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OXALOBACTER FORMIGENES COLONIZATION NORMALIZES OXALATE EXCRETION IN A GASTRIC BYPASS MODEL OF HYPEROXALURIA

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Abstract

Background—Hyperoxaluria and oxalate kidney stones frequently develop after Roux-en-Y gastric bypass (RYGB). Oxalobacter formigenes can degrade ingested oxalate.

Objectives—Examine the effect of Oxalobacter formigenes wild rat strain (OXWR) colonization on urinary oxalate excretion and intestinal oxalate transport in a hyperoxaluric RYGB model.

Setting—Laboratory, United States.

Methods—At 21 weeks of age, twenty-eight obese male Sprague-Dawley rats survived Sham (n=10) or RYGB (n=18) surgery and were maintained on a 1.5% potassium oxalate, 40% fat diet. At 12 weeks post-operatively, half the animals in each group were gavaged with OXWR. At 16 weeks, % dietary fat content was lowered to 10%. Urine and stool were collected weekly to determine oxalate and colonization status, respectively. At week 20, [14C]-oxalate fluxes and electrical parameters were measured in vitro across isolated distal colon and jejunal (Roux limb) tissue mounted in Ussing Chambers.

Results—RYGB animals lost 22% total body weight while Shams gained 5%. On a moderate oxalate diet, urinary oxalate excretion was 4-fold higher in RYGB than Sham controls. OXWR colonization, obtained in all gavaged animals, reduced urinary oxalate excretion 74% in RYGB and 39% in Sham and was further augmented by lowering % dietary fat. Finally, OXWR colonization significantly enhanced basal net colonic oxalate secretion in both groups.

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CONFLICT OF INTEREST

All authors made substantial contributions to scientific conception and design, article draft and revisions, and agree to be accountable for all aspects of the work.

Conclusions—In our model, OXWR lowered urinary oxalate by luminal oxalate degradation in concert with promotion of enteric oxalate elimination. Trials of *O. formigenes* colonization and low fat diet are warranted in calcium oxalate stone formers with gastric bypass and resistant hyperoxaluria.

Keywords

gastric bypass surgery; hyperoxaluria; steatorrhea; *Oxalobacter formigenes*

INTRODUCTION

Oxalate is an end product of human liver metabolism and an organic acid found in a variety of plant-based foods (spinach, bran, leafy greens) and plant products (chocolate, almonds, peanut butter). Enteric hyperoxaluria (EH), a pathologic hyperabsorption of dietary oxalate, is a spectrum of gastrointestinal disorders characterized by fat malabsorption due to reduced functional small intestinal surface area. As fat-soluble vitamins and calcium ions are saponified by excessive luminal fat, less calcium is available for oxalate binding, leading to increased intestinal absorption of oxalate via passive or active gut pathways. Roux-en-Y gastric bypass (RYGB), one of the most effective surgical procedures for sustained weight loss, is the most common cause of EH in the US, with RYGB-associated hyperoxaluria as high as 90% in some reports [1, 2]. Combined with decreased urine volume and low urinary citrate, hyperoxaluria can raise kidney stone risk in RYGB patients up to 4-fold that of non-RYGB patients [3, 4].

Oxalobacter formigenes is an anaerobic, commensal bacteria of the human colon that utilizes oxalate as its sole energy source[5]. Controlled diet studies have shown that colonized individuals, estimated between 38–77% of the world's population, excrete significantly less oxalate than non-colonized individuals[6]. Furthermore, lack of *O. formigenes* colonization has been demonstrated to be a risk factor for both hyperoxaluria and recurrent calcium oxalate stone disease[7, 8] but has not been studied as a therapy in the bariatric population. Our group has previously reported elevations in fecal fat and urinary oxalate in an obese rodent model of RYGB[9]. We hypothesized that *O. formigenes* colonization could reverse RYGB-associated hyperoxaluria and examined the effect of *O. formigenes* colonization on urinary oxalate excretion and intestinal oxalate transport in this established hyperoxaluric model.

MATERIALS AND METHODS

Animals and surgical procedures

Animal studies were conducted in accordance with the University of Florida and the NIH Guide for the Care and Use of Laboratory Animals using approved animal protocols. Male Sprague-Dawley rats purchased from Charles River at 3 weeks of age were given free access to water and a high-fat diet (D12492, Research Diets Inc., New Brunswick, NJ, USA) containing 60% fat (casein-based), 20% protein and 20% carbohydrate (total 5.2 kcal/gm) for 18 weeks in order to establish dietary-induced obesity (DIO).

At 21 weeks of age the animals were randomly assigned to either RYGB (n=21) or Sham surgery (n=10). As described previously for RYGB animals [10, 11], a 4 cm mid-line incision was made below the xyphoid process. The terminal ileum was identified at the ileocecal valve and followed orally 35 cm where a 4 mm enterotomy was made to construct the common channel. The jejunum was then followed another 10 cm proximally and completely transected to configure the Roux limb (this segment in Sham is defined as the “predestined” Roux limb). A hand-sewn, interrupted, end-to-side anastomosis was performed by sewing the proximal portion of biliopancreatic limb (25–35 cm) to the enterotomy using 5-0 PDS. The vagal nerves of the stomach were identified and mobilized laterally, and the left gastric artery suture ligated. The stomach was then transected 2–3 mm below the level of the gastro-esophageal junction, and an end-to-end hand-sewn gastrojejunostomy was performed using 5-0 PDS, creating a small stomach pouch. The gastrotomy on the defunctionalized stomach was oversewn, fascia was closed using a running 4-0 Vicryl suture, and the skin re-approximated. All Sham animals received a gastric enterotomy, bowel mobilization, operative time, and closure as RYGB.

Dietary regimens

Following their respective procedures, the animals were allowed return of bowel function for two weeks and then placed on to ad lib 0.6% calcium, 40% fat (40% carbohydrate, 20% protein; D11021102, Research Diets, New Brunswick, NJ) with 1.5% potassium oxalate, providing 4.5 kcal/gm. The day after experimental week 16, all animals were changed to ad lib 0.6% calcium, 10% fat (70% carbohydrate, 20% protein; D11021101, Research Diets, New Brunswick, NJ) with 1.5% potassium oxalate supplementation, providing 4.6 kcal/gm. Weekly body weights and daily food were recorded for a total of 20 weeks, and total body weight loss was defined as pre-operative weight minus body weight (gm)/pre-operative weight (gm).

Urine and fecal collections and analytical methods

Rats were housed individually in metabolic cages and 24-h urine collections were made under hydrated mineral oil (3 ml, to prevent evaporative loss of urine volume) placed into vessels containing 20 µl of 2% sodium azide as a preservative. Urinary oxalate was determined in acidified (HCl) samples collected from all of the animals over a 24-h period using a kit assay (Trinity Biotech #591, St. Louis, MO). Urinary creatinine was determined using a modification of the Jaffé reaction as previously described [13].

Colonization studies

OXWR, a wild rat strain of *Oxalobacter formigenes* [14, 15] was used in the colonization study which has been previously detailed [14, 16] and, prior to study, all rats were confirmed non-colonized. Briefly, ~ 20 mg of freshly collected fecal material was inoculated into anaerobically sealed vials containing a 20 mM oxalate medium and, after incubation at 37° C for ~ 7 days the loss of oxalate in the medium, which was determined spectrophotometrically, was indicative of the colonization status of each rat [16, 17]. At week 12 post-operatively and under momentary isoflurane anesthesia, non-colonized rats were administered an esophageal gavage of a 1.5 ml inoculum containing an average of 150 mg wet weight of bacteria from a 24-h culture of OXWR. Rats were similarly inoculated again

two days later. Approximately 7 days after the second gavage, fresh fecal specimens were collected for the detection of OXWR, and all rats gavaged with OXWR were found to be colonized with the oxalate degrader. Luminal contents were also collected from the specified intestinal segments when rats were euthanized for flux studies to confirm the localized segment-specific colonization status at that time.

Oxalate flux measurements

Intestinal tissues were removed from rats anesthetized with isoflurane, and the animals were then euthanized by complete exsanguination. Transmural fluxes of oxalate across isolated segments of the rat from mid-Roux limb and distal colon (~ 10 cm segment measured proximal to the base of the urinary bladder) were determined using ^{14}C -oxalate (Amersham, Piscataway, NJ, USA) as previously described [18]. For Sham animals, we obtained small bowel 45 cm proximal to the ileocecal valve ("predestined" Roux limb), corresponding to the location of the Roux limb in RYGB animals. All transport studies were conducted 20 weeks following the surgical procedure. The magnitude and direction of the net flux ($J_{\text{net}}^{\text{Ox}}$) was determined by calculating the difference between two unidirectional fluxes (mucosal to serosal, $J_{\text{ms}}^{\text{Ox}}$ and serosal to mucosal, $J_{\text{sm}}^{\text{Ox}}$) measured for a period of 45 min at 15 min intervals, under short-circuit conditions. The specific activity of the radioisotope, which was constant throughout the flux period, was determined both at the beginning and end of the experiment by sampling (50 μl) from the labeled ("hot") compartment. At each 15 min interval, a 1 ml sample was removed from the opposing ("cold") compartment with replacement of 1 ml of unlabeled buffer and a correction was applied for this dilution effect. The electrical parameters of the tissue were also recorded at 15 min intervals throughout the entire experiment. Tissue conductance (G_T , $\text{mS}\cdot\text{cm}^{-2}$) was calculated as the ratio of the open-circuit potential (mV) to the short-circuit current (I_{sc} , $\mu\text{A}\cdot\text{cm}^{-2}$), according to Ohm's Law and net fluxes were determined on conductance matched (G_T 20%) tissues.

Statistical analyses

Results are presented as the mean $\pm$ one standard error (SE) for "n" the number of matched tissue pairs as well as for the urinary biochemical analyses for the different groups of animals. A statistical comparison of more than two means was performed by a one-way analysis of variance (ANOVA) followed by Bonferroni's t-test for multiple comparisons with the control group. A paired t-test was used for the comparison of two normally distributed means while non-parametric data was compared using Mann-Whitney U tests. In all three cases, differences were considered significant if $p < 0.05$.

RESULTS

Weight loss and food intake

After 18 weeks on high fat diet, mean DIO rat pre-operative weight was 762 ± 31 gm with a mean daily caloric intake of ~102 kcal. No deaths occurred in Sham controls whereas 3 RYGB animals died post-operatively, leaving 18 RYGB animals and 10 Sham for analysis. By post-operative week 3 and thereafter, RYGB animals had significantly lower % body weight differences than their Sham counterparts (Figure 1A). Mean daily caloric intake was

similar to baseline in Shams by post-operative week 3 and in RYGB by post-operative week 8 (Figure 1B).

Urine oxalate and creatinine

Compared to baseline, mean urinary creatinine excretion was significantly lower at end of study for all RYGB animals ($n=18$; mean 172.6 ± 14 vs 149.1 ± 16 $\mu\text{mol/d}$, $p=0.037$) but not when all Shams were compared ($n=10$; mean 165.4 ± 12 vs 168.9 ± 13 $\mu\text{mol/d}$, $p=0.89$; Supplemental Table 1). The hyperoxaluric phenotype previously reported for the RYGB rat model was confirmed in the present study^[9]. At 12 weeks post-surgery and despite similar food intake, the daily mean urinary oxalate excretion of all RYGB rats ($n=18$) fed an oxalate-supplemented, 40% fat diet was ~ 4-fold higher (Supplemental Table 1) compared to their Sham ($n=10$) counterparts (RYGB = 20.41 ± 2.1 $\mu\text{moles/24 h}$ versus Sham = 5.0 ± 0.5 $\mu\text{moles/24 h}$, $p<0.001$). OXWR-colonized RYGB animals normalized their urinary oxalate to that of Sham by week 20 (5.8 ± 0.9 $\mu\text{moles/24 h}$ vs 5.45 ± 0.5 $\mu\text{moles/24 h}$, $p=0.76$; Supplemental Table 1). Furthermore, a non-significant reduction (39%) in urinary oxalate excretion was noted in Sham OXWR animals compared to Sham at the same time point (5.45 ± 0.5 $\mu\text{moles/24 h}$ vs 3.32 ± 0.3 , $p=0.27$; Supplemental Table 1). To accurately compare renal biochemical parameters between animals of different weight and lean muscle mass, 24 hour urinary oxalate to urinary creatinine (UOC) ratios were calculated and plotted (Figure 2). RYGB OXWR-colonized animals had significantly lower UOC ratios than RYGB animals at week 14, a mere two weeks after OXWR colonization, that was sustained through week 20. Similarly, Sham OXWR animals had consistently lower UOC ratios than their matched Sham counterparts week 14–20, although not statistically significant due to their low baseline oxalate excretion.

RYGB and Sham flux studies

Both RYGB and Sham animals were readily colonized by OXWR gavage, and no animals lost colonization throughout the follow-up. At end of study, OXWR was detected in all RYGB distal colonic segments and also detected within luminal contents of the Roux limb. The presence of OXWR in sham animals (Figure 3A) significantly enhanced the basal net secretory flux of oxalate, by 2-fold (grey box), across this segment by a significant decrease in active oxalate transport J_{ms} and concomitant increase in J_{sm} . Colonization of RYGB animals with OXWR revealed a marked increase in active oxalate transport J_{sm} only (Figure 3B). Electrical parameters were not altered by colonization ($I_{sc} = 0.52 \pm 0.17$ $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ and 0.40 ± 0.09 $\mu\text{Eq}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$; $GT = 19.1 \pm 2.2$ $\text{mS}\cdot\text{cm}^{-2}$ and to 20.7 ± 1.2 $\text{mS}\cdot\text{cm}^{-2}$) in the absence or presence of OXWR, respectively.

Oxalate fluxes in RYGB animals measured across the Roux limb segment (Figure 3C) were clearly affected by the presence of the bacteria, and a robust net secretory flux of oxalate was promoted by OXWR driven primarily by a 6.5-fold increase in J_{sm} with no significant effects on J_{ms} , I_{sc} , or GT . While OXWR was detected in the Roux limb of RYGB animals, it was not detected in the “predestined” Roux limb counterpart segment of the OXWR-gavaged Sham rats. These Sham animals were OXWR positive in the distal colonic segments. Finally, there were no differences in oxalate flux or electrical characteristics of the

“predestined” Roux limb segment in either gavaged or non-gavaged Sham group (data not shown).

DISCUSSION

Since its discovery in the 1980's, *Oxalobacter formigenes* has been studied for its ability to colonize the gut and degrade oxalate. Lack of colonization can pose as a potential risk factor for hyperoxaluria and calcium oxalate stones in non-colonized individuals^[7, 8]. Early studies in animals ingesting a high oxalate diet demonstrated marked reductions in urinary oxalate excretion when colonized with *O. formigenes* – a result primarily driven by altered intestinal oxalate handling. As further proof, two case series in humans taking either probiotics or *O. formigenes* were published in 2005 and 2006, respectively, each demonstrating the potential oxalate-lowering ability of gut bacteria in hyperoxaluric patients^[15, 16]. However, in 2011, a multi-center randomized trial of orally administered *O. formigenes* in Primary Hyperoxaluria (PH) patients, a rare genetic disease characterized by abnormally high hepatic oxalate synthesis and high urinary oxalate excretion, showed no difference in urinary oxalate levels between treated and untreated groups^[17]. To date, no human studies have been published on the effect of *O. formigenes* colonization in RYGB or enteric hyperoxaluric patients.

The DIO rodents and RYGB procedure used in this experiment are validated models of human nutrition and bariatric surgery. In our hands, this model mimics human weight loss, food intake, fat malabsorption, and enteric hyperoxaluria on a moderate oxalate diet^[9]. In this experiment, OXWR colonization resulted in a 74% decrease in urinary oxalate excretion after 4 weeks of colonization (Figure 2, week 16). Moreover, this study confirms that OXWR not only readily colonizes the intestine of our RYGB rat model but also produces the same beneficial oxalate-lowering effects previously reported in other non-obese murine models^[19–21]. This data conflicts with a much older human study (1986) that showed that patients with jejunoileal bypass either had either lower rates of *O. formigenes* activity or lacked the bacteria because of an altered luminal environment^[25]. More modern human studies suggest that it is a lack of colonization, not lack of activity. Two separate studies have reported low *O. formigenes* colonization rates in morbidly obese individuals who were either being evaluated for or just after bariatric surgery^[22, 23]. Of the 61 individuals tested, only 12 were colonized with *O. formigenes* (12/61, 19.7% positive status), an especially low rate when one considers that roughly half of US citizens are colonized^[24].

Mechanistically, in addition to reducing the amount of free oxalate available for gut absorption, we found that OXWR interacts with intestinal mucosa to increase active secretion of oxalate into the gut lumen by either inducing or enhancing net oxalate secretion^[19]. This finding was reproducible within RYGB distal colon and also, surprisingly, within the Roux limb, where colonization endured throughout the 8 week experiment. In contrast, the bacterium was not detected in the corresponding segment (the “pre-destined” Roux limb) when colonized Shams were euthanized for flux studies, despite the fact that their large intestines were all confirmed to be colonized at that time point. It is possible that this segment was temporarily colonized in these animals but was lost over time, as was previously reported in the upper intestine of mice gavaged with *O. formigenes*^[20].

We have previously shown that lowering % dietary fat content can significantly reduce urinary oxalate excretion in our RYGB model^[18]. In this particular study, reducing dietary fat to 10% lowered urinary oxalate another ~20% in our RYGB animals, such that renal oxalate excretion in colonized RYGB rats was comparable to that in Sham rats that were not colonized (Figure 2, week 20). Even in non-colonized RYGB animals, lowering % dietary fat after the week 16 collection resulted in a 32% decrease in urine oxalate excretion (mean 19.7 ± 3.1 $\mu\text{moles}/24 \text{ h}$ to 13.43 ± 2.9 $\mu\text{moles}/24 \text{ h}$, $p < 0.001$). This again highlights the fact that a high fat diet may increase luminal free oxalate available for absorption following the RYGB procedure.

CONCLUSIONS

Using the hyperoxaluric, DIO-RYGB model, we were able to reduce urinary oxalate excretion to that of obese controls by colonizing RYGB animals with *Oxalobacter formigenes* and by lowering % dietary fat. For human RYGB patients with resistant hyperoxaluria and recurrent calcium oxalate stones who have failed conservative measures, our data suggests that clinical trials of *O. formigenes* colonization are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Park AM, Storm DW, Fulmer BR, Still CD, Wood GC, Hartle JE. A prospective study of risk factors for nephrolithiasis after Roux-en-Y gastric bypass surgery. *J Urol*. 2009; 182:2334–9. [PubMed: 19762043]
2. Duffey BG, Alanee S, Pedro RN, et al. Hyperoxaluria is a long-term consequence of Roux-en-Y Gastric bypass: a 2-year prospective longitudinal study. *J Am Coll Surg*. 2010; 211:8–15. [PubMed: 20610243]
3. Valezi AC, Fuganti PE, Junior JM, Delfino VD. Urinary evaluation after RYGBP: a lithogenic profile with early postoperative increase in the incidence of urolithiasis. *Obes Surg*. 2013; 23:1575–80. [PubMed: 23519632]
4. Canales BK, Hatch M. Kidney stone incidence and metabolic urinary changes after modern bariatric surgery: review of clinical studies, experimental models, and prevention strategies. *Surg Obes Relat Dis*. 2014; 10:734–42. [PubMed: 24969092]
5. Allison MJ, Dawson KA, Mayberry WR, Foss JG. *Oxalobacter formigenes* gen. nov., sp. nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. *Arch Microbiol*. 1985; 141:1–7. [PubMed: 3994481]
6. Jiang J, Knight J, Easter LH, Neiberg R, Holmes RP, Assimios DG. Impact of dietary calcium and oxalate, and *Oxalobacter formigenes* colonization on urinary oxalate excretion. *J Urol*. 2011; 186:135–9. [PubMed: 21575973]
7. Kaufman DW, Kelly JP, Curhan GC, et al. *Oxalobacter formigenes* may reduce the risk of calcium oxalate kidney stones. *J Am Soc Nephrol*. 2008; 19:1197–203. [PubMed: 18322162]

8. Siener R, Bangen U, Sidhu H, Honow R, von Unruh G, Hesse A. The role of *Oxalobacter formigenes* colonization in calcium oxalate stone disease. *Kidney Int.* 2013; 83:1144–9. [PubMed: 23536130]
9. Canales BK, Ellen J, Khan SR, Hatch M. Steatorrhea and hyperoxaluria occur after gastric bypass surgery in obese rats regardless of dietary fat or oxalate. *J Urol.* 2013; 190:1102–9. [PubMed: 23499748]
10. Green ML, Hatch M, Freel RW. Ethylene glycol induces hyperoxaluria without metabolic acidosis in rats. *Am J Physiol Renal Physiol.* 2005; 289:F536–43. [PubMed: 15855660]
11. Freel RW. Dihydroxy bile salt-induced secretion of rubidium ion across the rabbit distal colon. *Am J Physiol.* 1987; 252:G554–61. [PubMed: 3031996]
12. Hatch M, Cornelius J, Allison M, Sidhu H, Peck A, Freel RW. *Oxalobacter* sp. reduces urinary oxalate excretion by promoting enteric oxalate secretion. *Kidney Int.* 2006; 69:691–8. [PubMed: 16518326]
13. Hatch M, Gjymishka A, Salido EC, Allison MJ, Freel RW. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter*. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G461–9. [PubMed: 21163900]
14. Freel RW, Hatch M, Earnest DL, Goldner AM. Oxalate transport across the isolated rat colon. A re-examination. *Biochim Biophys Acta.* 1980; 600:838–43. [PubMed: 7407147]
15. Lieske JC, Goldfarb DS, De Simone C, Regnier C. Use of a probiotic to decrease enteric hyperoxaluria. *Kidney Int.* 2005; 68:1244–9. [PubMed: 16105057]
16. Hoppe B, Beck B, Gatter N, et al. *Oxalobacter formigenes*: a potential tool for the treatment of primary hyperoxaluria type 1. *Kidney Int.* 2006; 70:1305–11. [PubMed: 16850020]
17. Hoppe B, Groothoff JW, Hulton SA, et al. Efficacy and safety of *Oxalobacter formigenes* to reduce urinary oxalate in primary hyperoxaluria. *Nephrol Dial Transplant.* 2011; 26:3609–15. [PubMed: 21460356]
18. Hatch M, Canales BK. The mechanistic basis of hyperoxaluria following gastric bypass in obese rats. *Urolithiasis.* 2016; 44:221–30. [PubMed: 26584912]
19. Hatch M, Cornelius J, Allison M, Sidhu H, Peck A, Freel RW. *Oxalobacter* sp. reduces urinary oxalate excretion by promoting enteric oxalate secretion. *Kidney Int.* 2006; 69:691–8. [PubMed: 16518326]
20. Hatch M, Freel RW. A human strain of *Oxalobacter* (HC-1) promotes enteric oxalate secretion in the small intestine of mice and reduces urinary oxalate excretion. *Urolithiasis.* 2013; 41:379–84. [PubMed: 23959075]
21. Hatch M, Gjymishka A, Salido EC, Allison MJ, Freel RW. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with *Oxalobacter*. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G461–9. [PubMed: 21163900]
22. Duffey BG, Miyaoka R, Holmes R, et al. *Oxalobacter* colonization in the morbidly obese and correlation with urinary stone risk. *Urology.* 2011; 78:531–4. [PubMed: 21529902]
23. Froeder L, Arasaki CH, Malheiros CA, Baxmann AC, Heilberg IP. Response to dietary oxalate after bariatric surgery. *Clin J Am Soc Nephrol.* 2012; 7:2033–40. [PubMed: 23024163]
24. Kelly JP, Curhan GC, Cave DR, Anderson TE, Kaufman DW. Factors related to colonization with *Oxalobacter formigenes* in U.S. adults. *J Endourol.* 2011; 25:673–9. [PubMed: 21381959]
25. Allison MJ, Cook HM, Milne DB, Gallagher S, Clayman RV. Oxalate degradation by gastrointestinal bacteria from humans. *J Nutrition.* 1986; 116:455–60. [PubMed: 3950772]

Figure 1.

A. Percent body weight loss after surgery in RYGB- (n=18) and Sham- (n=10) operated rats. Solid black box corresponds to postoperative care period (~14 days) before the animals had full access to solid chow. Statistically significant % weight differences were noted post-operative week 3 and beyond (*). Data shown are mean $\pm$ SEM and displayed as continuous for Figure purposes only. * $p < .01$ vs Sham values by Mann-Whitney U tests. B. Changes in daily calorie intake after surgery. The dotted line represents mean calorie intake before surgery. Sham animals returned to baseline caloric intake by week 3–4 while RYGB animals took until week 7–8. Data shown are mean $\pm$ SEM. * $p < .01$ vs preoperative value by Mann-Whitney U tests.

Figure 2.

Urinary oxalate and creatinine ratios in RYGB- (n=18) and Sham- (n=10) operated rats at baseline and after *Oxalobacter formigenes* wild rat (OXWR) gavage (week 12, black arrow). All gavaged animals remained colonized. Diet was maintained at 40% fat, 1.5% potassium oxalate until week 16, when % fat decreased to 10%. Data shown are mean $\pm$ SEM and displayed as continuous for figure purposes only. Statistically significant differences (*) noted between RYGB group weeks 14–20.

Figure 3.

Unidirectional fluxes of oxalate (J , $\text{pmoles} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$) measured across isolated short-circuited bowel segments of Sham and RYGB animals ~20 weeks post-surgery on diet containing 10% fat and 1.5% oxalate supplementation, asterisk denotes a significant difference ($p < 0.05$) between groups. A. Sham colonic tissue ($n=10$ tissue pairs in each group) with and without OXWR colonization. No significant difference noted in transepithelial conductance ($G_T = 19.9 \pm 3.4$ and $23.9 \pm 2.9 \text{ mS} \cdot \text{cm}^{-2}$ between non-colonized and OXWR groups) or short-circuit current ($I_{sc} = 0.42 \pm 0.06$ vs $0.63 \pm 0.15 \text{ } \mu\text{Eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$ non-colonized and OXWR respectively). B. RYGB colonic tissue ($n=18$ tissue pairs) with and without OXWR colonization. No difference noted in conductance ($G_T = 19.1 \pm 2.2$ and $20.7 \pm 1.2 \text{ mS} \cdot \text{cm}^{-2}$) and current ($I_{sc} = 0.52 \pm 0.10$ vs $0.40 \pm 0.09 \text{ } \mu\text{Eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$). C. RYGB Roux limb ($n=18$ tissue pairs). No difference noted in conductance ($G_T = 5.9 \pm 0.6$ vs $6.9 \pm 0.4 \text{ mS} \cdot \text{cm}^{-2}$) and current ($I_{sc} = 0.30 \pm 0.03$ and $0.31 \pm 0.05 \text{ } \mu\text{Eq} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$).