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Intestinal resection-associated metabolic syndrome☆

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ABSTRACT

Background: Short bowel syndrome occurs following massive small bowel resection (SBR) and is one of the most lethal diseases of childhood. We have previously demonstrated hepatic steatosis, altered gut microbiome, and increased fat deposition in our murine model of SBR. These novel findings prompted us to investigate potential alterations in glucose metabolism and systemic inflammation following intestinal resection.
Methods: Male C57BL6 mice underwent 50% proximal SBR or sham operation. Body weight and composition were measured. Fasting blood glucose (FBG), glucose, and insulin tolerance testing were performed. Small bowel, pancreas, and serum were collected at sacrifice and analyzed.
Results: SBR mice gained less weight than shams after 10 weeks. Despite this, FBG in resected mice was significantly higher than sham animals. After SBR, mice demonstrated perturbed body composition, higher blood glucose, increased pancreatic islet area, and increased systemic inflammation compared with sham mice. Despite these changes, we found no alteration in insulin tolerance after resection.
Conclusions: After massive SBR, we present evidence for abnormal body composition, glucose metabolism, and systemic inflammation. These findings, coupled with resection-associated hepatic steatosis, suggest that massive SBR (independent of parenteral nutrition) results in metabolic consequences not previously described and provides further evidence to support the presence of a novel resection-associated metabolic syndrome.

Short bowel syndrome is a massive loss of small bowel due to surgical resection for acquired or congenital conditions in children and adults. Short bowel syndrome has been associated with several long-term changes including hepatic dysfunction and short stature [1,2]. Data regarding body composition in pediatric SGS patients are sparse. In a recent study of 34 children with intestinal failure from a variety of causes, those who required PN had a significant deficit in lean limb mass with greater fat mass indices, the latter effect related to the amount of PN needed [2]. In another report, the body composition of pediatric SGS patients who had all weaned successfully from PN were compared with reference standards [3]. In this cohort, height and lean body mass were significantly lower while % body fat was normal. These findings are analogous to our murine SBR model that demonstrates a perturbed body composition phenotype as mice recover from massive small bowel resection (SBR) in the absence of PN.

In mouse models, the combination of obesity and elevated fasting blood glucose is routinely used to study the renowned metabolic syndrome (MeS). The diagnosis of MeS has significant implications on morbidity and mortality with increased risk of cardiovascular events, type II diabetes (DMII), and death [4,5]. In addition to the diagnostic features and cardiovascular consequences, systemic inflammation and pathologic triglyceride accumulation in the liver is often seen in humans and mice with MeS [6]. Similar to MeS, data demonstrate that perturbations in glucose homeostasis are linked to non-alcoholic fatty liver disease (NAFLD) [7]. In addition to changes in body composition, previous studies from our lab have found that mice develop hepatic steatosis 10 weeks after small bowel resection [8]. Given our findings of altered body composition and development of steatosis resembling models of MeS and NAFLD, the purpose of this study was to test the hypothesis that massive intestinal resection results in perturbed glucose homeostasis due to a systemic proinflammatory response.

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1. Methods

1.1. Animals

C57BL6 mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 7 weeks of age. Mice were housed on arrival in a facility with a 12-h light/dark cycle. Male mice were used exclusively in order to limit known hormonal confounders on the timing and severity NAFLD [9]. Food and water were provided ad libitum to SBR animals and sham animals were provided the same amount of food as SBR cages. This study was approved by the Washington University Animal Studies Committee (Protocol #20130308) in accordance with the National Institute of Health laboratory animal care and use guidelines.

1.2. Operations and sample collection

All operated mice underwent a 50% proximal SBR or sham operation as we have previously described [10] .Briefly, through a midline laparotomy the bowel is exteriorized and then transected 12 cm from the terminal ileum and 2-3 cm from the pylorus. The intervening mesentery was ligated with silk suture and a hand sewn, end-to-end anastomosis was performed with interrupted 9–0 nylon sutures. For sham operations, the proximal bowel was transected 12 cm from the terminal ileum and reaanastomosis performed (no resection). Animals were fasted for the first 24 h post operatively then returned to standard liquid diet (LD; PMI Micro-Stabilized Rodent Liquid Diet LD 101; TestDiet, St. Louis MO). Animals were sacrificed at 5 or 10 weeks after operation. The small intestine, pancreas, and serum were collected.

1.3. Glucose and insulin tolerance testing

Insulin and glucose tolerance tests were performed as previously described [11,12]. Animals were acclimated to the tester by handling of the animals for 2 days prior to experimentation. Animals were fasted overnight (18 h) on wood chip bedding. Animals were weighed and tails clipped then rested for 1 h in a heated, low stimulation environment. Mice were then given a 2 mg/g glucose load either intraperitoneally (IPGTT) or via oral gavage (OGTT). For insulin tolerance testing an intraperitoneal injection of human regular insulin (Eli Lilly and Co., Indianapolis, Indiana, USA) at a dose of 0.75 U/kg body weight. Tail vein blood glucose was measured at times 0, 15, 30, 60, and 90 min. Animals were excluded from GTT if they failed to demonstrate a glucose peak or demonstrated a late persistent peak (>500) consistent with a stress response. Tests were performed at both 5 and 10 weeks following SBR.

1.4. Body composition

Given that the hepatic steatosis we observed occurs at a later time point than we have previously studied, we chose to look at the late changes in body composition by comparing measurements at 10 weeks to those at 5 weeks. Body composition was measured by MRI as previously described [13].

1.5. Histology and islet quantification

Pancreas sections were fixed in formalin, embedded in paraffin, and sections stained with Hematoxylin and eosin. Slides were scanned at 10X magnification. Quantification of total pancreas area and islet area was performed in Image-J software. All slides were analyzed by the same investigator and blinded for group.

1.6. Inflammatory cytokines

The Mesoscale Discovery VPLEX Proinflammatory Panel 1 (mouse) kit (Catalog#K15408) with reagents for the detection of interleukin 1-beta (IL-1β), IL-6, and tumor necrosis factor-alpha (TNFα) was used to investigate serum cytokine levels at 10 weeks after small bowel resection. Samples were prepared according to the manufacturer protocol. Electrochemiluminescence (ECL) was measured and reported as emitted fluorescence intensity. Data analysis was performed using the MSD Discovery Workbench analysis software.

1.7. Statistics

Graphs and statistical analysis were performed using Graphpad-Prism. Glucose tolerance tests were analyzed using 2-way repeated measures analysis of variance (ANOVA) with Sidak's multiple comparisons. We chose this statistical method because we obtained multiple measures from the same subjects for tolerance testing. Fasting blood glucose, serum cytokines, body composition and pancreatic islet mass were analyzed using Student's t-test. A p value <0.05 was considered significant. All analyses presented were calculated using G*Power software and had a measured power of 0.80 or greater [14].

2. Results

2.1. Weight

Resected mice gained 13% less body weight compared with shams after 5 weeks (100.7% vs. 115.1% of baseline weight $p < 0.0001$;). Postoperatively sham mice lost 3% of their total body weight while SBR mice lost 10%. Sham mice surpassed their baseline starting weight by week 2 while SBR mice did not achieve this level until four weeks post operatively (Fig. 1).

2.2. Body composition

Sham operated mice gained significant fat (75.5%, $p < 0.001$) and lean mass (10.9% $p = 0.001$) when comparing body composition 5 weeks to body composition at 10 weeks after operation. On the other hand, following SBR, mice maintained their fat mass over the later time period but lost lean mass (8.4% $p < 0.05$). (Fig. 1B).

2.3. Blood glucose and glucose tolerance testing at 5 weeks postoperatively

Fasting blood glucose levels in resected mice were 40% higher than in sham operated animals (101.6 ± 4.12, $n = 17$ vs. 67.4 ± 2.67, $n = 18–19$, $p < 0.0001$, Fig. 2A). After IP administration of glucose, SBR mice demonstrated significantly higher blood glucose at fifteen, thirty, and sixty minutes when compared to sham operated mice (Fig. 2B). The same trend was further confirmed with oral glucose administration as indicated in Fig. 2C. Two-way ANOVA demonstrated significance for both GTT studies when analyzing operation type ($p < 0.002$ for OGTT, $p < 0.0001$ for IGTT) demonstrating a significant difference in the overall trend of glucose tolerance between the groups (Fig. 2B-C). Additionally, we found that fasting blood glucose positively correlates with 15 min ($r = 0.60$, $p < 0.002$) and peak blood glucose levels ($r = 0.85$, $p < 0.001$, Fig. 2D).

2.4. Insulin tolerance testing at 10 weeks postoperatively

Elevated fasting blood glucose levels persisted after SBR when compared with sham operated mice at 10 weeks following operation (Fig. 3A). We next investigated insulin tolerance. We found no change in peripheral insulin resistance after SBR since there were no differences in blood glucose levels after insulin administration between the two groups at any time point. (Fig. 3B)