect of SG on (A) weekly body weight changes and (B) cholesterol level. Data are represented as mean ± SD. *P < 0.05; **P < 0.001.

Figure 4. Effect of SG on (A) IPGTT (B) Hba1c and (C) HOMA-IR at the end of the study (n = 5 VSG; n = 7 sham); Data are represented as mean ± SD. *P < 0.05; **P < 0.001.

DISCUSSION:

Most of the prospective cohort studies have confirmed that increasing the body mass index is related to increasing the mortality. Obesity is associated with a higher risk of diabetes, cardiovascular diseases (CVD), and death in both sexes. The specific pathologic processes have established a link between diabetes, obesity, and mortality[14]. The systematic reviews indicate the benefits of bariatric surgery in reducing risk factors for CVD with evidence for ventricular hypertrophy regression and improved diastolic function as well[15]. Bariatric/metabolic surgery can improve traditional CVD risk factors in obese diabetic patients, and the 10-year CHD and fatal CHD risk have shown to be reduced by up to 50% after various bariatric/metabolic surgical procedures in obese patients with T2D[4].

Bariatric surgery is the most effective long-term therapeutic strategy for obesity. The significant improvements of metabolic parameters from bariatric surgery such as lipid profile, inflammatory biomarkers, blood pressure, and heart failure are also noted especially in the unexpected type 2 diabetic control. SG has reduced and modified adipose tissue inflammation and development and has possibly reduced the atherosclerosis risk.

Changes of gastro-intestinal hormones might play a role in long-term weight control[16,17]. The SG has also reduced caloric intake and normalized the glycemic control[18]. The gastric bypass surgery related glycemic tolerance effects are related to the foregut hormonal effects evoked by bypassing the proximal bowel from the current theories[19]. However, the SG has demonstrated the comparable antidiabetic effect, suggesting further non-bypassing related unknown mechanisms even beyond the effect of glucagon-like peptide 1 receptor[16,20]. Lopez et al. have shown that SG promotes weight loss, improves the cholesterol and glucose profile, leptin insensitivity, and decreases insulin resistance, which is independent of the ghrelin level changes in obese Zucker rats[21]. All these above issues need further animal studies to clarify the connection with diabetes, obesity and diseases. SG is a bariatric procedure that does not involve intestinal bypass, which has become increasingly popular in recent decades in the US and globally due to low long term complications[22].

The bariatric surgical procedure is technically difficult and time consuming with high surgical mortality and long-term complication and is the main critical step for small animal experiments. The SG is currently the most used surgery for weight reduction in the US because it is a simple procedure with high effectiveness, low co-morbidity, and low mortality. This protocol has similar advantages mimicking the weight reduction and metabolic surgeries practiced routinely in humans. In general, surgical techniques are quite difficult in mice because of the small body size, and a dissecting microscope is frequently needed with high surgical mortality.

Schlager et al. have developed a mouse model of SG with a surgical clip application instead of a hand sewn closure for the stomach. This animal model has demonstrated similar effects of body weight reduction and glucose control, but has failed to reveal significant pancreatic islet cell proliferation[10]. This effect on the metabolism from this mice model is similar to the human bariatric/metabolic surgery with a very low operative mortality.

This current study demonstrates constant weight reduction after the SG in the HFD fed mice with improved glycated hemoglobin, IPGTT, HOMA-IR (homeostatic model assessment for insulin resistance), and cholesterol level. This surgical procedure is safe and easy to perform in mice.

The surgical survival rate of this protocol in our laboratory is around 90% after a learning period, and the procedure takes less than 15 minutes. This protocol may provide significant value in future small rodent studies with surgical feasibility, safety, reproducibility, and clinical relevance. This method may also be used to highlight physiological changes observed after bariatric surgery in humans. The difficulty of SG surgery plays an important factor in the success of animal studies. This protocol is easy to perform, and imitates the SG as commonly practiced in other animal and human studies.

This animal model can investigate the clinical relevance of diabetes and obesity using a brief surgical procedure with sleeve gastrectomy. These procedures can shorten the operation time and improve the surgical survival rate. The main advantage of this study is to mimic the clinical outcomes of bariatric surgery in human patients using a simple surgical model.

DISCLOSURES

The authors declare no conflict of interest.

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Fig 1 whole procedure

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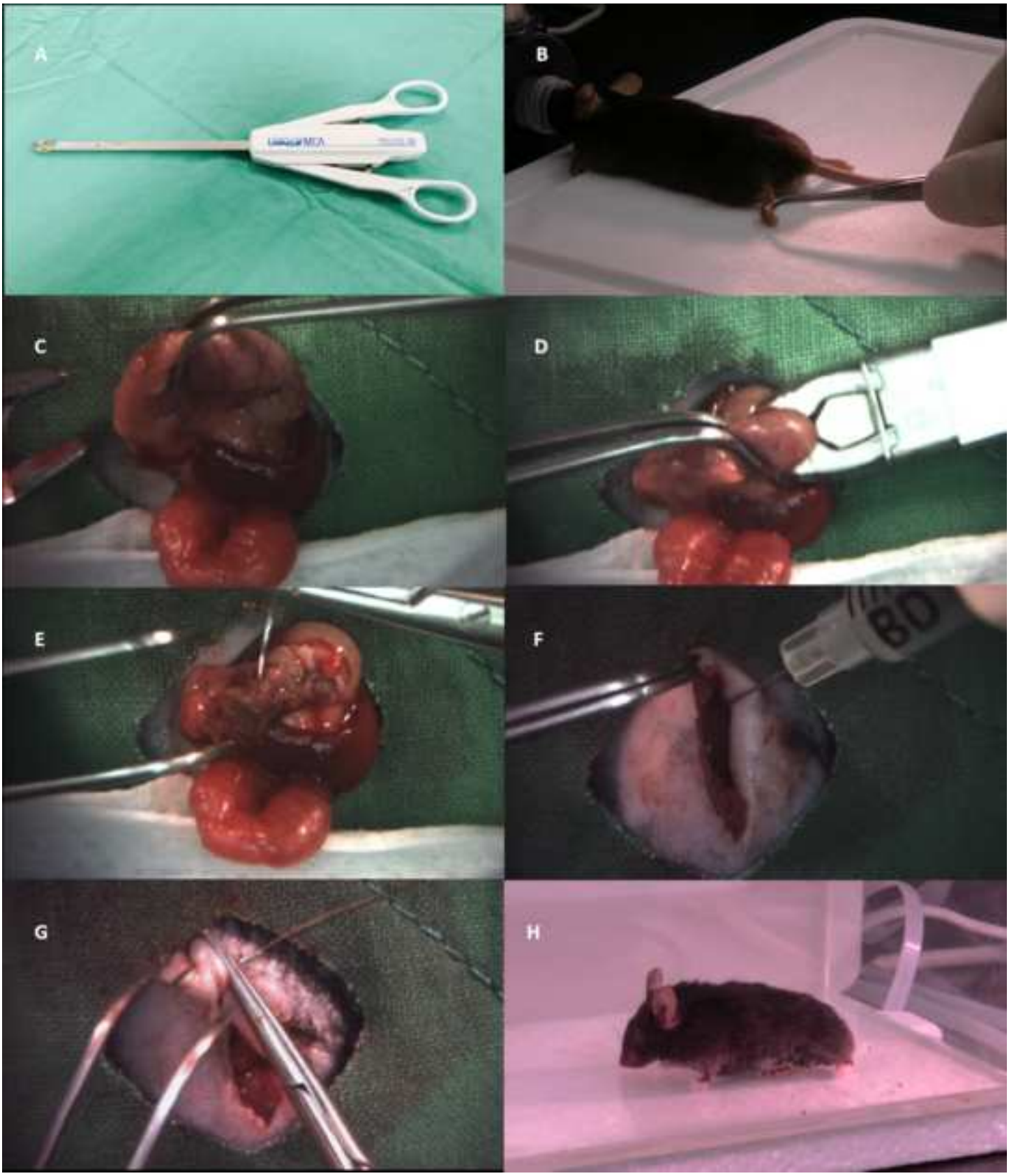

Fig 2

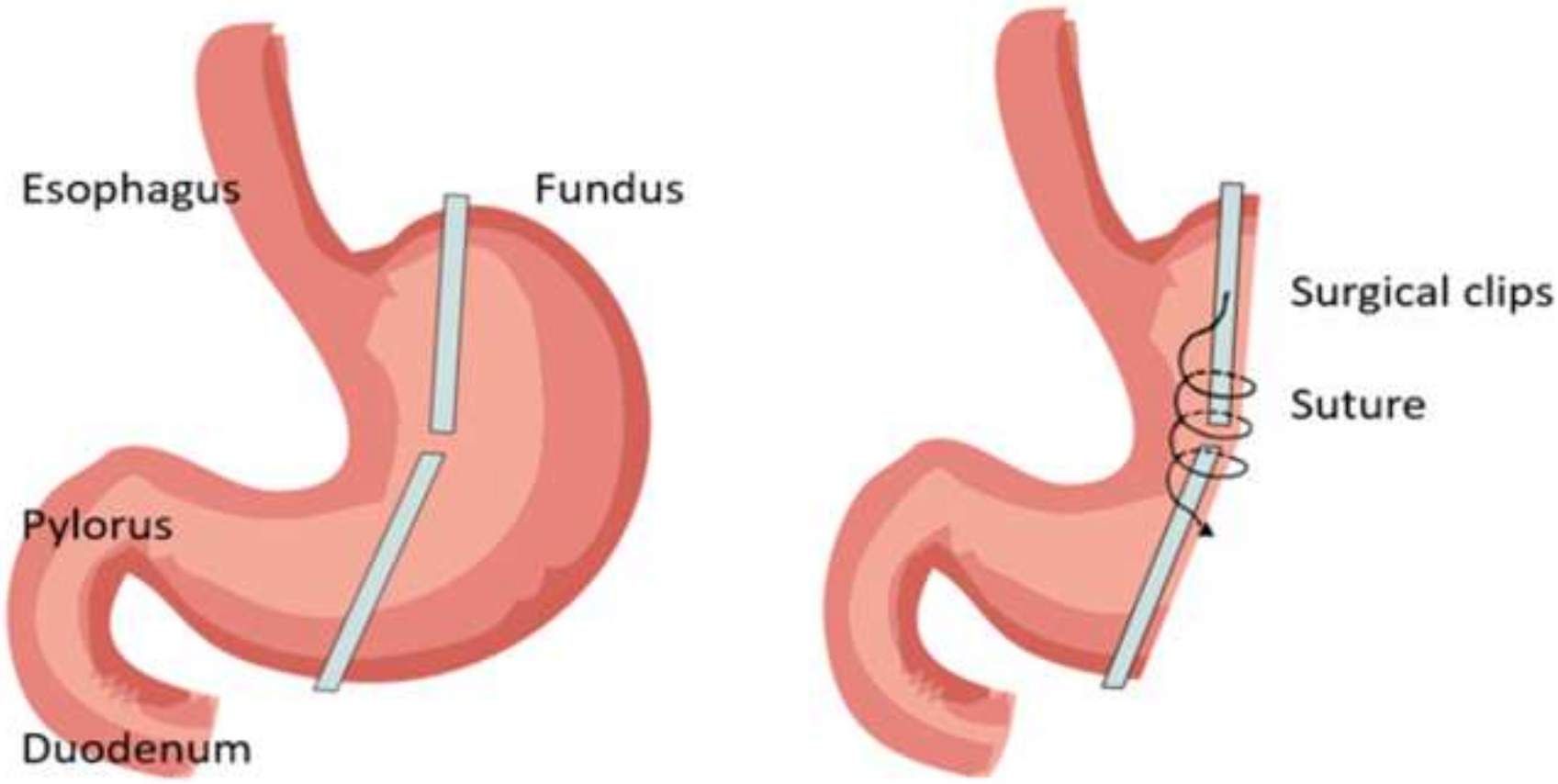

Figure 3

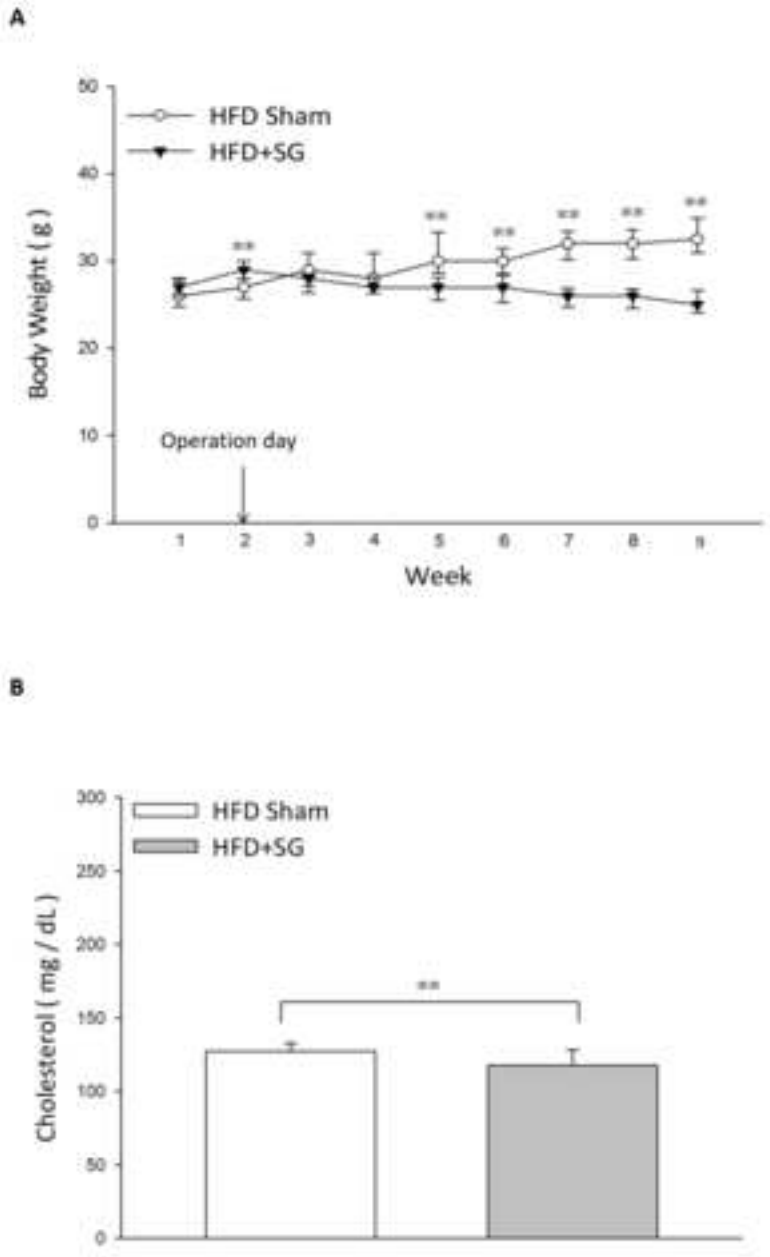

Figure 4

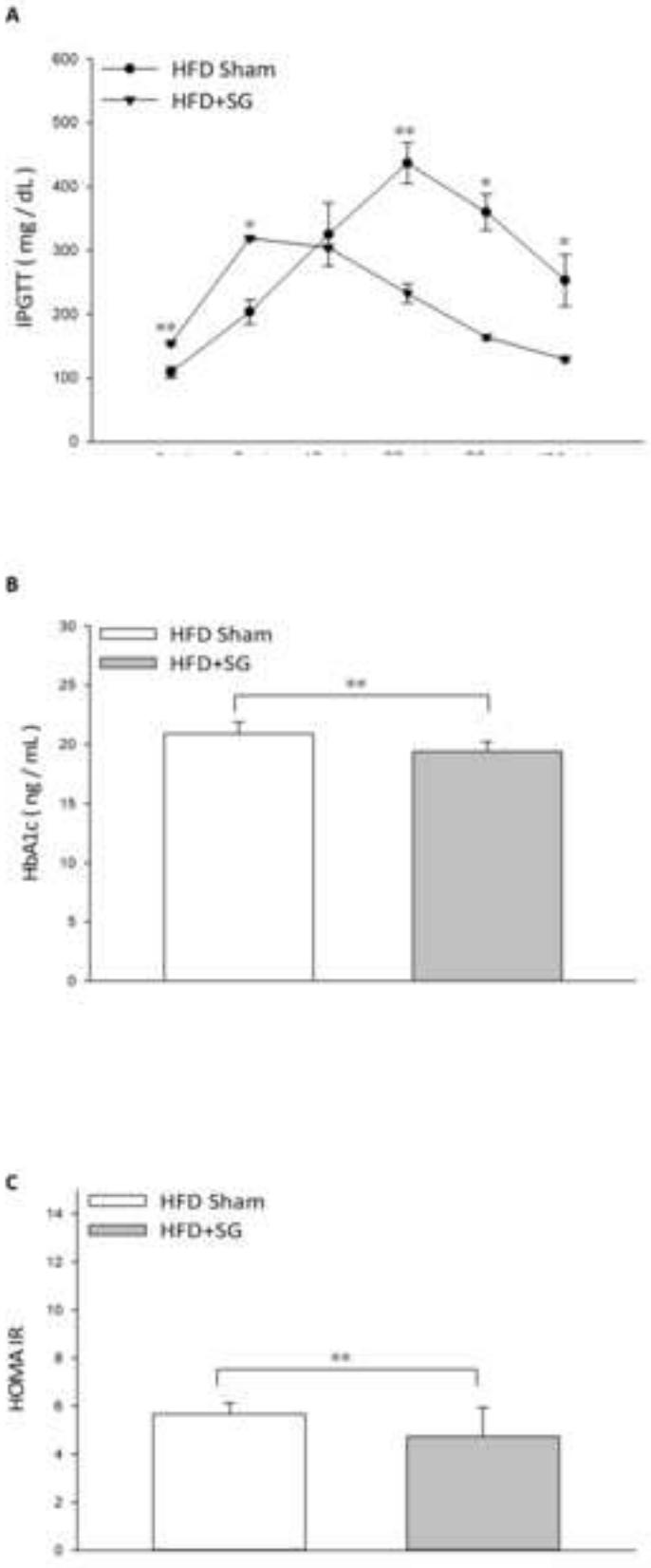

Table of Materials

Name	Company	Catalog Number
0.9% normal saline	China Chemical & Pharmaceutical Co., Ltd.	
5-0 monofilament suture	Shineteh Inc. Taiwan	
Alm	Shineteh Inc. Taiwan	ST-A072PK
Animal Anesthesia	Step Technology	0712VAP11076
Barraqer-Troutman forceps	Shineteh Inc. Taiwan	ST-N309(H-4410)
cefazolin	Taiwan veteran pharmacy company	
Chow diet	Research Diets	
Glucose strips and glucometer	BeneCheck	BKM13-1
HbA1c kit	Level	OKEH00661
Hematology system	Fuji	FUJI DRI-CHEM 4000i
High Fat diet	Research Diets	1810724
Iris Forceps	Shineteh Inc. Taiwan	ST-1210
Iris Scissors	Shineteh Inc. Taiwan	ST-S011
Isoflurane	Panion & BF biotech INC	8547
Ketoprofen	Sigma	22071154
Stereo microscope	MicroTech	SZ-5T
Surgical clip (M)	Echicon Inc., Somerville, NJ	
Vidisic gel	Dr. Gerhard Mann Chem.-pharm. Fabrik GmbH	

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Matrx
UNIK
BeneCheck
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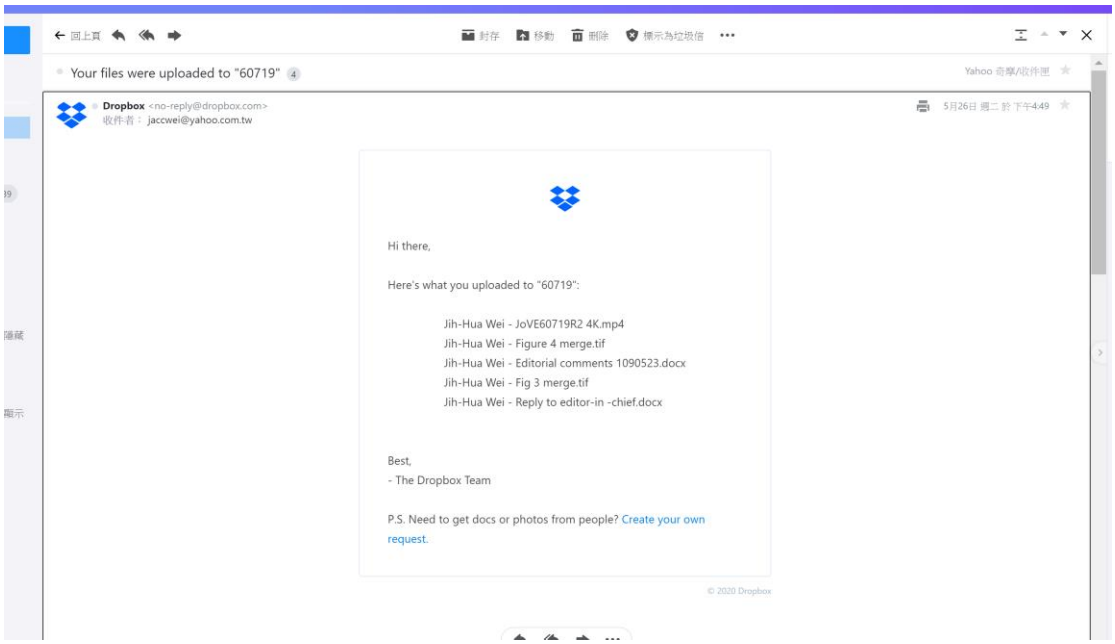

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Changes to be made by the author(s) regarding the video:

1. Please change the title of the video to match the title of the written manuscript.

Reply: Yes, we have changed the title and the end of the video to “Sleeve gastrectomy in mice using surgical clips “.

2. xxx

Reply: ???, Sorry, I don’t understand.

3. There are still significant issues with the editing style. For the previous submission, I listed all of the jump cuts and asked for them to be replaced by crossfades. In this revision, some of those jump cuts were replaced by partial fades to black, and the rest of the jump cuts remained. Below I am listing three things: 1. The edits that are still jump cuts. These needs to be replaced with crossfades (where one clip fades directly into the next clip). 2. The edits that are these fades to black. These also need to be replaced by crossfades. 3. Small sections of the video where there are too many jumps cut edits within a short time span. The edits need to be spaced out or removed to make these actions easier for the audience to follow.

Reply: Sorry for the incomplete corrections. We have corrected all these problems. We used the PowerDirector 17 crossfade and fades techniques for the editing. Due to the re-editing and changes, the following corrected timing has changed as well with a great extent.

Jump cuts (to be replaced by crossfades)

0:46: corrected with crossfades

0:50: corrected

0:55: corrected

1:02: corrected

1:26: corrected

1:33: corrected

2:14: corrected

2:33: corrected

2:52: corrected

2:56: corrected

3:01: corrected

3:11: corrected

3:34: corrected

Partial fades to black (to be replaced by crossfades)

1:09: corrected

1:30: corrected

1:41: corrected

1:45: corrected

1:55: corrected

2:16: corrected

2:27: corrected

2:46: corrected

3:26: corrected

3:32: corrected

3:36: corrected

Sections where there are too many edits (edits should be spaced out or removed)

0:50-0:52: re-edited

1:05-1:06: re-edited

2:46-2:50": re-edited

4. Video Framing

1:10-1:16 - There are thin black borders on the right and bottom sides of the frame. The video clip should be resized and/or repositioned to eliminate this.":

Reply: The section has been deleted.

Breslow Western Diet

5TFH

DESCRIPTION

Modification of TestDiet® AIN-76A Semi-Purified Diet 5800-B, Breslow Western . This formula originally known as TD88137.

Storage conditions are particularly critical to TestDiet® products, due to the absence of antioxidants or preservative agents. To provide maximum protection against possible changes during storage, store in a dry, cool location. Storage under refrigeration (2° C) is recommended. Maximum shelf life is six months. (If long term studies are involved, storing the diet at -20° C or colder may prolong shelf life.) Be certain to keep in air tight containers.

*Product Forms Available**	*Catalog #*
1/2" Pellet	1810724

**Other Forms Available By Request*

INGREDIENTS (%)

Sucrose	34.1460
Milk Fat	21.0000
Casein - Vitamin Free	19.5000
Dextrin	15.0000
Powdered Cellulose	5.0000
AIN-76 Mineral Mix	3.5000
AIN-76A Vitamin Mix	1.0000
Calcium Carbonate	0.4000
DL-Methionine	0.3000
Cholesterol	0.1500
Ethoxyquin (a preservative)	0.0040

FEEDING DIRECTIONS

Feed ad libitum. Plenty of fresh, clean water should be available at all times.

CAUTION:
Perishable, upon receipt store in a cool dry place, refrigeration recommended.

For laboratory animal experimental use only, NOT for human consumption.

5/23/2005

NUTRITIONAL PROFILE [1]

Protein, %	***17.8***
Arginine, %	0.68
Histidine, %	0.50
Isoleucine, %	0.93
Leucine, %	1.69
Lysine, %	1.42
Methionine, %	0.80
Cystine, %	0.07
Phenylalanine, %	0.93
Tyrosine, %	0.99
Threonine, %	0.75
Tryptophan, %	0.22
Valine, %	1.11
Alanine, %	0.54
Aspartic Acid, %	1.26
Glutamic Acid, %	3.98
Glycine, %	0.38
Proline, %	2.30
Serine, %	1.08
Taurine, %	0.00
Fat, %	***20.2***
Cholesterol, ppm	2,056
Linoleic Acid, %	0.53
Linolenic Acid, %	0.10
Arachidonic Acid, %	0.03
Omega-3 Fatty Acids, %	0.00
Total Saturated Fatty Acids, %	12.58
Total Monounsaturated Fatty Acids, %	4.60
Fiber (max), %	***5.0***
Carbohydrates, %	***50.5***
Energy (kcal/g) [2]	***4.55***

From:	kcal	%
Protein	0.710	15.6
Fat (ether extract)	1.815	39.9
Carbohydrates	2.022	44.4

Minerals	
Calcium, %	0.68
Phosphorus, %	0.55
Phosphorus (available), %	0.55
Potassium, %	0.36
Magnesium, %	0.05
Sodium, %	0.10
Chlorine, %	0.16
Fluorine, ppm	0.0
Iron, ppm	36
Zinc, ppm	35
Manganese, ppm	59
Copper, ppm	6.0
Cobalt, ppm	0.0
Iodine, ppm	0.21
Chromium, ppm	2.0
Molybdenum, ppm	0.00
Selenium, ppm	0.11

Vitamins	
Vitamin A, IU/g	12.0
Vitamin D-3 (added), IU/g	1.0
Vitamin E, IU/kg	50.0
Vitamin K (as menadione), ppm	0.50
Thiamin Hydrochloride, ppm	6.0
Riboflavin, ppm	6.0
Niacin, ppm	30
Pantothenic Acid, ppm	15
Folic Acid, ppm	2.0
Pyridoxine, ppm	5.8
Biotin, ppm	0.2
Vitamin B-12, mcg/kg	10
Choline Chloride, ppm	0
Ascorbic Acid, ppm	0.0

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly. Nutrients expressed as percent of ration on an As Fed basis except where otherwise indicated.
2. Energy (kcal/gm) - Sum of decimal fractions of protein, fat and carbohydrate x 4,9,4 kcal/gm respectively.

ISO 9001:2000 CERTIFIED

TestDiet
www.testdiet.com

Here we declare all animals were used humanely and appropriately anesthetized. The pain control and prevention were also performed with ketoprofen as needed for potentially painful procedures and distress under animal welfare.

Jih-Hua Wei, M.D., Ph.D.

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Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

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Author(s):	Jih-Hua Wei, M.D., Ph.D., Che-Hung Yeh, M.S., Wei-Jei Lee, M.D., Ph.D., Shing-Jong Lin, M.D., Ph.D., Po-Hsun Huang, M.D., Ph.D.

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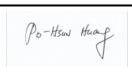
Signature:	
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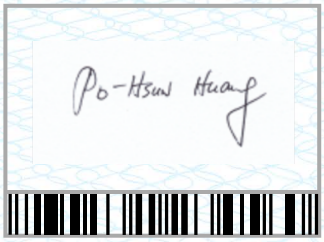

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