ILEAL INTERPOSITION SURGERY DID NOT PREVENT THE ONSET OF TYPE 2 DIABETES IN RATS

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ABSTRACT

Background: Bariatric surgeries are effective in resolving type-2 diabetes independent of body weight loss. We have used ileal interposition (IT) surgery, a special type of bariatric surgeries, to study the role of the lower intestine in metabolic improvement. The surgery effectively improved glucose tolerance after the rats were treated by low-dose streptozotocin (STZ). However, it is not known whether the surgery could have a similar effect if performed before STZ treatment. Methods: Fourteen male Long–Evans rats received either sham or IT surgery first and then were treated with STZ (35 mg/kg) eleven weeks after the surgeries. Body weight, food intake, body composition and glucose tolerance were measured before and after the surgery. Results: IT surgery improved glucose tolerance before STZ treatment. However, IT surgery did not delay the onset of diabetes as glucose tolerance was not improved four weeks after STZ treatment. No significant difference was found in either body weight or body composition during the experiment. Conclusion: IT surgery can improve glucose tolerance in euglycemic rats without STZ treatment, but IT surgery cannot prevent the onset of diabetes caused by low-dose streptozotocin (STZ).

INTRODUCTION

Surgical procedures referred to as Roux-en-Y in the USA and biliopancreatic diversion in Europe are effective in resolving type-2 diabetes (Sjostrom et al. 2004; Buchwald et al. 2004). Such surgeries produced restriction, malabsorption and increased stimulation of the distal (lower) small intestine (i.e., mainly ileum). The main effect of both restriction and malabsorption is to inhibit food intake and reduce body weight. Early and increased nutrient diversion to the ileum activates endocrine changes because the lower intestine has several unique characteristics. First, the ileum is a major site of producing gut peptides, such as glucagon like peptide-1 (GLP-1) and Peptide YY (PYY) from the enterocytes called "L" cells (Stanley et al. 2004). Second, the peptides, secreted from L cells in the ileum, slow gastrointestinal motility and gastric emptying, a phenomenon known as "ileal brake" (Spiller et al. 1984; Read et al. 1984). Third, 95% of bile acids are taken up at the ileum, and bile acids transporters are exclusively expressed in the ileum (Stelzner et al. 2000; Dawson et al. 2009). Also, bile acids have been shown to improve glucose tolerance both in vitro and in vivo (Thomas et al. 2009; Katsuma et al. 2005; Watanabe et al. 2006; Gerhard et al. 2013). These unique features of the ileum led us to propose the "hind-gut" hypothesis that an increased nutrient delivery to the lower small intestine, mainly ileum, results in the secretion of hormones or factors that act as mediators in diabetic improvement. To isolate the effects of lower intestinal stimulation in the absence of restriction and malabsorption, we utilized a novel surgical model called ileal interposition (or IT surgery).

IT surgery involves the relocation of a short portion of the lower intestine (10 cm in rats), primarily ileum, to a more proximal region at the beginning of the jejunum (Atkinson et al.

1982). The surgery is one type of metabolic/bariatric surgery, but it usually does not cause significant loss of body weight in rats (Cummings et al. 2010; Strader et al. 2009). Previous studies on human subjects with low BMI (below 30) showed improved glucose homeostasis and regression of dyslipidemia when IT surgery was combined with sleeve gastrectomy (De Paula et al. 2006; De Paula et al. 2010; De Paula et al. 2011). IT surgery also improved glucose tolerance and decreased insulin resistance in Long-Evans rats fed with either high-fat diet and STZ (Strader et al. 2005) or chow and STZ (Strader et al. 2009).

Although the remission of diabetes after IT surgery has been documented, there are only a few studies examining the effect of surgery on the prevention of the onset of diabetes. IT surgery can delay diabetes onset in UCD-T2DM (the University of California at Davis type-2 diabetes mellitus) rats (Cummings et al. 2010). Bariatric surgery, as compared with usual care, reduces the long-term incidence of type 2 diabetes by 78% in obese human subjects (Carlsson et al. 2012). However, no one has performed bariatric surgeries on healthy, non-diabetic rats or people to see if the surgeries could prevent the onset of diabetes. Therefore, we performed IT surgery on healthy, non-diabetic rats before the onset or induction of diabetes by STZ to determine if the procedure could delay or prevent the onset of diabetes.

METHODS

Timeline of Experiment

Fourteen male Long-Evans rats (about three months old, Harlan, Indianapolis, IN, USA) were provided with water and chow ad libitum during the experiment. The surgeries (as described below) were done at week 0. An EchoMRI was done (as described below) twice for each rat, one week before and six weeks after the surgeries. At eleven weeks after the surgeries, all rats were injected with streptozotocin (STZ), which is a nitrosourea analog and selectively induces pancreatic beta cell death by alkylation of DNA (Delaney et al. 1995; Elsner et al. 2000). A dose of 35mg/kg STZ intraperitoneally, which specifically destroys 80% of pancreatic beta cells, was used to create type 2 diabetes (Junod et al. 1969; Srinivasan et al. 2005). Oral Glucose Tolerance Test (OGTT) was done twice (as described below), nine weeks and fifteen weeks after the surgeries. OGTT coincided with two weeks before and four weeks after STZ treatment (Fig. 1). The rats were then sacrificed seventeen weeks after the surgeries. For each rat, body weight, food intake and glucose concentration were measured every day after STZ treatment.

Ileal Interposition Surgery

Rats were treated with either sham (n = 6) or ileal interposition (n = 8) surgery as described by Strader (Strader et al. 2009). Rats were anesthetized with isoflurane anesthesia (2%) during the procedure. Briefly, a midline abdominal incision was made and the caecum was externalized. Intestinal transections were made at 5 and 15 cm proximal to the ileocecal valve to isolate a 10-cm segment of ileum. A single anastomosis was made using 7-0 silk suture (Ethicon, Cincinnati, OH) at the site of the segment removal. The segment was laid aside and kept moist with warmed 0.9% saline while the remaining intestines were externalized to locate the Ligament of Treitz. The jejunum was transected 5 cm distal to the ligament of Treitz and the segment was interposed using anastomosis in an iso-peristaltic direction. The intestines were bathed in 0.9% saline and re-inserted into the abdominal cavity. Sham-operated rats were treated with three transections in the same locations as the ileal interposition group, which were immediately re-joined by anastomosis.

Oral Glucose Tolerance Test

The rats were not fed overnight for 16 hrs and then given an oral gavage of glucose (20% D-glucose; 1g/kg). After glucose ingestion, blood glucose in a blood sample from the rat tail was measured by handheld glucometers in duplicate (TheraSense Freestyle Glucometers) at 0, 15, 30, 45, 60 and 120 min.

EchoMRI

Body composition (fat and lean mass) was determined by nuclear magnetic resonance (EchoMRI-900 3-in-1, Echo Medical Systems, Houston, TX). Live conscious rats were inserted into an appropriate Plexiglas animal tube and placed into the EchoMRI machine.

Statistics

All statistics were performed with Prism Statistical Software, and significance was set as * $p < 0.05$. Body weight, food intake and blood glucose concentrations were analyzed by two-way ANOVA with repeated measures. Body composition (fat and lean mass) from EchoMRI was analyzed by *t*-test.

RESULTS

1. Effect of ileal interposition on body weight

Body weights for sham and IT surgery groups were not significantly different during the experiment (two-way ANOVA, F = 0.42, DFn = 7, DFd = 84, P > 0.05; Fig. 2). As we expected, before STZ treatment (at 11th week after surgery), both groups of rats kept increasing their body weights. After STZ treatment, both groups of rats had a trend of decreasing body weights. The statistical powers for ANOVA during the weeks were 0.06, 0.07, 0.13, 0.09, 0.11, 0.11, 0.07 and 0.07 at -1, 6, 9, 11, 12, 13, 14 and 15 weeks, respectively.

2. Effect of ileal interposition on body composition

Fat mass for sham and IT surgery groups was not significantly different either before the surgeries (*t*-test, $t = 1.81$, DFn = 5, DFd = 7, P > 0.05; Table 1) or after the surgeries (*t*-test, $t = 1.54$, DFn = 5, DFd = 7, P > 0.05; Table 1). Lean mass for sham and IT surgery groups was also not significantly different either before the surgeries (*t*-test, $t = 4.17$, DFn = 5, DFd = 7, P > 0.05; Table 1) or after the surgeries (*t*-test, $t = 1.50$, DFn = 5, DFd = 7, P > 0.05; Table 1).

3. **Effect of STZ treatment on food intake**

There was no significant difference in food intake between Sham and IT groups within thirteen days after STZ treatment (two-way ANOVA, F = 0.02, DFn = 1, DFd = 120; Fig. 3). The statistical powers during the weeks were 0.06, 0.13, 0.05, 0.23, 0.11, 0.06, 0.46, 0.05, 0.18, 0.07 and 0.1 at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 days after STZ, respectively. As we expected, the food intake of both Sham and IT groups increased from 17g/day to 40g/day after the STZ treatment (Fig. 3).

4. Effect of ileal interposition on glucose

The IT surgery group showed significantly improved glucose tolerance at the 60 min point during OGTT test (two-way ANOVA, F = 2.39, DFn = 1, DFd = 60, P < 0.05); however, no

significant difference was found at other time points (two-way ANOVA, $F = 2.39$, $DFn = 1$, $DFd = 60$, $P > 0.05$; Fig. 4a). However, no improved glucose tolerance was detected between Sham and IT after STZ treatment, which was also 15 weeks after the surgery (two-way ANOVA, $F = 1.49$, $DFn = 1$, $DFd = 60$, $P > 0.05$; Fig. 4b). The statistical powers for ANOVA were 0.285, 0.336, 0.11, 0.07, 0.05 and 0.07 at 0, 15, 30, 45, 60 and 120 minutes at 15th week after surgery, respectively.

DISCUSSION

"Ileal brake" has been proposed previously (Pironi et al. 1993; Spiller et al. 1984) and is associated with increased secretion of peptide hormones, including GLP-1 and PYY from L-cells located in the ileum. Increased secretion of these hormones may be responsible for the improved glucose tolerance (Strader 2006). The IT surgery has been shown to delay the onset of type-2 diabetes in University of California at Davis Type-2 Diabetes Mellitus (UCD-T2DM) rats. This delay of onset may be related to increased nutrient-stimulated secretion of GLP-1 (7–36) and PYY and improvements of insulin sensitivity, β-cell function and lipid metabolism (Cummings et al. 2010).

We examined whether ileal interposition (IT) surgery can prevent the development of diabetes in healthy, non-diabetic rats. As expected, IT surgery improved glucose tolerance before STZ treatment (Fig. 4a). However, IT surgery did not delay the onset of diabetes as glucose tolerance was not improved four weeks after STZ treatment (Fig. 4b). Our lab previously documented that IT surgery improves glucose tolerance in the same strain of rats (Long-Evans) fed with the same food (chow) (Strader et al. 2009). The only difference between the previous study and this study is which occurs first: STZ or IT surgery. It seems that IT surgery improves glucose tolerance in rats with STZ-induced diabetes, but not in healthy rats. Thus, our results suggest that IT surgery, a special type of bariatric surgery, on a healthy, non-diabetic person may not offer any protective effect from the onset of diabetes.

The mechanism for the lack of a protective effect of IT surgery on healthy rats is unknown. It has been shown that the "jejunized" ileum and ileum adaptation protects against obesity-related comorbidities following IT surgery (Kohli et al. 2010). The lack of a protective effect of IT surgery on the onset of diabetes might be related to adaptation of the interposed ileum. Because healthy Long-Evans rats do not have any glucose tolerance problem, the interposed ileum might be adapted in the nearby healthy jejunum. Therefore, the interposed ileum, if IT surgery occurs before STZ treatment, may not secrete any protective peptide hormones such as GLP-1 and PYY. By contrast, if IT surgery occurs after STZ treatment, the interposed ileum is close to the diabetic jejunum, which keeps activating the interposed ileum, "the brake", to secrete long-term protective hormones. Further studies could be PCR and western blot on the segments of the intestine to examine the gene adaptation and protein secretion levels. The limitations of the study are the small sample sizes of the experiment and the low statistical powers for the tests, and thus we emphasize that the negative results should be interpreted with caution.

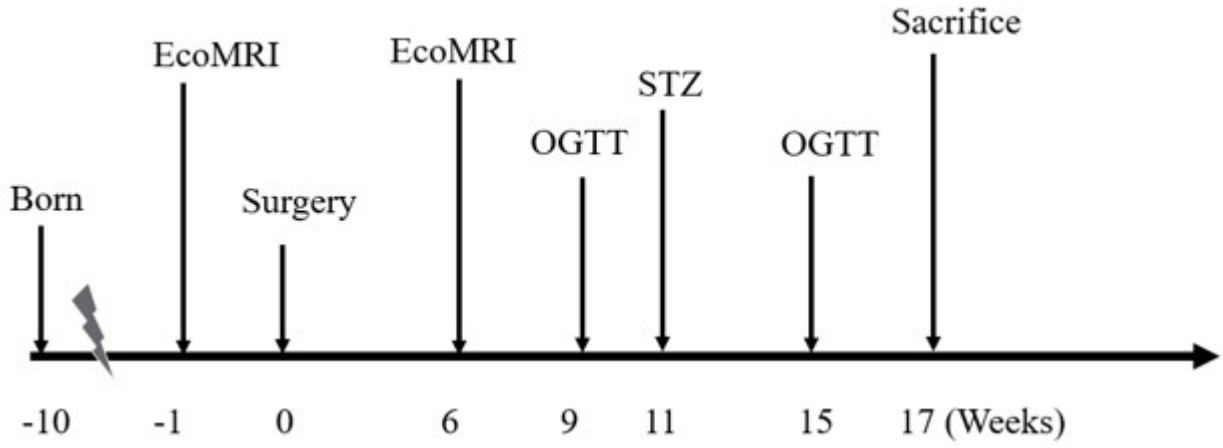

Figure 1: Timeline of experiment (OGTT = Oral Glucose Tolerance Test, STZ = streptozotocin)

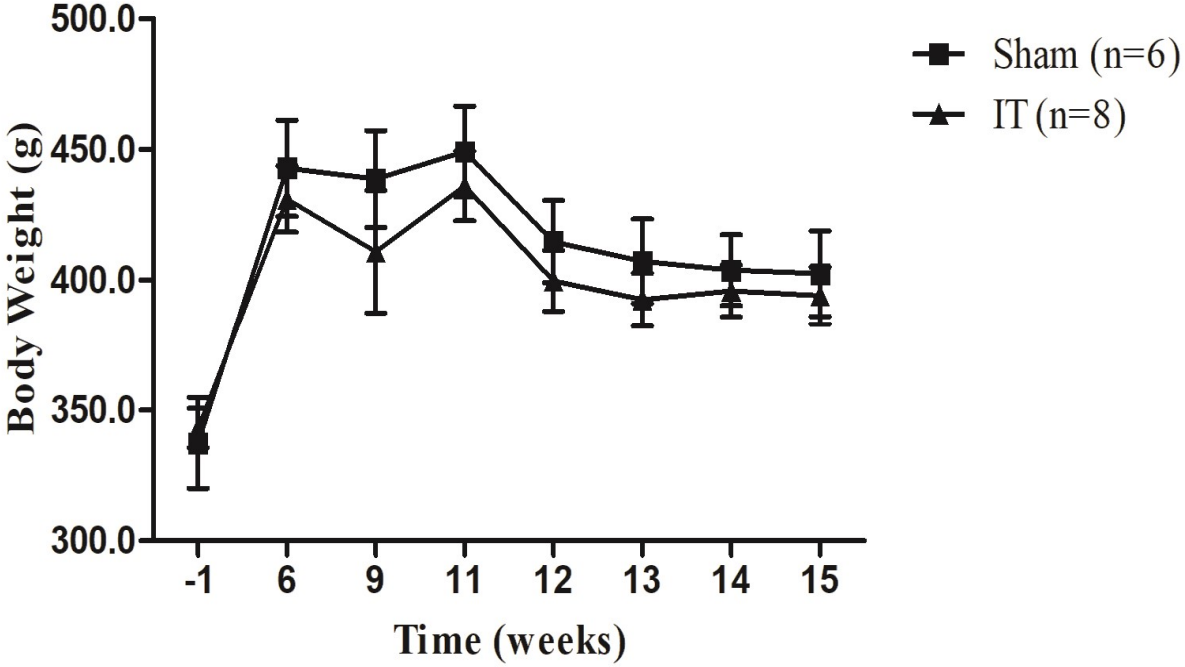

Figure 2: Body weight of Sham and IT rats before and after surgeries.

At 0^{th} week, rats received either Sham or IT surgery, as described in methods. Body weights for sham and IT surgery groups were not significantly different during the experiment (two-way ANOVA, $F = 0.42$, $DFn = 7$, $DFd = 84$, $P > 0.05$). The statistical powers during the weeks were 0.06, 0.07, 0.13, 0.09, 0.11, 0.11, 0.07 and 0.07 at -1, 6, 9, 11, 12, 13, 14 and 15 weeks, respectively. Error bar represents ± standard error of the mean.

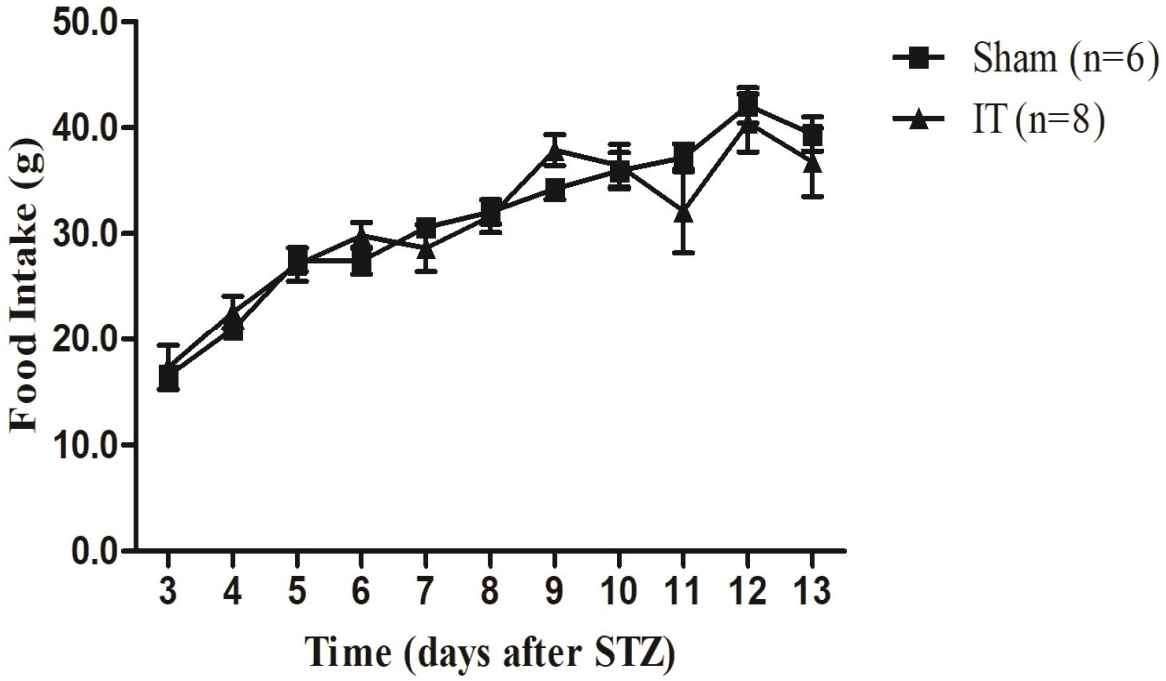

Figure 3: Food intake post STZ treatment (at 11th after surgery)

Food intake was measured daily after STZ treatment in rats for about fifteen days. Food intake amounts for sham and IT surgery groups were not significantly different during the experiment (two-way ANOVA, $F = 0.02$, $DFn = 1$, $DFd = 120$, $P > 0.05$). The statistical powers during the weeks were 0.06, 0.13, 0.05, 0.23, 0.11, 0.06, 0.46, 0.05, 0.18, 0.07 and 0.1 at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 days after STZ, respectively. Error bar represents ± standard error of the mean.

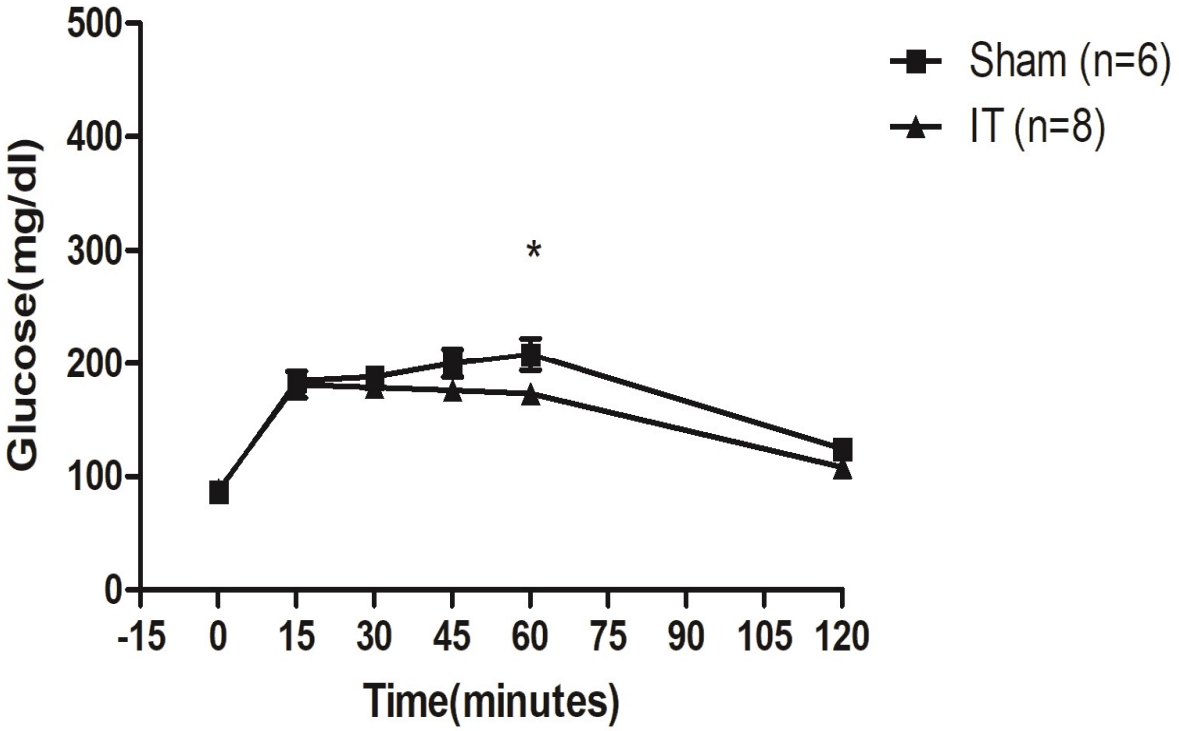

Figure 4a: Blood glucose levels two weeks before STZ treatment (at 9th week after surgery)

At 9th weeks following surgery, an oral glucose tolerance test was performed using 1g/kg oral glucose gavage. Glucose in tail blood was measured by handheld glucometers in duplicate at 0, 15, 30, 45, 60 and 120 min. The IT surgery group showed significantly improved glucose tolerance at 60 min, but not at other times (two-way ANOVA, $F = 2.39$, $DFn = 1$, $DFd = 60$, $P > 0.05$). Error bar represents ± standard error of the mean.

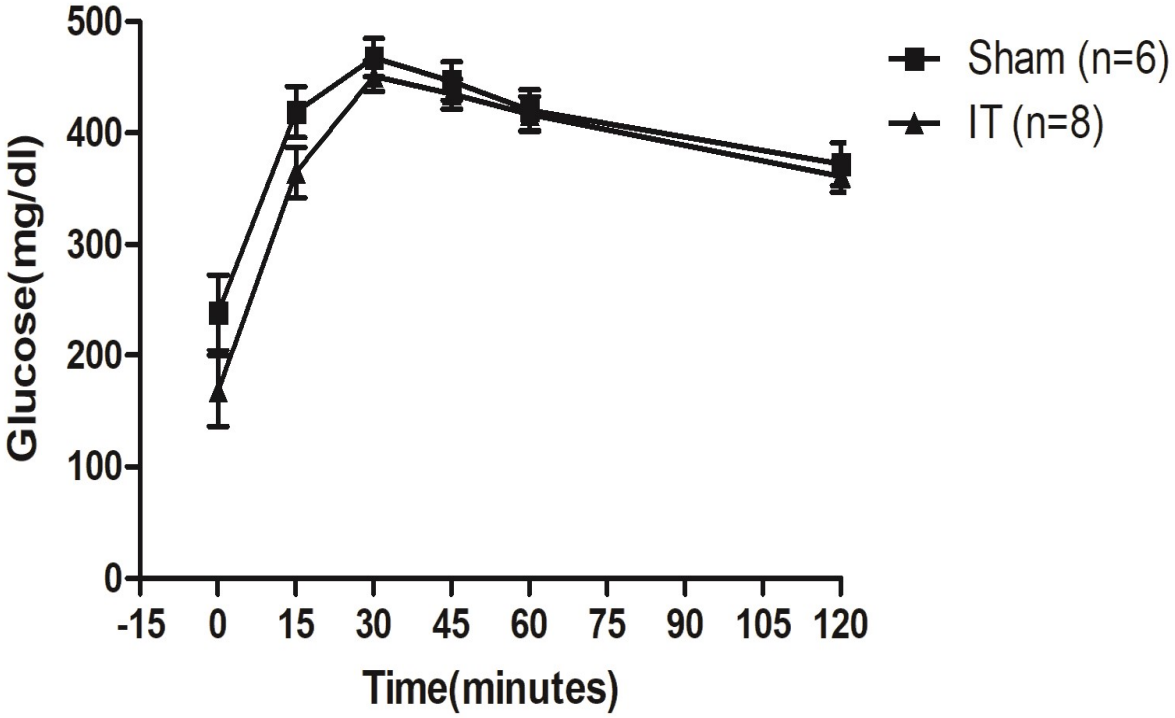

Figure 4b: Blood glucose levels four weeks after STZ treatment (at 15th week after surgery)

At 15th week following surgery an oral glucose tolerance test was performed using 1g/kg oral glucose gavage. Glucose levels for sham and IT surgery groups were not significantly different at 0, 15, 30, 45, 60 or 120 min (two-way ANOVA, $F = 1.49$, $DFn = 1$, $DFd = 60$, $P > 0.05$). The statistical powers were 0.285, 0.336, 0.11, 0.07, 0.05 and 0.07 at 0, 15, 30, 45, 60 and 120 minutes, respectively. Error bar represents ± standard error of the mean.

Table 1: Fat and lean mass (g)

		Sham (Mean ± SD)	IT (Mean ± SD)	P value	*t* value	Statistical power
Fat mass	Before surgery	26.91 ± 3.81	28.55 ± 2.83	0.37	1.81	0.21
	After surgery	50.6 ± 10.53	55.32 ± 13.08	0.48	1.54	0.09
Lean mass	Before surgery	258.8 ± 37.21	264.1 ± 18.22	0.73	4.17	0.17
	After surgery	309.43 ± 30.15	300.75 ± 24.65	0.56	1.50	0.14

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