Research Article

Thyrotropin Releasing Hormone (TRH) Restores Pancreatic Islet Function in Diabetic Male Mice by Facilitating Bone Marrow Migration

LuGuang Luo1,3*, William Newton1, Joseph Kim1, and John Luo2

1The Center of Stem Cell Biology, Department of Medicine, Roger Williams Hospital, Boston University School of Medicine, USA
2Insure Health Inc., USA
3The Center for Natural Healing in Rhode Island, USA

*Corresponding author: LuGuang Luo, The Center of Stem Cell Biology, Department of Medicine, Roger Williams Hospital, Boston University School of Medicine, Providence, RI 02908, USA, E-mail: lgluo@BU.edu, mycenterfornaturalhealing@gmail.com

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Abstract

Diabetes mellitus type 1 (T1D) is characterized by the loss of pancreatic islet β-cells. Thyrotropin-releasing hormone (TRH) is a small neuropeptide classically known for its role as a regulator of thyroid function. We and other groups have previously found that TRH is expressed in the pancreas and can correct hyperglycemia through pancreatic islet β cells. Previous in vivo and in vitro experiments have shown that β cell viability and function are improved after bone marrow transplant. Based on previous data, we hypothesize that TRH regulates blood glucose by stimulating bone marrow (BM) mediated islet β cell recovery. STZ-induced T1D rats were treated with weekly TRH injections for 65 days. We tracked their blood glucose levels and later excised their pancreases for anti-insulin staining to assess islet morphology. T1D mice were transplanted with GFP-labeled bone marrow and treated with TRH. Immunofluorescence was used to assess if BM infiltrated the islets and restored their function. Our results confirmed that TRH injections reduced hyperglycemia, and increased islet cross sectional area in T1D rats. In the mouse model, TRH caused BM to migrate to the pancreatic islets and reduced fasting blood glucose levels in T1D treatment groups. This suggests that TRH may improve islet function by recruiting BM stem cells to stimulate β cell regeneration. TRH could perhaps be used as a novel treatment from T1D. Treating damaged islets with TRH could improve β cell function, reducing a patient’s dependence on exogenous insulin.

Keywords: diabetes mellitus, thyrotropin-releasing hormone, islets of langerhans, bone marrow mesenchymal stem cells, islet neogenesis

Introduction

Diabetes mellitus type-1 is an endocrine disorder characterized by the autoimmune destruction of insulin-producing islet β cells, and consequently chronic hyperglycemia. This incurable disease presents itself during childhood, and its sufferers are dependent on daily treatments of insulin and other medications for the remainder of
their lives to control the disease’s deadly complications [1]. As a result, the financial burden of T1D on patients and the global healthcare system is enormous and increasing as the disease becomes more and more prevalent. Despite the significant cost of these treatments, in many cases, T1D’s symptoms still cannot be fully controlled, and the global mortality rate of this disorder remains high [2,3]. The increasing global incidence of T1D has placed the development of novel treatment techniques that reduce patient dependence on insulin and pharmaceutical medications in high demand. One potential avenue of treatment is the regeneration of islet β cell tissue to increase insulin secretion.

Thyrotropin-releasing hormone (TRH) is a small neuropeptide first found in the hypothalamus. TRH is classically known for its role as a stimulator of thyroid stimulating hormone (TSH) release as part of the pituitary-thyroid axis [4]. TRH has also been found to play an active role in blood glucose regulation, alleviating hyperglycemia in animal models of diabetes mellitus [5-8]. Due to the initial discovery of TRH within the hypothalamus, it was first believed that this effect is mediated by the central nervous system (CNS). However, later experiments using hypophysectomized animals showed similar TRH associated glucose regulation [9], suggesting an alternative mechanism for TRH’s involvement in pancreatic function. Another study made the observation that targeted disruption of the TRH gene in mice causes hyperglycemia that is not reversed by exogenous thyroid hormone injections [10]. This suggests that TRH regulates blood glucose via a mechanism distinct from TSH activation. TRH has since been shown to be expressed in a number of different organs outside of the CNS, including the pancreas [11,12], where it is thought to play a role in islet development [13,14].

In our previous study we demonstrated that treating STZ induced diabetic rats with TRH preserved β cell insulin secretion and significantly reduced hyperglycemia. Histochemical analysis of the rats’ islets also revealed that TRH reduced β cell apoptosis and stimulated regeneration of the islet tissue [7]. This data supports a previous report suggesting that TRH has a protective effect on islet function [9], while also implying that TRH has the ability to regenerate islet β-cell loss. Brdu and nestin staining revealed that cellular proliferation was occurring in pancreatic islets treated with TRH [7]. In previous studies, we found that BM stem cells are able to similarly stimulate islet β cell recovery, increasing insulin secretion [15-18]. We propose that the delivery of TRH might stimulate BM stem cells migrate to sites of pancreatic damage and promote islet β cell regeneration.

The goal of this study was to confirm TRH’s regenerative effect on islet β cell function, and to determine whether BM migration is the cause of this effect. To this end, we injected streptozotocin (STZ)-induced T1D rat and mouse models with TRH and monitored their fasting blood glucose levels (BGL) over the course of two months. Pancreatic tissue was excised and evaluated for any evidence of β cell recovery and BM co-localization within the islets.

Materials and Methods

Animal models

All use of live animals was approved by the Roger Williams Medical Center Institutional Animal Care and Use Committee.

Rats: 8-week-old male Sprague–Dawley rats weighing 180-200 g were obtained from Charles River Labs following IACUC-approval. The rats were randomly sorted into 4 categories: saline control (C, N=4), TRH only (T, N=2), STZ only (S, N=2), and TRH with STZ (TS, N=5). Rats were housed in individual cages in the Roger Williams Medical Center Animal Care Facility (ACF) with cage cards identifying each specimen’s assigned treatment group, and ear punches denoting the individual animals. Note that the number of rats in each experimental group is not uniform because some of the rats passed away during the course of the experiment.
**Mice:** 8-week-old male C57B1/6NCrl (C57B1) and C57B1/6-TgN (ACTb EGFP)1Osb (C57B1-EGFP) mice, weighing 15-25 g were obtained from Jackson Laboratories (Bar Harbor, ME). The C57B1 mice were randomly sorted into 3 categories: control (C, N=5), STZ only (S, N=5), and TRH with STZ (TS, N=5). All mice were housed in cages in the Roger Williams Medical Center Animal Care Facility (ACF) with cage cards identifying each specimen’s strain and assigned treatment group, and ear punch denoting each individual animal.

C57B1-EGFP is a transgenic strain of C57B1 mouse that expresses enhanced green fluorescent protein (EGFP) cDNA, causing all tissues, with the exception of erythrocytes and hair, to fluoresce green under excitation. All C57B1-EGFP mice (N=4) were sacrificed and used as BM donors for the C57B1 mice.

**Bone marrow transplantation**

The donor C57B1-EGFP donor mice were sacrificed by cervical dislocation under a dose of 60 mg/kg body weight (BW) pentobarbital solution (Sigma: St. Louis, MO) anesthesia. The femur, tibia, and pelvic bones were excised. BM was flushed out of the bones using a syringe and a 22-gauge needle containing PBS and 5% heat-inactivated fetal calf serum (HI-FCS). After re-suspension of the BM in PBS without HI-FCS, cells were passed through a 40 µm cell strainer. Cell numbers were counted in crystal violet and viability was assessed by trypan blue staining [17].

Prior to BM transplantation, all C57B1 mice were subjected to fractional total body irradiation (TBI) at a total dosage of 500 cGy (100 cGy/min) to induce total bone marrow destruction. TBI was applied using a photon producing linear accelerator (Elekta: Stockholm, Sweden). 20 million EGFP labeled BM cells harvested from the C57B1-EGFP donor mice were transplanted into each of the irradiated C57B1 mice via the tail vein.

**Induction of type-I diabetes**

**Rats:** Rats assigned to the S and TS groups were injected intraperitoneally (IP) with STZ (65 mg/kg of BW) [19]. Rats in the T and C groups were injected IP with normal saline. On day 3 following injection, a diabetic condition was confirmed in all rats of the STZ injected groups based on a fasting BGL of > 300 mg/dL.

**Mice:** 10 days after BM transplantation, mice assigned to the S and TS groups were injected with STZ (200 mg/kg BW, IP) to induce a T1D-like state of pancreatic β cell destruction and hyperglycemia. On day 3 following injection, a diabetic condition was confirmed in all mice of the STZ injected groups as defined by a fasting BGL > 300 mg/dL.

**TRH and glucose**

**Rats:** All rats assigned to the T and TS groups were injected IP with TRH (pGlu-His-ProNH2: SIGMA: St. Luis, MO) at a dosage of 10 µg/kg BW once per week, for 65 days starting on day 3 after the initial STZ or saline injection. All injections were given IP. The BGLs of all rats were measured at 13-time points from day 0 to day 65 following STZ injection. Blood glucose was measured after 12 hours of fasting using an Accucheck Blood Glucose Meter (Roche Diagnostics Corporation, IN) from approximately 10 µl of peripheral blood drawn from the tail vein of each animal.

**Mice:** All mice assigned to the T and TS groups were injected IP with TRH (pGlu-His-ProNH2, Sigma: 20 µg/kg bwt) once per week, from day 18 to day 49 following BM transplant. All injections were given IP. The BGLs of all rats were measured at 10-time points from day 3 to day 49 following BM transplant. Blood glucose was measured after 12 hours of fasting using an Accucheck Blood Glucose Meter (Roche Diagnostics Corporation, IN) from approximately 10 µl of peripheral blood drawn from the tail vein of each animal.
Animal sacrifice and sample collection

On day 65 and 50 of each respective study, all rats and mice were anesthetized by cervical dislocation under sodium pentobarbital anesthesia (60 mg/kg BW, Sigma, St. Louis, MO) injected IP. Following confirmation of death, each animal’s pancreas was collected through an abdominal incision.

Excised pancreatic specimens were placed in freshly prepared PLP fixative solution (balanced phosphate solution with 2% paraformaldehyde, sodium m-periodate and L-lysine) for 2 hours at 4°C, with frequent agitation. Samples were then washed in a 7% sucrose buffer overnight followed by a 15% sucrose buffer wash for 2–3 hours and 25% sucrose plus 10% glycerol buffer wash for another 2 hours, all at 4°C. They were then rinsed in PBS and embedded in tissue freezing medium (OCT), frozen and stored at −80°C until ready for analysis. Before analysis, pancreases were sectioning into 5-micron cryosections [7,17].

Islet immunohistochemistry

Rat pancreas samples were analyzed by immunoperoxidase staining of paraformaldehyde-fixed slides using avidin–biotin-peroxidase detection (ABC Elite, Vector Laboratories, Burlingame, CA) as previously described [7]. Normal goat blocking serum at 1:100 dilutions in 3% PBS–BSA (Sigma Chemical Co., St. Louis, MO) was applied. The primary mouse anti-insulin monoclonal antibody 1:50 in 1% PBS–BSA (CHEMICON, Temecula, CA) was incubated with each section overnight at 4°C. After washing, the sections were incubated with the biotinylated secondary antibody (1:5000 dilution) for 30 min at room temperature, followed by avidin–biotin-peroxidase complex (diluted 1:25 in PBS) for 60 min. 3,3’-Diaminobenzidine tetrahydrochloride and hydrogen peroxide (Sigma, St. Louis, MO) provided the peroxidase. Once desired stain intensity was reached, the slides were washed with dH₂O.

Islet immunofluorescence

Mouse pancreases were sectioned as above. Pancreatic sections underwent immunofluorescence to evaluate changes in islet morphology and to assess BM migration. Islet immunofluorescence was done according to a previously described protocol [20]. Briefly, antigen retrieval was accomplished using citrate buffer, and blocked with ammonium chloride, 2% glycine, and phosphate-buffered saline, pH 7.4 (PBS). Pancreatic sections were incubated with mouse anti-insulin monoclonal antibody (CHEMICON, Temecula, CA) diluted 1:50 in blocking buffer (PBS/1% bovine serum albumin, BSA). Incubations were conducted overnight at 4°C. The samples were then incubated for 1 h at room temperature with goat anti-guinea pig IgG Texas Red (Abcam: Cambridge, UK) secondary antibody diluted 1:50 in PBS/1% BSA. After rinsing in PBS, the slides were mounted with DAPI for nucleic acid staining (Sigma-Aldrich; St. Louis, Missouri) and rinsed clear with distilled H₂O.

Image analysis

GFP expression and antigens labeled by fluorescence-conjugated antibodies were visualized by fluorescent microscopy (Axiovert w 135, Carl Zeiss, Oberko-chen, Germany). NIH ImageJ was used to digitally isolate individual islets and calculate their cross-sectional areas within each rat pancreatic section slide. In Immunofluorescence-labeled mouse pancreatic cross sections, the number of GFP positive islets and the total islets in each section were counted. The ratio between GFP positive islets and the total number of islets was determined.

Statistical analysis

All data is presented as means ± their standard deviations. Student’s t test with a two-tail distribution was used to determine the significance of differences in BGLs at each time point between the different animal treatment groups. Significance is defined by a p-value <0.05. χ² tests were performed to determine the significance of any detected BM migration. The data for blood glucose was analyzed by ANOVA statistical program. Analysis was
Results

TRH reduces hyperglycemia in diabetic rats

On the third day after STZ injection, all SD rats in the S and TS groups displayed BGLs greater than 400 mg/dL, indicating a diabetic state. Meanwhile, as expected, the T and C groups displayed serum glucose levels around 100 mg/dL. Weekly TRH injections significantly ameliorated hyperglycemia in the TS rat group, bringing the rats’ mean BGLs down from a mean concentration of 454.2 ± 99.7 mg/dL on day 3 post STZ to 218.2 ± 66.8 mg/dL on day 65 post STZ. The reduction in blood glucose was first significant on day 9 and continued to decrease for the duration of the experiment. The S group ended the experiment with a mean BGL (482 ± 40.7 mg/dL) significantly higher than the STZ & TRH group (218.2 ± 66.8 mg/dL) (p<0.05), remaining over 400 mg/dL for the entire duration of the study. The T group and saline control groups showed no significant change in blood glucose over the course of the experiment. Neither group’s mean blood glucose concentration was significantly different from one another at any point in the study, but both maintained significantly lower glucose levels compared to the S and ST groups at all time points during the study. Figure 1 tracks the mean BGLs of each rat over the course of the experiment after STZ injection. Figure 2 displays this data as a table and cites the significance of differences between each group. These observations confirm our previous observations that can ameliorate hyperglycemia in the rat model [17].

Figure 1: Average Serum Glucose in Rat Exposed to TRH, STZ, and STZ & TRH. Rats are placed in four groups: control (C), TRH (T), STZ only (S), and streptozotocin with TRH (TS). The serum glucose of the rats was measured on specific days for a total of 65 days. Groups C and T maintained similar serum glucose levels within the healthy range. Rats exposed to streptozotocin experienced hyperglycemia. TS mice also experience hyperglycemia, but the hyperglycemia was statistically lower than that of the S group.

TRH has a protective and proliferative effect on pancreatic islets

Cross sections of rat pancreases were observed under 40X magnification. Immunohistochemistry was performed on the cross-sections using anti-insulin antibodies. From the portions of each image that were stained red, we were able to identify individual islets. A random selection of islets was chosen for analysis using NIH ImageJ to calculate the cross-sectional islet area. Islet cross-sectional area was measured because it roughly correlates with islet mass, which correlates with insulin release. The results are presented in figure 2.

The mean cross-sectional islet area of group C, which was treated with neither STZ nor TRH, was 370.03 ± 314.97 µm². The mean cross-sectional islet area of each treatment group relative to C is displayed in figure 2. As expected, rats from the S group displayed the smallest mean cross-sectional area (164.23 ± 156.19 µm²), presumably
due to the majority of the islet cells having undergone necrosis as a result of STZ exposure. The T group had the highest mean cross-sectional area \( (2093.21 \pm 1591.63 \mu m^2) \), with a \( 566 \pm 440\% \) increase relative to the untreated control. The magnitude of this improvement is surprising and supports our earlier findings that TRH not only protects the islets from damage but promotes \( \beta \) cell proliferation as well [7]. The TS group had a mean cross-sectional area of \( 672.5 \pm 425.34 \mu m^2 \), a significant increase relative to the islet cross-sectional areas of C \( (p<0.02) \) and S \( (p<0.001) \), but reduced relative to T. This signifies that TRH was able to preserve the islet cells from STZ-induced damage and promote even further growth. Morphologically, islets from the TS group appeared fragmented, made up of smaller clusters of IPCs, as opposed to a single mass (Figure 2D). This could be indicative of islet neogenesis, or simply the result of islet disassociation from the STZ.

![Image](image_url)

**Figure 2:** Insulin immunohistochemistry was conducted on islets from each rat treatment group. A) An islet from a control rat. B) An islet from a rat treated with STZ only. C) An islet treated with TRH only. D) An islet treated with STZ and TRH. The table compares the mean islet cross sectional areas of each treatment group relative to the untreated control group along with the significance of any differences.

**TRH combined with a bm transplant reduces hyperglycemia in diabetic mice**

Although radiation and BM transplantation initially heightened BGLs in subjected mice (not shown), by day 3 after transplant the mean glucose levels of each treatment group had normalized to \( \sim 100 \) mg/dL. On day 10 after BM transplant, STZ was injected into the mice of the S and TS groups. By day 18 both groups displayed mean blood glucose concentrations of \( >300 \) mg/dL, indicating a diabetic state. Starting on day 18, TRH was administered to the mice in the TS group once per week. Similarly, to the T1D rats, the weekly TRH injections corresponded with a reduction of serum glucose in the TS group. By day 49 the BGL of the TS group had fallen to \( 264 \pm 25.3 \) mg/dL, compared to \( 384 \pm 37.4 \) mg/dL on the day of initial TRH injection. By contrast, no overall BGL decrease was observed in mice of the S group, which exhibited a final BGL of \( 395 \pm 38.5 \) mg/dL, a value significantly \( (p<0.05) \) higher than that of the TS group. As with the rats, TRH did not completely cure the hyperglycemia of the TS mice. The day 49 BGL of the TS group was significantly \( (p<0.05) \) higher than that of the C group (156). A longer study would reveal whether or not continued TRH injection would eventually completely eliminate hyperglycemia in STZ-injected animals. Table 1 and Figure 3 show the BGL of each mouse group at each time point from day 3 to day 49 post BM transplant.
Table 1: Mean Serum Glucose Levels Over Time in BM Transplanted Mice Treated with STZ (S), TRH+STZ (TS), and Neither (C)

<table>
<thead>
<tr>
<th>Mean Serum Glucose (mg/dL)</th>
<th>Day 3</th>
<th>Day 10 (STZInj)</th>
<th>Day 12</th>
<th>Day 18 (TRH)</th>
<th>Day 21</th>
<th>Day 23</th>
<th>Day 24</th>
<th>Day 26</th>
<th>Day 29</th>
<th>Day 33</th>
<th>Day 38</th>
<th>Day 40</th>
<th>Day 49</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>85.5</td>
<td>108</td>
<td>147</td>
<td>119</td>
<td>112</td>
<td>111</td>
<td>147</td>
<td>79.5</td>
<td>163</td>
<td>156</td>
<td>106</td>
<td>163</td>
<td>156</td>
</tr>
<tr>
<td>S</td>
<td>84</td>
<td>80</td>
<td>293</td>
<td>359</td>
<td>447</td>
<td>486</td>
<td>534</td>
<td>497</td>
<td>412</td>
<td>395</td>
<td>385</td>
<td>412</td>
<td>395</td>
</tr>
<tr>
<td>TS</td>
<td>88</td>
<td>101</td>
<td>439</td>
<td>384</td>
<td>451</td>
<td>445</td>
<td>449</td>
<td>297.5</td>
<td>277</td>
<td>264</td>
<td>387</td>
<td>277</td>
<td>264</td>
</tr>
</tbody>
</table>

TRH influences BM localization and transdifferentiation within mouse pancreatic islets

Through the transplantation of EGFP-tagged BM into the C57B mice, we were able to detect the localization of BM within mouse pancreatic islets in slide-fixed pancreas sections. Anti-insulin staining with Texas Red was used to identify islets, and the slides were treated with DAPI to label cellular nuclei. In the resulting fluorescent images, green = EGFP transfected BM; blue = cellular nuclei; red = insulin; yellow = EGFP positive cells that express insulin. Figure 4 shows example images of one islet from each treatment group. We counted the number of islets co-localized with BM in pancreatic cross sections taken from each treatment group and compared them to the total number of islets within each cross section.

Our results show very little BM infiltration within islets from the C group (Figure 3B). Of the islets detected within the C cross sections, only 20% showed GFP expression indicative of BM localization. No yellow staining appeared in either of the GFP+ islets which would indicate BM transdifferentiation. In the S group (Figure 3C), a very small number of islets were detected (3) with red insulin staining, and no significant BM co-localization or transdifferentiation was observed in any detectable islets. Finally, in the pancreatic cross section of a mouse from the TS group (Figure 3D), very few insulin expressing islets remained after STZ treatment, but GFP staining was detected in 100% of islets identified. The presence of yellow staining within these islets indicated the presence of BM-derived cells that expressed insulin. This suggests that the transplanted BM had transdifferentiated into IPCs.
Figure 3: Effect of TRH on Blood Glucose of Damaged Pancreatic Mice with Bone Marrow Transplantation. BM transplanted mice are placed in 3 groups: control (C), STZ only (S), and streptozotocin with TRH (TS). The serum glucose of the rats was measured on specific days for a total of 65 days. Group C maintained serum glucose levels within the healthy range. Rats exposed to streptozotocin on day 10 displayed hyperglycemia. TS mice’s hyperglycemia stabilized after the first TRH injection on day 18 and began dropping on day 24 to a level significantly lower than the S group by day 49.

Figure 4: Islets from each treatment group were stained for immunofluorescent imaging. Green = Bone Marrow (BM), Red = Insulin, Blue = Cellular Nuclei Yellow = Insulin Producing BM. A) An islet from a healthy mouse that was not subjected to GFP+ Bone Marrow (BM) transplantation. A large amount of insulin producing cells were present (white arrow). B) An islet from a mouse transplanted with EGFP+ BM. Both Insulin (large arrow), and EGFP+ BM (small arrow) were present. C) An islet from a STZ-induced T1D mouse without TRH treatment. Insulin producing cells are nonexistant, and minimal BM localization (white arrow) has occurred. D) STZ-induced T1D mice treated with TRH injections. Yellow staining is present which suggests BM transdifferentiation (white arrow). The table shows the proportion of EGFP+ cells to EGFP- islets in a pancreatic cross section from each treatment group.

χ² analysis of the total data set indicates that there is an association between the experimental group and the percentage of islets that are GFP positive (p<0.05). A comparison between STZ & TRH and STZ indicates that there is an association (p<0.05). A comparison between STZ and control did not reveal an association.
Discussion

TRH has been established as an important mediator of pancreatic islet development, with its expression in the developing pancreas directly correlating with insulin secretion [14]. The mechanism by which TRH influences islet development is unknown. One possibility is that TRH promotes the mobilization of stem cells and progenitor cells into β cells, which support islet β cell formation by inducing replication or by transdifferentiation. Experiments using BM have demonstrated that stem cell transdifferentiation into IPCs can occur in adult islets [17]. TRH has been shown to increase insulin secretion and reduce hyperglycemia in diabetic animal models by promoting islet recovery through a mechanism involving cellular differentiation [6,7]. The results of our study demonstrate a clear correlative link between TRH and islet neogenesis by way of BM transdifferentiation.

In the T1D SD rat model used in this study, we confirmed our previous findings [7] that TRH could ameliorate hyperglycemia caused by STZ-induced islet damage. We also demonstrated that this effect can be maintained and further improved through weekly TRH injections delivered over the course of 65 days (Figure 1, Table 2). In addition, anti-insulin stained pancreatic cross-sections taken from these rats showed a highly significant increase in islet cross sectional area in all groups treated with TRH compared to the control islets (Figure 3). Even in rats exposed to STZ, TRH recovered the islets to a mean cross-sectional area greater than that of the control (p<0.02). This increased islet area, coupled with enhanced glucose sensitivity, is indicative of β cell regeneration. The appearance of multiple small islet-like masses in the TS group islets is a characteristic suggestive of islet neogenesis [13]. This supports our hypothesis that neogenesis, rather than β cell replication, is the cause of this regeneration [13].

Table 2: Mean Serum Glucose Levels Over Time in Rats Treated with STZ (S), TRH (T), STZ+TRH (TS), and Neither (C)

<table>
<thead>
<tr>
<th>Day</th>
<th>C (mg/dL)</th>
<th>T (mg/dL)</th>
<th>S (mg/dL)</th>
<th>TS (mg/dL)</th>
<th>T vs. C</th>
<th>S vs. C</th>
<th>S&amp;T vs. S</th>
<th>S&amp;T vs. C</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>100.75± 8.26</td>
<td>101.5 ± 2.1</td>
<td>99.8 ± 5.1</td>
<td>101.6 ± 8.7</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>N/S</td>
<td>N/S</td>
</tr>
<tr>
<td>3</td>
<td>104.5 ± 5.68</td>
<td>107.5 ± 7.8</td>
<td>433.6 ± 47.3</td>
<td>454.2 ± 99.7</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>N/S</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>9</td>
<td>95.75 ± 15.92</td>
<td>95.5 ± 10.6</td>
<td>320 ± 52.5</td>
<td>286.4 ± 181.8</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
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<tr>
<td>16</td>
<td>97 ± 7.44</td>
<td>82 ± 1.4</td>
<td>393.8 ± 30</td>
<td>298 ± 136</td>
<td>N/S</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
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<tr>
<td>23</td>
<td>90.5 ± 6.03</td>
<td>89 ± 5.6</td>
<td>463.8 ± 62.6</td>
<td>376.8 ± 37.6</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>30</td>
<td>78.25 ± 5.12</td>
<td>74.5 ± 0.7</td>
<td>522.4 ± 26.9</td>
<td>386.4 ± 27</td>
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<td>&lt;0.001</td>
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<tr>
<td>40</td>
<td>96.75 ± 13.7</td>
<td>80 ± 4.2</td>
<td>537.2 ± 23.5</td>
<td>269.2 ± 23.5</td>
<td>N/S</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
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<tr>
<td>44</td>
<td>81.5 ± 9.7</td>
<td>90.5 ± 2.1</td>
<td>466.8 ± 17</td>
<td>259.6 ± 17.8</td>
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<td>48</td>
<td>83.75 ± 9.7</td>
<td>89 ± 11.3</td>
<td>489.4 ± 41</td>
<td>289.8 ± 26.3</td>
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<td>485.2 ± 5</td>
<td>287.8 ± 70.8</td>
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<td>&lt;0.001</td>
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<td>460.75 ± 50.7</td>
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<td>453.5 ± 42.9</td>
<td>173.6 ± 28.2</td>
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<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>61</td>
<td>90.5 ± 8.4</td>
<td>67.5 ± 0.7</td>
<td>541.75 ± 42.8</td>
<td>200 ± 32.1</td>
<td>N/S</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>65</td>
<td>79.25 ± 4.5</td>
<td>85 ± 14.1</td>
<td>482 ± 40.7</td>
<td>218.2 ± 6.8</td>
<td>N/S</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

To determine whether islet neogenesis occurred, we used STZ-induced T1D C57Bl mice transplanted with EGFP+ BM. We changed to the mouse model because of the unavailability of EGFP-labeled rat strains and because mouse BM transplantation is a more well characterized procedure. The EGFP label allowed us to observe BM co-localization with islets and to detect any transdifferentiation that might result in β cell neogenesis. The STZ diabetic
mice followed the same general pattern of BGL reduction following weekly TRH injections, although it did take longer for this reduction to occur, perhaps due to the 8 day wait between STZ injection and the first TRH treatment. This data supports the hypothesis that TRH can restore islet function in cases of T1D.

As our results show, the pancreatic section examined from the TS group only possessed 2 detectable islets (stained with red anti-insulin), but both islets expressed EGFP indicative of BM co-localization. In these islets, yellow fluorescence was visible, indicating the presence of BM-derived cells that produced insulin (Figure 3D). This is an expected characteristic of BM stem cells that had transdifferentiated into IPCs. The islet population of the S group cross-section was similarly low (3), but none of the islets in that group contained transdifferentiated insulin producing BM cells. In the pancreatic cross section examined from the C group, 10 islets were detected, 2 of which were EGFP+. Of these 2 EGFP+ islets, none expressed yellow fluorescence that would indicate transdifferentiation.

The presence of EGFP fluorescence within the islets of the TS group was expected if our hypothesis that TRH induces BM migration to pancreatic islets is correct. Our hypothesis is further supported by the low frequency of BM co-localization in islets from the other two groups, neither of which was treated with TRH. The low population of detectable islets in the TS group can be explained by the 8-day delay between STZ administration and the first injection of TRH. During this time period, the majority of the mice’s islets would have undergone necrosis. The same was expected and observed in the S group. The morphology of the differentiated BM cells in the TS group was interesting. The IPCs were arranged in several small clusters, rather than a single mass. Such morphology has been proposed to be characteristic of islet neogenesis [13]. It is possible that, given time, each IPC cluster would develop into a new pancreatic islet.

The expression of yellow fluorescence within the TS mouse islets was interesting. Yellow fluorescence is indicative of BM derived cells that express insulin. In conjunction with the improved glucose metabolism observed in these mice, this suggests that TRH might cause islet neogenesis by promoting BM transdifferentiation into β cells. In nature, islet neogenesis is known to be one of the leading drivers of embryonic and neonatal islet development [21]. Islet neogenesis in the adult pancreas, however, is controversial [13]. Our lab has previously observed the transdifferentiation of BM stem cells in to IPCs, though in that instance cytokines were used to induce the process [17]. Despite improved blood glucose regulation in the T1D mice, it is not clear from our results whether or not the IPCs transdifferentiated from the BM cells are glucose sensitive, and thus as functional as β cells. Further studies testing the response of TRH-treated T1D islets to high glucose challenge would prove useful in that regard. In addition, the mechanism used by TRH to incite stem cell migration and transdifferentiation remains unknown.

In summation, we found that TRH can regenerate the pancreatic islets of STZ-induced diabetic rats and mice. Furthermore, through unknown means, TRH caused BM to migrate to STZ-damaged pancreatic islets and transdifferentiate into IPCs. It is possible that this transdifferentiation results in β cell neogenesis, restoring β cells lost due to T1D. If its effect on islet function can be fully established, TRH could prove to be an effective therapeutic approach to regenerate the islets of T1D patients, reducing their dependence on exogenous insulin.

**Conflict of Interest**

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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