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Adaptation of Intestinal and Bile Acid Physiology Accompany the Metabolic Benefits Following Ileal Interposition in the Rat

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Abstract

Purpose—Ileal interposition recapitulates many of the metabolic improvements similar to Roux-en-Y gastric bypass. We aimed to determine whether the metabolic improvements seen following ileal interposition were conferred solely by the interposed segment by examining changes in neighboring intestinal segments as well as the composition of the bile acid pool.

Materials and Methods—Adult male rats were treated with either Sham or Ileal Interposition surgeries. Glucose tolerance tests, body composition analysis, polymer chain reaction, enzyme-linked immunosorbent assay and mass spectrometry were done after the surgeries.

Results—This study showed that ileal interposition improved glucose tolerance and enhanced both fasting and glucose-stimulated GLP-1 secretion in diabetic rats. Total bile acid pool was similar between groups but the composition favored glycine-conjugation in rats with ileal interposition. Insulin secretion was highly correlated with the 12-alpha hydroxylase index of activity. The interposed ileum exhibited an increase in mRNA for Preproglucagon and Peptide-YY; however, the bile acid transporter, apical sodium bile acid transporter, was dramatically reduced compared to sham rats. The interposed segment becomes jejunized in its new location as indicated by an increase in Glut2 and Pepck mRNA, genes predominantly synthesized within the jejunum.

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Conflict of Interest Statement

All authors in the manuscript have nothing to disclose.

Ethical Approval

>All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The welfare of animals used for research has been respected. All applicable institutional and/or national guidelines for the care and use of animals were followed. All animal procedures were approved by the Institutional Animal Care and Use Committee of Southern Illinois University-Carbondale.

Informed Consent

Does not apply.

Conclusion—Ileal relocation alone can significantly alter the bile acid pool to favor a more insulin sensitive metabolism in association with intestinal wide alterations in mRNA for a variety of genes. Ileal interposition may confer metabolic improvement via both the interposed segment and the associated intestinal changes in all segments of the intestine, including the colon.

Introduction

Bariatric surgery results in massive weight loss in addition to physiological improvement of many obesity-associated co-morbid conditions. While it has become increasingly clear that bariatric surgery offers metabolic benefit that cannot be explained by weight loss alone; however the mechanisms underlying these effects have yet to be identified. One notable and particularly interesting effect is the rapid and durable remission of Type-2 diabetes following Roux-en-Y gastric bypass (RYGB) (1). Some hypothesize that one key anatomical feature of RYGB that mediates euglycemia in diabetic patients may be over stimulation of the lower intestine by relatively unmodified nutrients traversing through the lower gastrointestinal tract. To examine this, we utilized a rodent model of ileal interposition. Interposition surgery offers some investigatory benefit as it involves a single gastrointestinal manipulation unlike the multifaceted surgical procedure RYGB (2, 3). Importantly, diabetic rats that receive ileal interposition show improved glucose tolerance and increased ileal-produced hormone secretion independent of body weight (3); ileal transposition is still in the early stage of animal and human trials (4).

The ileum is a key contributor to the enterohepatic biliary system since the vast majority of bile salts are reabsorbed at this location. Ileal interposition involves the relocation of an ileal segment within the duodenojejunal segments of the small intestinal tract. Because the ileum is in a more proximal location, it is likely that IT surgery may result in alterations in bile salt uptake, composition, and signaling. It has been shown that the ileal segment can become morphologically jejunized once placed within the proximal small intestine (5). In this study, we aimed to determine whether neighboring small intestinal segments and colon are functionally altered as a result of proximal ileal placement by examining intestinal gene expression and bile salt composition in diabetic and non-diabetic rats that have received ileal interposition and sham surgery.

Materials and Methods

Animals

Twenty-nine adult male (5 months of age) Long-Evans rats (250–350g) were fed *ad libitum* throughout each of the studies. One cohort of rats (Diabetic, n = 14) was treated with a single low dose of streptozotocin (STZ) (35mg/kg; intraperitoneally) thirty days prior to surgery. In addition, rats were given *ad libitum* access to a high-fat diet (HF) (40% fat) (Research diets, New Brunswick, NJ). The second cohort of rats (Non-Diabetic; ND, n = 15) was fed standard Purina rodent chow throughout the study. All rats were age matched and randomly separated into either Sham or Ileal Interposition surgical groups (Diabetic Sham, n=7; Diabetic IT, n = 7; ND Sham, n = 7; ND IT, n = 8). All animal procedures were approved by the Institutional Animal Care and Use Committee of Southern Illinois University-Carbondale.

Experimental Design

Rats in the Diabetic group (HF + STZ) were given *ad libitum* access to the high-fat diet at the start of the study. After four weeks of feeding on the high-fat diet rats were then given a single i.p. injection of STZ (35 mg/kg) (6). The diabetic rats continued to feed freely on the high-fat diet for an additional four weeks prior to surgery. Chow-fed (Non-Diabetic; ND) and Diabetic (HF + STZ) rats were given either a Sham or Ileal Interposition operation as previously described (2, 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Results

Body weight and composition

At no time during the experiment did the diabetic rats differ based on the surgical procedure they received (Figure 1A). In addition, shortly before termination of the study, body composition was determined using EchoMRI (Houston, TX) and showed no significant changes in lean or fat percentage between the surgical groups (Figure 1B and Figure 1C).

Glucose Tolerance Test and Plasma GLP-1

Rats that received interposition surgery showed improved glucose tolerance at 15, 30, 45, 60 and 120 minutes (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; Figure 2A). Plasma insulin during the glucose tolerance test was not found to be significantly different at any time point (Figure 2B). Similarly, there was no difference in total insulin secretion during the OGTT (Figure 2C). Active plasma GLP-1 was significantly elevated during the OGTT in diabetic rats that received interposition surgery as compared to sham-operated rats at 15 and 30 minutes (* $p < 0.05$; Figure 2D). A significant interaction was determined between surgery and time; therefore, a Student's *t*-test was performed on the fasting levels of active GLP-1 and rats with interposition surgery had higher levels as compared to sham-operated rats (** $p < 0.01$; Figure 2E). Again, rats that were chow fed showed significant improvements in glucose tolerance and GLP-1 secretion during their OGTT and these data have been previously published (2).

Bile Acid Profiles in Diabetic Rats

Fasting total bile acids were not significantly different between the diabetic surgical groups (Figure 3B). Total bile acid levels were significantly different between chow-fed (Non-Diabetic) rats with interposition and sham surgery. Those data have been reported elsewhere (2). Despite no change in total bile acids, the make-up of the bile acid composition was significantly different in diabetic rats who received interposition surgery. Specifically, glycocholic acid and tauro-beta-muricholic acids were significantly higher in diabetic rats with interposition as compared to diabetic sham-operated rats (* $p < 0.05$; Figure 3A). While the total bile acids were not significantly different, the diabetic rats with interposition showed a four-fold decrease in the ratio of taurocholate:glycocholate (TCA/GCA) (* $p < 0.05$; Figure 3C). The ratio of TCA/GCA was decreased because rats with interposition had

significantly elevated glycocholate levels (* $p < 0.05$; Figure 3D). Lastly, when glucose-stimulated insulin concentrations at 15 minutes into the oral glucose tolerance test from all rats were plotted against an index of 12- α hydroxylase activity (or CYP8b1) involving the tauro-conjugated bile pool, there was a highly significant correlation ($p < 0.0001$; Spearman correlation = -0.88 ; Figure 3E). In other words, when plasma insulin was elevated or restored rats showed a significant decrease in a sensitive index of 12- α hydroxylase activity.

Intestinal Gene Expression in Diabetic Rats

Figure 4 (top) is an illustration of the locations from which the intestinal segments were collected during sacrifice. Small 3–5mm segments of intestine were collected from the duodenum, jejunum, ileum and colon in rats that received sham surgery. Similarly, segments of duodenum, interposed ileum, jejunum (distal to the interposed segment of ileum), remnant (or native) ileum, and colon were collected from rats that received ileal interposition. For purposes of the two-way ANOVA data analysis, which requires equal numbers of group comparisons, the ileal segment from the sham-operated rats was utilized twice (sham-ileum vs. remnant ileum of IT rats and sham-ileum vs. interposed ileum of IT rats). In diabetic rats with ileal interposition L-cell gene products were significantly elevated compared to sham-operated controls. Specifically, *Pre-proglucagon* gene expression was not only elevated in the ileal segment that had been interposed within the duodenal-jejunal region but also elevated in the remnant ileum and colon (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; Figure 4A). No difference in *Pre-proglucagon* was found between the duodenum and jejunum comparisons. Although not the exact pattern, gene expression for *Peptide YY* was also elevated in rats with ileal interposition compared to sham-operated rats (* $p < 0.05$; Figure 4B). The duodenal hormone glucose independent polypeptide (GIP) was not different within any segment between sham and interposition-treated rats (Figure 4C). Gene expression for *Apolipoprotein A-IV* was significantly increased in the interposed ileum of rats with ileal interposition surgery as compared to sham (*** $p < 0.001$; Figure 4D). In the diabetic rats, *Glut2* gene expression did not reach significance for any segment following interposition surgery, although there was a trend for significance within the interposed ileum (Figure 4E). In contrast to *Glut2*, the rate-limiting enzyme in glucose production, *Pepck* was significantly affected by ileal interposition. Gene expression for *Pepck* was elevated within the interposed ileum and colon in rats that received IT surgery (*** $p < 0.001$; Figure 4F).

The apical sodium bile transporter (*Asbt*) gene expression was dramatically reduced within the interposed ileum compared to that of the sham ileum (** $p < 0.001$; Figure 6A). No difference for *Asbt* expression was found between any other intestinal segments. In contrast to *Asbt*, gene expression for the ileal bile acid binding protein (*Ibabp*) was found to be significantly increased in the remnant ileum of rats which had ileal interposition surgery (*** $p < 0.001$; Figure 6B). Gene expression for *Tgr5* was not different between any intestinal segments (Figure 6C). Fibroblast growth factor 15 (*Fgf15*) was significantly higher within the colon of rats that received ileal interposition surgery (* $p < 0.05$; Figure 6D).

Intestinal Gene Expression in Non-Diabetic Rats

In a previously published study we found that chow-fed rats benefited from the glucose-lowering effect of ileal interposition surgery and that upon sacrifice they showed elevated plasma bile acids (2). Intestinal tissue from these same rats was harvested and saved for future examination and gene expression analysis. No significant changes in gene expression within intestinal segments were found for *Pre-proglucagon* (Figure 5A), *Apo-lipoprotein A-IV* (Figure 5C) or *Pepck* (Figure 5E). However, non-diabetic rats showed a greater change in the amount of gene expression throughout the intestines for *Peptide YY* as compared to diabetic rats. *Peptide YY* gene expression was significantly elevated in the intestine of rats that received ileal interposition compared to sham (** $p < 0.001$, * $p < 0.05$; Figure 5B). Lastly, *Glut2* mRNA was found to be increased within the interposed ileum as compared to the sham ileum (** $p < 0.01$; Figure 5D).

In contrast to the results of diabetic rats, *Asbt* mRNA was not affected in the interposed ileum but showed a significant increase within the remnant ileum of rats that received ileal interposition surgery (** $p < 0.001$; Figure 7A). No changes were found between any intestinal segments (Figure 7B) for the expression of *Ibabp*. *Tgr5* was not changed in non-diabetic rats following ileal interposition (Figure 7C). Finally, *Fgf15* mRNA was found to be significantly reduced within the remnant ileum of rats that received ileal interposition (* $p < 0.05$; Figure 7D). The difference in liver gene expression between non-diabetic rats that received either sham or ileal interposition surgery has been reported in supplementary Figure 2.

Discussion

The present study examined mechanisms underlying the metabolic improvement following ileal interposition. Similar to our previous studies, IT surgery improved glucose tolerance in diabetic rats (2, 3). Interestingly, diabetic rats that received IT surgery were no different in body weight or body composition as compared to sham-operated rats. These findings are in contrast to those by Kohli *et al.* who showed that rats which undergo IT surgery increase lean body mass and decrease fat mass (7). A potential explanation for this disparity is that our diabetic model involved rats subjected to a high-fat diet followed by low-dose streptozotocin treatment. While IT surgery in the present study improved glucose tolerance, insulin secretion between the two groups was not significantly different, suggesting that insulin sensitivity was improved in IT-treated rats (Supplementary figure 1). It is noteworthy that fasting plasma GLP-1 (7–36) was also increased in rats that had received IT surgery. Previous work has shown that another ileal-produced hormone PYY to be altered (higher) following RYGB, however; the increase in basal or fasting active GLP-1 in this model may be novel (8).

We examined the role of ileal interposition on changes in bile acids and intestinal bile acid transporters. There are many physiological consequences following the relocation of distal ileum into the duodenojejunal region. First, the interposed ileum will be exposed to more concentrated nutrients and biliopancreatic secretions. Second, the relocated ileum will be placed in a region of small intestine with a dramatically different microbial milieu and pH. Together, the new intestinal environment may alter the functional nature of the ileal segment.

The present findings support previous studies that show an increase in the relative amounts of conjugated bile salts following IT surgery (7). Rats that had IT surgery increase the relative amounts of glycocholic acid compared to those in sham-operated rats, however, the total plasma bile salt pool was not significantly different between the two groups, suggesting that taurine may not be limiting in bile salt disposition in the liver. These data differ from our previous study and by that of Kohli *et al.* in non-diabetic rats in which total bile salts were elevated following ileal interposition surgery. One limitation of the study is that we did not measure the fecal loss of bile acids after the surgeries. Initial studies of 12 α -hydroxylase regulation in rat revealed insulin as the primary regulator of the enzyme via gene expression in a physiological context (9–11). Recent studies suggest that insulin sensitivity is highly correlated with 12- α hydroxylase activity (12, 13). In fact, when insulin concentrations were plotted against an index of 12- α hydroxylase activity a very strong correlation was determined. It is reasonable to suggest that insulin signaling may be restored in rats that received IT surgery which then suppresses 12- α hydroxylase activity. Farnesoid nuclear receptor (FXR) is another negative regulator of 12- α -hydroxylase expression in rat and mouse. Our data reconcile observations of improved insulin sensitivity in the 7 α -hydroxylase-transgenic mouse (14).

When the ileum is surgically relocated in the IT model, a small (~5cm) portion of ileum remains in its' native location connected to the ileocecal valve, rendering the IT animal with potentially two functional sites for bile salt uptake. Diabetic rats that receive IT surgery show a 95% decrease in the relative amount of *Asbt* mRNA within the interposed ileum when compared to the intact ileum of sham-operated rats. Suppression of *Asbt* expression is an established action of FXR engagement and so this observation could indicate additional consistency with the FXR constitutive engagement hypothesis due to the proximity of highly enriched bile to the transposed ileum (15). In view of the observed reductions in *Asbt* expressed in the interposed ileum, dihydroxylated bile salts would thermodynamically out-compete trihydroxylated bile salts for uptake. Thus, *Asbt* expressed at normal levels is rarely saturated; however, the combination 95% suppression of *Asbt* in the IT segment and subsequently greater uptake of dihydroxylated species results in a disproportionate drop in plasma cholate levels. This, in turn, may translate downstream to a lower than normal level of FXR engagement in the distal ileum. Since the FXR dependent regulation of ASBT involves LRH1 and SHP, and the regulation of IBABP is directly regulated by FXR, a differential (reciprocal) pattern of transporter expression could ensue. Following uptake by ASBT, IBABP escorts bile salts to the basolateral surface. It has been suggested that increased IBABP reflects increased transport and activation of FXR. While the proximally located ileum in IT rats did not show a significant increase in *Ibabp* mRNA, the remnant ileum showed very high levels of *Ibabp* mRNA compared to the ileum of sham-operated rats. Bile is highly concentrated proximal to the interposed ileum and it is possible that elevated concentrations may down-regulate the *Asbt*. However, data from Kohli *et al* and the present data in non-diabetic rats show no difference in *Asbt* mRNA in the interposed ileum compared to sham animals. It is possible that the induction of diabetes induced by streptozotocin plus high fat diet alters bile transport differently than that in non-diabetic or high-fat fed only animals. Alternatively, animals were sacrificed between 3–4 months following surgery in the present study while those in the study by Kohli *et al* were studied at

six weeks, offering a possible difference in time-course of expression for *Asbt* as the animal's physiology adapts to the surgery (7).

It is unknown how the surgical relocation of the ileum within the proximal small intestine may alter neighboring intestinal gene expression. We determined that the interposed ileum does, in fact, synthesize more *Preproglucagon* mRNA, but that the remnant ileum and colon also synthesize even more *Preproglucagon* than that of the interposed ileum. Interestingly, chow-fed non-diabetic rats that received ileal interposition did not show a significant difference in *Preproglucagon* mRNA between any segment although we have previously reported that they do show improved glucose tolerance. Gene expression for *Pyy* showed a similar trend in diabetic rats and even a greater difference in non-diabetic rats. These data question whether the metabolic improvement that is conferred by ileal interposition is in fact mediated by the interposed ileum or the remnant/neighboring intestinal segments following anatomical relocation. Once relocated, the ileal segment may functionally assume jejunal characteristics (5). In support of this is the finding that genes primarily expressed within the jejunum such as *Glut2*, *Pepck*, and *Apo-AIV* mRNA are increased in the interposed ileal segment. It seems paradoxical that IT rats with improved glucose tolerance would increase intestinal gluconeogenesis. However, the increase in *Pepck* may be signaling via the portal circulation to inhibit glucose production as suggested by (16). These data are in contrast to those by Cooney *et al.* which showed a decrease in PEPCK in rats that have had RYGB (17).

In summary, these data suggest that the metabolic improvement following ileal interposition is multifaceted and may include changes in gene expression in other segments of small intestine and colon. Additionally, the improved glucose control is highly correlated with reduced 12-alpha hydroxylase activity and the altered conjugated bile acid composition may be another critical component. Finally, the metabolic state of the animal (diabetic vs. non-diabetic) significantly affects the pattern of intestinal gene expression seen following ileal interposition surgery; this finding warrants further investigation.

Supplementary Material

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Acknowledgments

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Figure 1.

Body weight and composition changes following ileal interposition or sham surgery in diabetic Long-Evans rat. No changes in body weight were found before or after surgery between rats with ileal interposition or sham surgery (A). In addition, thirteen weeks following surgery rats were examined for changes in body composition and no differences in body fat (B) or lean (C) percentages were noted.

Figure 2.

Glucose tolerance test following ileal interposition or sham surgery in diabetic Long-Evans rats. Rats that received ileal interposition showed an improvement in glucose clearance during the OGTT as compared to sham-operated rats (A) (two-way ANOVA with repeated measures; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$). Neither the time course plasma insulin levels (B), or the insulin area under the curve (C) (determined by trapezoidal analysis) was significantly different at any time point. Plasma levels of active GLP-1 (7–36) were significantly elevated in rats with ileal interposition as compared to sham rats during the OGTT at baseline, 15 minutes and 30 minutes (* $p < 0.05$; two-way ANOVA with repeated measures) (D). Fasting GLP-1 (7–36) was significantly higher in rats with ileal interposition compared to sham-operated rats (* $p < 0.05$; Student's *t*-test) (E).

A.

Figure 3.

Bile acid composition and relationship to oral glucose-stimulated insulin secretion following ileal interposition and sham surgery in diabetic Long-Evans rats. Rats that received ileal interposition surgery showed a significant increase in percent glycocholate and tauro- β -muricholic acid levels compared to sham-operated rats (Student's *t*-test; * $p < 0.05$) (**A**). Total bile acids were not significantly different between ileal interposition and sham-operated rats (**B**). However, the taurocholate/glycocholate ratio (**C**) was significantly decreased in rats with ileal interposition as compared to sham (Student's *t*-test; * $p < 0.05$). Similarly, glycocholate percent levels were elevated in rats with ileal interposition (Student's *t*-test; $p < 0.05$) (**D**). During the OGTT blood was collected and plasma insulin and bile acids were measured at 15 minutes. Plasma insulin secretion was highly correlated with 12- α -hydroxylase activity. The ^{tauro}12- α hydroxylase activity was determined by calculating the ratio of taurodeoxycholic acid/taurocholic acid. A Spearman correlation coefficient of -0.88 shows a highly significant negative relationship (Spearman correlation = -0.88 ; $p < 0.0001$). As plasma insulin secretion increased, 12- α -hydroxylase activity decreased (**E**). Data are shown for all rats from both surgical groups.

Figure 4.

Intestinal gene expression changes following ileal interposition or sham surgery in diabetic Long-Evans rats. During sacrifice, a 1 cm segment of intestinal tissue was collected from duodenum, jejunum, ileum, “interposed ileum”, “remnant ileum” and colon. The diagram above illustrates the location from which each of the segments was collected for each surgical group. For analysis, ileal samples from sham-operated rats were used twice for comparison against “interposed ileum” and “remnant or native ileum” of rats with ileal interposition surgery. The intestinal segments were immediately placed in RNALater and processed for semi-quantitative real-time PCR. For each gene, a n=5–6 was used for gene expression analysis. Gene expression was analyzed using the Livak 2^{-Ct} method. Relative changes are expressed relative to the expression within the duodenum for each surgical group. The values were then analyzed using a two-way ANOVA with repeated measures to identify significance within each corresponding segment. Some of the changes appear very high because the gene analyzed was found at very low levels within the duodenum. Because some genes may be restricted to the ileum, the relative change (as compared to the duodenum) may appear great. Gene expression for *Preproglucagon* was significantly elevated in rats that received ileal interposition compared to sham-operated rats. Specifically, the ileum (* $p < 0.05$), remnant ileum (** $p < 0.01$) and colon (*** $p < 0.001$) were significantly higher than the corresponding segment of sham-operated rats (A). *Pyy* gene expression was also significantly elevated within the remnant ileum of rats that received ileal interposition

compared to the ileum of sham-operated rats (* $p < 0.05$) (**B**). Gene expression was also examined for genes not typically abundant within the ileum. *Gip* gene expression, which is primarily synthesized within the duodenum, was not found to be different between the surgical groups for any segment (**C**). Gene expression for *Apolipoprotein A-IV* was significantly increased within the interposed ileum as compared to the ileum of sham-operated rats, suggesting that the newly re-located ileum may become jejunalized since *Apolipoprotein A-IV* is primarily synthesized within the jejunum (** $p < 0.001$) (**D**). Two other genes involved in glucose transport and synthesis were measured. The primary glucose transporter *Glut2* did not show any significant change between any segment; although a trend towards significance appeared between the ileal segments (**E**). However, *Pepck*, the rate limiting step in gluconeogenesis, was significantly increased in the interposed ileum (** $p < 0.001$) and colon (* $p < 0.01$) of rats that received ileal interposition as compared to sham-operated rats (**F**).

Figure 5.

Intestinal gene expression changes following ileal interposition or sham surgery in non-diabetic rats. In contrast to diabetic rats, no change in *Pre-proglucagon* gene expression was identified between segments (A). *Pyy* gene expression, however, was significantly increased in the ileum (*** $p < 0.001$), remnant ileum (*** $p < 0.001$), and colon (* $p < 0.05$) of in non-diabetic rats that received ileal interposition compared to sham-operated rats (B). *Gip* was not measured in non-diabetic rats but was previously shown to be unchanged (ref). *Apolipoprotein A-IV* was not significantly different between any segments in non-diabetic rats (C). However, *Glut2*, which did not show significance in diabetic rats, was significantly elevated within the ileal segment (** $p < 0.01$) for non-diabetic rats with ileal interposition surgery as compared to non-diabetic sham-operated rats (D). Lastly, no significant change was found for *Pepck* gene expression in non-diabetic rats (E).

Figure 6.

Intestinal bile-related gene expression following ileal interposition or sham surgery in diabetic rats. Gene expression was found to be approximately 95% suppressed (** $p < 0.01$) within the interposed ileal segment in rats with ileal interposition as compared to the non-relocated ileum of sham-operated rats (**A**). There was no difference in *Asbt* expression between the remnant ileum of rats with interposition surgery compared with the native non-relocated ileum of sham-operated rats. However, the cytosolic bile acid transporter, *Ibabp* was significantly elevated within the remnant ileum in rats with ileal interposition as compared to sham-operated rats (** $p < 0.001$) (**B**). Bile acids activate a G-protein receptor TGR5 or a nuclear receptor, FXR. Gene expression for *Tgr5* did not show any significant change between surgical groups in diabetic rats (**C**). However, *Fgf15*, a target of bile acids, was found to be significantly elevated within the colon of rats with ileal interposition as compared to sham-operated rats (* $p < 0.05$) (**D**). For each gene a $n = 5-6$ rats was used.

Figure 7.

Intestinal bile-related gene expression following ileal interposition or sham surgery in non-diabetic rats. In contrast to the gene expression profile for diabetic rats, non-diabetic rats showed a significant increase in *Asbt* gene expression within the remnant ileum after rats received ileal interposition as compared to sham-operated rats (**A**) (** $p < 0.001$; two-way ANOVA with repeated measures). However, *Ibabp* was not significantly different between surgical groups for any intestinal segment (**B**). The bile acid receptor *Tgr5* was also not significantly changed following ileal interposition in non-diabetic rats (**C**). Lastly, *Fgf15* was shown to be significantly decreased within the remnant ileum of rats that received ileal interposition as compared to sham-operated controls (**D**) (* $p < 0.05$; two-way ANOVA with repeated measures). Sample size was $n = 7-9$ rats for gene expression analysis.