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Original Research

Severe Anemia in Sprague–Dawley Rats after Roux-en-Y Gastric Bypass Surgery

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Roux-en-Y gastric bypass (RYGB) surgery is one of the most commonly performed bariatric procedures for weight loss in humans. However, this procedure is not risk-free, and patients may experience complications that include small bowel obstruction, gastrointestinal bleeding, chronic diarrhea, ulcers, malnutrition, and anemia. In particular, anemia is a recognized long-term complication and can be severe. Rats have been used as a model to study the effects of gastric bypass surgeries. They can experience similar complications as people, but the development of severe anemia has not previously been reported in rats. We observed 2 cases of severe anemia in female Sprague–Dawley rats after RYGB surgery. These cases prompted us to further investigate the frequency and severity of anemia after RYGB in rats. Blood work and necropsies were performed on 9 additional female Sprague–Dawley rats (5 with RYGB, 4 with sham surgery). In these 9 rats, only one had signs of clinical anemia. These 3 anemic rats displayed moderate to severe pallor of the eyes and ears. Necropsy findings in anemic RYGB rats included pale internal organs and eccentric heart enlargement, which led to a significantly higher heart:body weight ratio in RYGB rats as compared with sham controls. Anemic rats had either a macrocytic normochromic anemia, consistent with vitamin B_{12} or folate deficiency, or microcytic hypochromic anemia, indicative of iron deficiency. Researchers who perform RYGB surgery in rats should be aware of the potential complication of severe anemia. Plans for the diagnosis and management of this complication and the development of criteria for humane endpoints for severe anemia are recommended as a refinement to these studies.

Abbreviations: RYGB, Roux-en-Y gastric bypass

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Introduction

Obesity and its comorbidities, including type 2 diabetes mellitus and cardiovascular disease, have become increasingly prevalent in the United States, with approximately 30% of adults having a body mass index above 25% or 30%; these values are considered to indicate overweight and obese status, respectively.[7,9] To control weight gain and aid in weight loss, many people undergo bariatric surgery, with Roux-en-Y gastric bypass (RYGB) being the most commonly performed procedure. This restrictive–malabsorptive procedure reduces the size of the stomach and bypasses a majority of the small intestine, successfully reducing the amounts of fat and calories absorbed. Despite the procedure's success, several associated complications have been noted in humans, including small bowel obstruction, gastrointestinal bleeding, chronic diarrhea, gastric ulcers, nutritional deficiencies, and anemia.[8,9]

Anemia develops in as many as 45% to 50% of patients after bariatric surgery.[9] Several factors may contribute to this condition. Surgical bypass of the distal stomach and proximal sections of the small intestines leads to malabsorption of micronutrients, most notably iron, vitamin B_{12}, and folate. All of these micronutrients are essential for the normal development of erythrocytes.[14]The small intestine is also important in the absorption of dietary iron. Therefore, a large proportion of dietary iron is not absorbed after gastric bypass surgery. In addition, the newly formed gastric pouch contributes less hydrochloric acid to the small intestines. This less acidic environment reduces the bioavailability of iron by hindering the conversion of iron into the absorbable ferrous state. Finally, the majority of dietary iron comes from the consumption of red meat, but many bariatric patients become intolerant to red meat after surgery.[14]

Compared with iron, deficiencies in vitamin B_{12} and folate are less prevalent causes of anemia. Their absorption and bioavailability depend on the acidity of the remaining gastric environment and the presence of intrinsic factors produced by gastric parietal cells. Body stores of these nutrients are usually sufficient to prevent acute deficiencies in folate or vitamin B_{12} after surgery, with most patients becoming clinically anemic about 2 to 4 y after surgery.[14]

RYGB surgery is performed in rats to create a model for investigation of the multifactorial nature of obesity and RYGB-related complications.[3] Here, we describe 3 cases of severe anemia in female Sprague–Dawley rats (Crl:CD(SD); *Rattus norvegicus*, Charles River Laboratories), that underwent RYGB surgery as part of an alcohol-use study.

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Experiment Overview

This case study retrospectively highlights clinical and diagnostic findings in 2 female Sprague–Dawley rats that developed clinical signs of anemia after RYGB surgery. These 2 rats were involved in a research protocol studying the neural mechanisms underlying how RYGB and other potential contributing factors may increase and sustain motivation for alcohol use. In brief, obesity was induced by the provision of a commercial high-fat diet (D12492, Research Diets, New Brunswick, NJ). Once the target adiposity was reached, RYGB surgery was performed. Several weeks after surgery, rats received intermittent access to alcohol followed by either continuous alcohol access or self-administration via operant behavioral testing. A subset of rats also received a subcutaneous radiotelemetric implant for monitoring heart rate variability.

Case Reports

Case 1 (rat 20-126). A 7-wk-old, female Sprague–Dawley rat was placed on a high-fat diet to produce dietary obesity, defined by body adiposity > 25%, which develops after 6 to 8 wk on high-fat diet in Sprague-Dawley rats. After which, they underwent RYGB surgery and subcutaneous implantation of a telemetry device (TSE Systems, Data Sciences International, St Paul, MN) to measure blood pressure and heart rate from the femoral artery. Approximately 2 wk after surgery, the rat presented with a hunched appearance and pallor, with porphyrin staining around the eyes, urine staining on the fur, and a greasy appearance associated with high-fat diet consumption. The rat had a thin body condition score, which is a score of 2 out of 5 when using a conventional rat body condition scoring system.[6] Weight loss can be an expected outcome after the gastric bypass surgery. The rat was given an analgesia (carprofen, 5 mg/kg, SC), and a CBC count was performed. The bloodwork revealed a nonregenerative anemia and low hemoglobin. A plan was made to continue carprofen (5 mg/kg once a day) and to recheck the bloodwork in 1 wk. While the rat was being handled at the time of the second blood collection, the surgical incision over the telemetric device dehisced. The rat was then anesthetized with isoflurane and the dehiscence was cleaned and aseptically closed. CBC results from the second blood collection showed essentially no improvement in the anemia. The rat also showed mild leukocytosis that was characterized by neutrophilia with a left shift, lymphocytosis, and monocytosis. Because of a lack of improvement of clinical signs, continued anemia, and potential infection, this rat was given a poor prognosis, removed from the study, and euthanized with carbon dioxide (CO_2). Necropsy was not requested or performed.

Case 2 (rat 20-193). Another 7-wk-old, female Sprague–Dawley rat underwent similar procedures as described above except for implantation of a telemetric device. After RYGB surgery, this rat had a decreased appetite and was reluctant to eat solid food. With the provision of additional doses of carprofen and provision of moistened crushed diet, the rat recovered and continued in the study. The rat then received intermittent access to 10% alcohol for 4 wk. After 23 d of alcohol exposure, the rat was reported to be moderately lethargic and pale, with an unkempt, greasy coat and loose feces, which can be expected experimental outcomes. Once the alcohol consumption period was complete, alcohol was removed for 2 wk. During this time, the diarrhea resolved, but the paleness and greasy haircoat became progressively worse. Due to the poor prognosis, euthanasia was elected. The rat was euthanized with CO_2 and was immediately necropsied. All mucous membranes, skin, and internal organs were pale. The gastric bypass surgical sites had healed without complication. The right and left ventricles of the heart appeared to be moderately enlarged eccentrically, with the right and left ventricular walls appearing to be thinner than normal. The rest of the gross necropsy was unremarkable. A blood smear showed a microcytic hypochromic anemia.

Materials and Methods

Animals. To further investigate the frequency and severity of anemia in this model, we collected data from 9 additional female Sprague–Dawley (Crl:CD(SD)) rats at the end of their study. These rats were obtained from Charles River Laboratories (Wilmington, MA; weight, 200 to 225 g) and based upon vendor reports were free from Kilham rat virus, rat Theiler virus, rat coronavirus, rat minute virus, Toolan H1 virus, rat parvovirus, reovirus type 3, lymphocytic choriomeningitis virus, murine adenovirus types 1 and 2, Sendai virus, *Mycoplasma pulmonis*, pneumonia virus of mice, *Pneumocystis carinii*, and endo- and ectoparasites. All rats were used as approved by the Pennsylvania State University College of Medicine IACUC.

Rats were housed singly in wire-bottom caging, which was IACUC-approved and necessary to prevent injury during recovery from surgery, prevent of eating solids (essential for RYGB surgery), and aid in the measurement of food intake. Room temperatures were maintained between 70 to 72 °F (21 to 22 °C), relative humidity of 30% to 70%, and a 12:12-h light:dark cycle, with lights on at 0700 and off at 1900. All rats were fed a high-fat diet (D12492, Research Diets, New Brunswick, NJ) to induce obesity. Preoperatively, the rats each received a single dose of ceftriaxone (30 mg/kg SC). Surgical procedures and perioperative care were performed as described previously.[5] In brief, all rats were anesthetized with isoflurane; 5 of the 9 rats underwent RYGB bypass surgery, and the remaining 4 underwent a sham surgical procedure that involved creating and closing a midline laparotomy incision. For analgesia, sustained-release buprenorphine (1 mg/kg SC; Wedgewood Pharmacy, Swedesboro, NJ) and carprofen (5 mg/kg SC; Zoetis, Parsippany-Troy Hills, NJ) were administered. At approximately 14 wk after surgery, all rats were gently restrained by using a towel, and each rat's face was photographed to capture the coloration of the ears, mucous membranes, and eyes. Immediately after the photograph, each rat was euthanized with CO_2 and necropsied. Blood was collected from the heart to perform CBC counts and serum iron analysis. CBCs were performed in house while serum was sent to IDEXX Bioanalytics (Columbia, MO) for serum iron analysis. The rats and their hearts were weighed.

Statistics. Data for CBC count, serum iron content, and heart weight:body weight ratio were analyzed by using Prism (version 9, GraphPad Software, San Diego, CA) and presented as violin plots to illustrate the density and distribution of the data. Statistical significance was determined by using the Mann–Whitney test, with statistical significance defined as a P value of less than 0.05. Where appropriate, data from the 2 retrospective cases (rats 20-126 and 20-193) and the 9 additional rats were combined.

Results

Necropsy findings. Before euthanasia, the faces of rats in the prospective study were photographed to document the coloration of their ears and eyes. Based on the CBC data, rat 20-197 was the only anemic rat in this cohort. At euthanasia, this rat had noticeably pale ears, eyes, lungs, and liver but did not display any other signs of illness. In addition, this rat's heart was markedly enlarged (Figure 1). The liver weight was comparable to all other rats in this cohort. These gross findings were similar

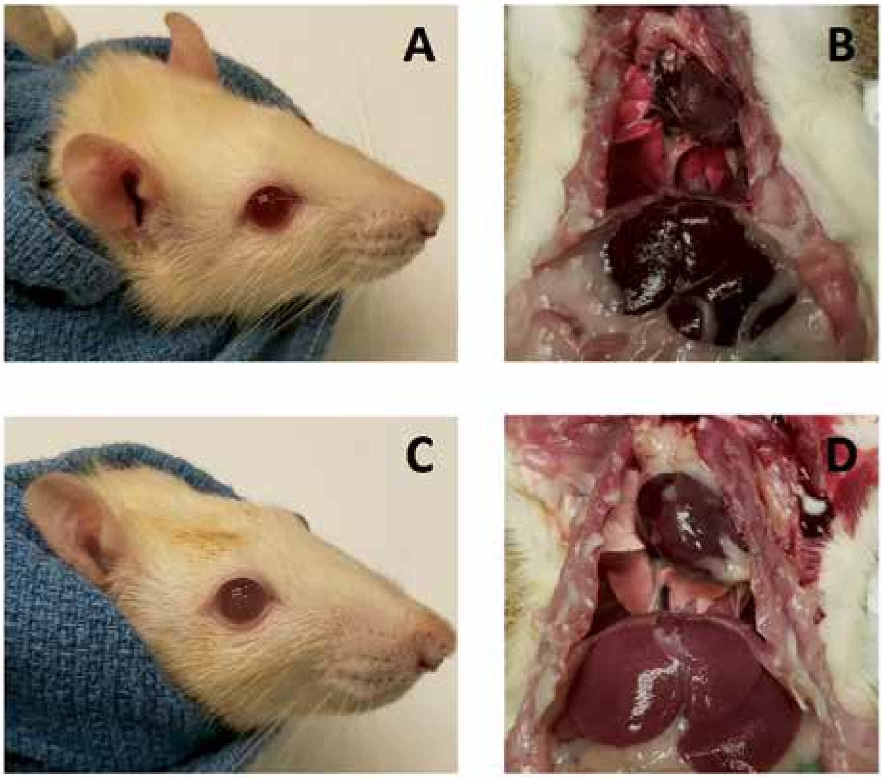

Figure 1. (A) Antemortem photograph of a nonanemic sham control rat, showing normal coloration of the eyes. (B) Antemortem photograph of anemic rat 20-197, displaying pale eyes. The pupil is easier to discern than the nonanemic rat pupil. (C) Postmortem photograph of a nonanemic sham control rat, showing normal coloration of the lungs and liver. (D) Postmortem photograph of anemic rat 20-197, displaying pale lungs and liver and an enlarged heart.

to those in rats 20-126 and 20-193, as mentioned above in the case reports, but we did not photograph those rats. Necropsy of the other 8 rats in this cohort was unremarkable.

CBC count results. The pallor of rats 20-126 and 20-197 paralleled their RBC counts and Hct values (Figure 2). Due to a sampling error, a CBC count could not be performed for rat 20-193.

Rats 20-126 and 20-197 had different types of anemia. Rat 20-126 had a macrocytic normochromic anemia, whereas rat 20-197 developed a microcytic hypochromic anemia. In addition, Hgb levels were severely decreased in both rats (8 and 5 g/dL, respectively). The RBC counts for the other RYGB rats were within the normal range reported[4] for female Sprague–Dawley rats (7.23 to $8.11 \times 10^6/\mu L$), but the values for other RBC parameters were more variable for RYGB rats than for sham control rats. In particular, RYGB rats had a significantly lower mean MCH value ($P \leq 0.01$), with some animals reaching levels as low as 8.4 pg, whereas sham control rats averaged 19.8 pg (Figure 2).

Serum iron measurements. Serum iron values were variable and widely distributed for both sham control and RYGB rats. The serum iron levels of sham control rats ranged between 190 and 472 µg/dL, whereas values for RYGB rats ranged between 22 and 460 µg/dL. Serum iron for female Sprague–Dawley rats has a normal reported range of 90 to 350 µg/dL.[11] The anemic rats 20-193 and 20-197 had serum iron levels of 22 and 32 µg/dL, respectively (Figure 2).

Heart:body weight ratios. RYGB rats weighed 242 to 352 g whereas the sham control rats weighed 372 to 524 g. Heart weights for RYGB rats ranged from 1.3 to 2.7 g and for sham control rats were 1.2 to 1.9 g. The ratio of heart to body weights was significantly greater ($p \leq 0.05$) in RYGB rats (median, 6.16) than in sham control rats (median, 3.3, respectively. (Figure 2).

Discussion

This study provides evidence that, like humans, rats may develop anemia after RYGB surgery. This anemia is likely due to a nutritional deficiency that is associated with the malabsorption of nutrients. As many as 45% to 50% of patients who have undergone RYGB develop anemia after surgery, making anemia the most likely postoperative complication.[9] As many as 11% of RYGB patients develop severe anemia, which is classified by the World Health Organization as Hgb of less than 8 g/dL.[10,15] According to this definition, 2 of the RYGB rats, 20-126 and 20-197, were severely anemic. Anemic human patients may present with clinical symptoms including fatigue, pallor, and dyspnea on exertion, much like the clinical signs we saw in the rats that became anemic.[9]

In humans, anemia after bariatric surgery is most often due to iron deficiency, with vitamin B_{12} or folate deficiency as a secondary cause.[9] Rat 20-126 had a macrocytic normochromic anemia, which is consistent with a vitamin B_{12} or folate deficiency, whereas rat 20-197 had a microcytic hypochromic anemia, which is indicative of iron deficiency. Anemia due to nutritional deficiencies of vitamin B_{12} or folate develops due to disrupted maturation of erythrocytes. This type of anemia should be distinguished from anemia caused by iron deficiency. Life-long, periodic monitoring of serum ferritin and hemoglobin is recommended for patients after RYGB surgery. Ferritin is a transport molecule that stores and releases iron. This protein is found in the liver, spleen, skeletal muscle, and bone marrow. Iron is necessary for the synthesis

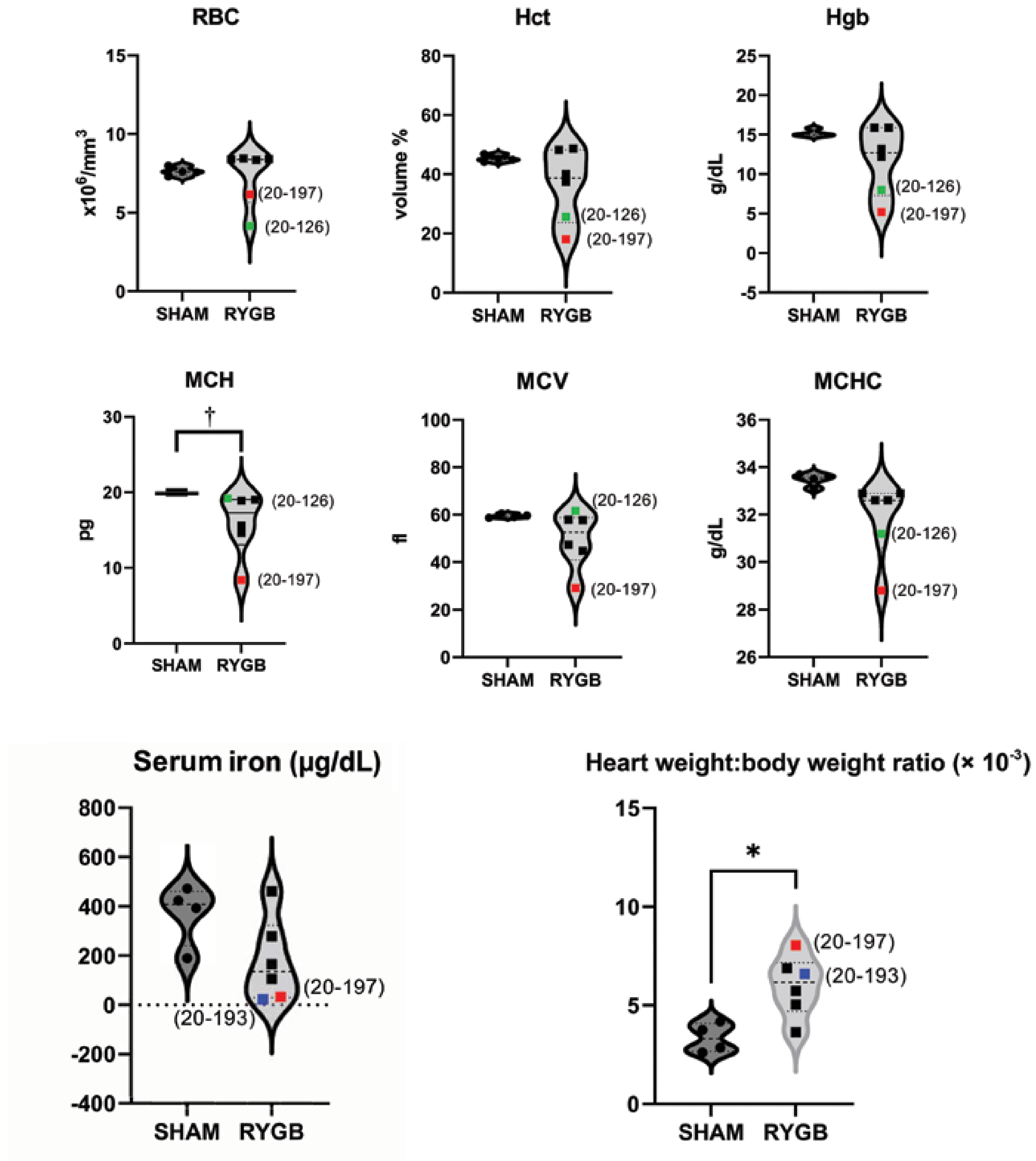

Figure 2. Assessed hematologic parameters for sham control and RYGB rats, including RBC count, Hct, Hgb, MCH, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), serum iron, and heart weight:body weight ratios. The data points for anemic rats 20-126 (green), 20-193 (blue), and 20-197 (red) are highlighted. Sham controls, $n = 4$; RYBG group (includes rat 20-126), $n = 6$. †, $P \leq 0.01$, *, $P \leq 0.05$.

of hemoglobin, and with iron deficiency, ferritin concentrations decrease before the development of other hematologic evidence of iron deficiency.[9] Commercial diagnostic laboratories do not currently measure rat serum ferritin, so we instead measured serum iron. The normal range for serum iron in rats can vary widely, with normal levels as low as 82 µg/dL in Sprague–Dawley rats.[12] However, the levels in rats 20-193 (22 µg/dL) and 20-197 (32 µg/dL) which underwent RYGB surgery, were well below this range. As expected with iron deficiency, hemoglobin synthesis was reduced, resulting in low Hgb and MCH values.

Potentially related to iron deficiency, values for the body weight:heart weight ratio were more variable in rats with RYGB surgery than in sham controls. In a previous study,[11] rats that were fed an iron-deficient diet to induce anemia also developed eccentric cardiac hypertrophy. These rats also had significantly elevated heart rates but showed no changes in any other measurement of cardiac function or blood pressure. Eccentric hypertrophy is the pathologic enlargement of the heart due to volume overload. Cardiomyocytes become elongated and hypertrophic to create larger cardiac chambers, which help to accommodate the increased blood volume and minimize stress on the cardiac walls. In rodent models of chronic volume overload-induced by arteriovenous fistula, rats develop heart arrhythmias that can result in sudden cardiac arrest.[2] Further investigation and larger sample sizes are necessary to determine whether the iron deficiency that arises due to RYGB surgery in rats results in the same cardiac hypertrophy and poses the same health risks.

Recognizing that severe anemia can be a complication of RYGB surgery in rats, we suggest that protocols should contain a plan for the medical management of anemic animals or criteria to remove them from study. In people, oral iron supplementation is prescribed as a preventative measure after surgery; however, this supplementation does not correct anemia once it has developed. Typically, these patients need intravenous iron administration combined with oral or intramuscular supplementation with vitamin B.[1] Administration of iron and vitamin B supplements could improve the postoperative care and maintenance of RYGB rats that develop anemia. In addition, consideration of the level of anemia may be a way to refine humane endpoints. Rats that develop severe anemia and cannot be medically managed should be removed from the study.

The diagnosis of anemia in rats is challenging. In our experience, obtaining antemortem blood samples from severely anemic rats was difficult when using less-invasive collection techniques, such as lateral or medial saphenous or tail veins. Likely due to poor circulation, low fluid volume, and vasoconstriction, these rats bled slowly, and several attempts were necessary to obtain the necessary amount of blood for a CBC analysis. Furthermore, physical restraint for blood collection from an anemic rat could cause stress and result in further deterioration of its condition. Therefore, developing a method that improves the ability to monitor for anemia with minimal stress and restraint would be helpful.

Pallor of the eyes was one of the clinical signs we saw that may be useful in monitoring for anemia. In small ruminants, a FAffa MAlan CHArt (FAMACHA) score is used to monitor the degree of anemia caused by parasite burden.[13] The FAMACHA score reflects the color of the conjunctiva, from the deep red of a healthy animal through shades of pink and ends at a near-white coloration, as a result of progressively worsening anemia.[13] These changes in conjunctival color relate to the severity of anemia and correlate with Hct values. A system has been established in which animals that score high on the FAMACHA (more pale mucous membrane coloration) are treated for anemia. Animals that score low are considered to be within a normal range and are not treated. Developing a similar scoring system for rats that correlates with mild, moderate, and severe anemia would be a significant advance for the detection and management of anemia in rats. A FAMACHA-like system would allow researchers and veterinary staff to estimate the anemia levels of a rat by observation rather than through blood collection. This would likely mitigate stress induced by handling and would circumvent the technical challenges associated with blood collection from anemic rats. Further research is needed to develop an accurate FAMACHA-like anemia scoring system for rats. We recognize that the use of eye pallor would be limited to albino rats and that other methods for scoring pallor would be needed for pigmented rats.

Because the development of clinically significant anemia is a current concern for people after RYGB surgery, more research on prevention and treatment strategies is necessary. Our current study provides evidence that rats may provide a model for these investigations. A recognized limitation is that as in people, only a small percentage of rats develops severe anemia after RYGB; this factor mandates the use of a large number of rats to achieve an adequate sample size. Thus, sample size was a limitation of our study. Another limitation of our study is that all of the rats were female. Whether male rats also develop anemia after RYGB surgery is unknown.

Our study highlights the importance of planning for anemia as a potential complication. As a refinement, researchers who use this surgical model should develop plans to diagnose and manage rats that become anemic. The severity of anemia should be considered when developing humane endpoints.

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