Effects of sleeve gastrectomy in leptin deficient (ob/ob) mice and diet induced obese mice

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Abstract

Background/ Aims: Vertical sleeve gastrectomy improves weight loss and metabolic complications in obese humans. The aim of the present study was to show the outcome of sleeve gastrectomy in obese and super obese mice.

Methods: In an animal study, male wild type (WT) C57BL/6 diet-induced obese (DIO) mice were used to mimic obesity, and ob/ob mice were used for leptin deficiency-induced super obesity. To investigate whether sleeve gastrectomy works in leptin-deficient mice, the two groups were compared in weight course for 7 weeks after sleeve gastrectomy.

Results: In WT mice, vertical sleeve gastrectomy induced weight loss over the initial study period followed by weight regain over the presurgery level. In leptin-deficient ob/ob mice body weight was as well clearly reduced during the first weeks with an increase thereafter to the presurgery level.

Conclusion: Those data demonstrate that ob/ob mice and DIO mice responded differently to sleeve gastrectomy. In leptin deficient super obesity restrictive sleeve gastrectomy promotes an effective weight loss procedure. Sleeve gastrectomy leads to leptin-independent changes with favourable weight course.

Introduction

The continuous rise of obesity in the world is leading to a growing burden for health care systems and individual health. Conservative methods to weight reduction are most often failing. Bariatric surgery is the most successful strategy for treating obesity. The underlying mechanisms for this success are not clearly understood.

The most widely used bariatric surgery procedures are Roux-en-Y gastric bypass (RYGB) and vertical sleeve gastrectomy (VSG) [1]. A large multicenter study showed that the vertical sleeve gastrectomy as a stand-alone procedure seems to be a safe and effective procedure for the treatment of obesity and obesity related comorbidities [2]. Previous studies showed the effectiveness of sleeve gastrectomy in regulating body weight and ameliorating diabetes mellitus type II [3]. The effects of VSG and RYGB regarding loss of body weight, fat mass and improved glucose tolerance in humans are similar. Due to the lower operative risks and the lower postoperative morbidity, VSG is increasingly preferred [4]. Beside the restrictive character, the exact mechanisms of weight reduction after sleeve gastrectomy remained unclear. Concerning this there are considerations that the removal of the gastric fundus may affect food intake and final weight loss through hormonal pathways [5,6].

We have recently established a valid sleeve gastrectomy mouse model, characterized by a small gastric pouch, low mortality and suppression of body weight [7]. As restriction alone can not be the explanation of the potent effects of VSG, hormonal pathways must additionally have a decisive role. Leptin and other hypothalamic hormones regulate the energy balance over command and feedback signals. Leptin and adiponectin count to the peripheral factors that are produced by adipose tissue and regulate via the hypothalamic mechanism the long-term energy status [8]. Leptin concentration correlates with body weight and leptin-resistance contributes to hyperphagia, hypometabolism and obesity. The impact of leptin-feedback in loss and controlling of weight after sleeve gastrectomy is not known.

The present study was conducted to explore mechanisms of weight reduction including the impact of leptin-deficiency in obesity and super obesity following restrictive sleeve gastrectomy.

Materials and methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the University Hospital Tübingen. The institutional guidelines for the care and use of laboratory animals were followed throughout the study.

The mice were housed in the small animal facility of the University hospital of Tübingen under a light dark cycle of 12 to 12 hours.

In the study design wild type male C65Bl/6 mice (n=19) were used in diet induced obesity (DIO) group. DIO mice were generated by feeding with a high-fat diet (D12451; 60% calories from fat, 20% proteins, 20% carbohydrates, vitamins and minerals. Research Diets, Inc., New Brunswick, NJ) and a fructose sweetened beverage (3%) and given free access to water for 12 weeks. After surgery DIO mice were continuously fed with high-fat diet.

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Male ob/ob mice (n=16) in C57Bl/6j gene background were purchased from the Charles River Laboratories (Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany) at 4 weeks of age and fed the regular chow diet ad libitum before and after surgery.

**Surgical procedures**

Prior to surgical procedures, the mice were fasted for 24 h with free access to water. Sleeve gastrectomy was performed in DIO mice with a body weight of about 29 g.

In ob/ob mice surgery was performed at 12 weeks in age with a body weight of about 63 g.

**Anesthesia**

Mice were maintained by inhalation anesthesia throughout the preparation and operation, using a scavenged mask circuit to effect 1 to 4% of isoflurane (Forane®, Baxter Healthcare Corporation, Deerfield, IL 60015 USA).

**Analgesia**

In general anesthesia, the achievement of preoperative analgesia was performed with 5 mg per kg bodyweight of Carprofen (Rimadyl®, Pfizer) subcutaneously administered. For postoperative analgesia, subcutaneous Carprofen was given for further 3 days in a dosage of 5 mg per kg bodyweight.

**Aseptic techniques**

Standard sterile procedures were followed in all operations. The shaped abdomen was prepared with 3 times skin disinfection with uncoloured alcohol solution (Softasept® N B. Braun Melsungen AG, Melsungen, Germany) and on the operating field a sterile coverage with a fenestrated drape was placed. Sterile surgical gloves and instruments were used. No prophylactic or therapeutic doses of antibiotics or antifungals were administered.

**Sleeve gastrectomy**

After the achievement of anesthesia, analgesia and aseptic conditions with sterile coverage, an upper midline laparotomy was performed over a length of 1.5 centimeter to the xiphoid cartilage and a self-retaining retractor was placed. The liver was gently retracted and the stomach was gently exposed out of the abdominal cavity. The short gastric vessels were ligated towards the spleen using 6/0 vicryl (Ethicon, Norderstedt, Germany) and the greater omentum was dissected along the greater omentum was dissected along the greater curvature of the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume.

The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume. The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume. The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume. The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume. The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume. The tubular sleeve was created setting a vascular (bulldog) clamp on the stomach from 3 mm proximal the pylorus to the gastroesophageal junction (Angle of His) with always the same volume. The excluded greater curvature was dissected along the bulldog clamp aiming a resection of over 80% of gastric volume.

**Postoperative period**

After the surgical procedure the mice were placed back into the cages. For postoperative analgesia subcutaneous Carprofen was given for further 3 days in a dosage of 5 mg/kg bodyweight. On the day of operation, mice were kept without food with free access to water. For the following three days, a liquid diet with glucose syrup and free access to water was provided with subsequent transition to free high-fat diet (60% calories from fat) in DIO mice and the regular chow diet in ob/ob mice for the following weeks, always depending on the observed health and behavior of each animal.

**Physiological parameters**

Body weight was measured daily in the first 2 weeks and then weekly for 7 weeks after the surgery.

**Euthanasia**

In general anesthesia with isoflurane (Forane®, Baxter) and analgesia with 5 mg/kg bodyweight subcutaneous Carprofen (Rimadyl®, Pfizer), the mice were sacrificed by cardiac puncture and exsanguination 8 weeks after the surgical procedure. On mice, which died prior to 6 weeks after surgery, a necropsy was performed.

**Statistical analysis**

In this study, values are presented as mean ± standard deviation. Graphics were done using GraphPad Prism (Version 5.0c).

**Results**

SG resulted in weight loss in ob/ob mice and wildtype DIO mice

**Initial weight**

- DIO mice: In DIO mice (n=19) SG was performed at 12 weeks on HFD with body weight around mean 28.6 g (standard deviation ± 1.86 g, range 25 g to 31 g).
- Ob/ob mice: In ob/ob mice (n=16) SG was performed with a mean body weight around 62.8 g (standard deviation ± 4.4 g, range 52 g to 70 g).

**Weight course**

DIO mice: DIO mice showed a clear weight loss for the first week with an immediate weight regain thereafter (Figure 1). Most weight reduction was in the first week post-surgery with a peak of the percentual weight loss of 40% in the first postoperative week.

ob/ob mice: ob/ob mice showed an immediate and clear weight loss in the first week with a slow weight increase thereafter (Figure 2a and 2b). Most weight reduction was in the first week post-surgery with a peak of the percentual weight loss of 15% in the first postoperative week.

Percentage weight loss was compared between DIO and ob/ob mice at 1, 3, 5 and 7 weeks postsurgery (Figure 3). Persistent weight reduction was observed in ob/ob mice, but not in DIO mice after sleeve gastrectomy.

**Intraoperative complications and peri- and postoperative mortality**

DIO mice

In DIO mice intraoperative mortality was 0%. No significant bleeding was seen from either gastric exposure out of the abdominal cavity, resection or closure. Perioperative mortality during the first
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Figure 1. a) Postoperative course of absolute mean weight after sleeve gastrectomy in DIO mice. b) Percentual course of weight after sleeve gastrectomy in DIO mice.

Figure 2. a) Postoperative course of mean weight after sleeve gastrectomy in ob/ob mice. b) Percentual course of weight after sleeve gastrectomy
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Food and to gain significantly more body weight. Therefore, surgical procedures still retain the capacity to consume substantially larger amounts of food, though RYGB and VSG reduce the amount of food, animals and humans have been challenged by recent data from both humans and rodents: Even for these findings beginning with gastric volume restriction. The restrictive hypothesis, gastric volume restriction triggers earlier satiety after SG and RYGB due to increased gastric pressure, has been challenged by recent data from both humans and rodents: Even though RYGB and VSG reduce the amount of food, animals and humans still retain the capacity to consume substantially larger amounts of food and to gain significantly more body weight. Therefore, surgical procedures are also existing [10,11].

The current study allowed us to compare DIO and ob/ob mice in sleeve gastrectomy surgery. Ob/ob mice exhibited a weight loss of 15% in the first 2 weeks post surgery with a slow increase thereafter. Nevertheless, the effect was sufficient to keep the body weight under the presurgery level during the observation period. DIO mice, however, after initial weight loss of 40% regained weight rapidly surpassing their preoperative mean weight by about 10%. Ob/ob mice ( ) showed a moderate mean weight decrease with a slow regain in the further course without exceeding the mean preoperative weight.

Week was 12.5% (n=2). The overall survival of the study period (7w) was 74% (n=14).

ob/ob mice

In ob/ob mice intraoperative mortality was 0%. No significant bleeding was seen from either gastric exposure out of the abdominal cavity, resection or closure. Perioperative mortality during the first week was 12.5% (n=2). The overall survival of the study period (7w) was 75% (n=12). The overall survival of all groups was 74%.

Discussion

In this manuscript, we demonstrate that the weight reducing effects of sleeve gastrectomy are preserved even in leptin-deficient ob/ob mice. This demonstrates that the biological effects of SG are leptin-independent. We used our already published model which is characterized by close similarity to human SG surgery, including postoperative weight reduction, fast operation times and low mortality [7]. Bruinsma et al are providing another standardized procedure for mouse and rat models of Roux-en-Y gastric bypass and sleeve gastrectomy with detailed protocols [9]. Other surgical models of sleeve gastrectomy are also existing [10,11].

The current study allowed us to compare DIO and ob/ob mice in sleeve gastrectomy surgery. Ob/ob mice exhibited a weight loss of 15% in the first 2 weeks post surgery with a slow increase thereafter. Nevertheless, the effect was sufficient to keep the body weight under the presurgery level during the observation period. DIO mice, however, after initial weight loss of 40% regained weight rapidly surpassing their preoperative weight early on. There are several possible explanations for these findings beginning with gastric volume restriction. The restrictive hypothesis, gastric volume restriction triggers earlier satiety after SG and RYGB due to increased gastric pressure, has been challenged by recent data from both humans and rodents: Even though RYGB and VSG reduce the amount of food, animals and humans still retain the capacity to consume substantially larger amounts of food and to gain significantly more body weight. Therefore, surgical procedures are also existing [10,11].

Conclusion

Taken together, the present study shows weight reduction in DIO and leptin-deficient mice following sleeve gastrectomy. Therefore, our results suggest that leptin-sensitivity is not required for the effects of sleeve gastrectomy. However, long and short term weight course of ob/ob mice and DIO mice were different indicating that intact leptin circuitry is at least a modulating factor in SG outcome. Future research is needed to determine whether leptin is a distinguishing mechanism between VSG and RYGB.

Conflict of interest

None of the authors has a financial interest with regard to the submitted manuscript to declare.

Authorship

RB: Planned the study. Did the animal study. Did literature search. Wrote the article. Approved the final version.

JL: Did part of the animal study. Did literature search. Approved the final version.

AK: Wrote part of the article. Approved the final version.

JL: Did part of the animal study. Did literature search. Approved the final version.

MAK: Wrote part of the article. Approved the final version.

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