

# Evaluating the Use of a Glucagon-Like Peptide-1 Agonist as a Means of Increasing Pancreatic Islet Viability



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## Abstract

Diabetes Mellitus is one of the most common endocrine disorders and affects individuals worldwide, it occurs in the form of Type 1 (T1D) or Type 2 (T2D) diabetes. Islet Amyloid Polypeptide (IAPP) or Amylin, is involved in the pathology of T2D and contributes to the failure of pancreatic islet grafts in T1D.<sup>1</sup> Through varying mechanisms, amylin formation partakes in the progressive loss of  $\beta$ -cell mass in islet grafts associated with T1D.<sup>1</sup> This project aimed to address the loss of  $\beta$ -cell mass by culturing pancreatic islets *ex vivo* in elevated glucose in order to stimulate amyloid formation, and treating them with exenatide (a glucagon-like peptide-1 receptor agonist).

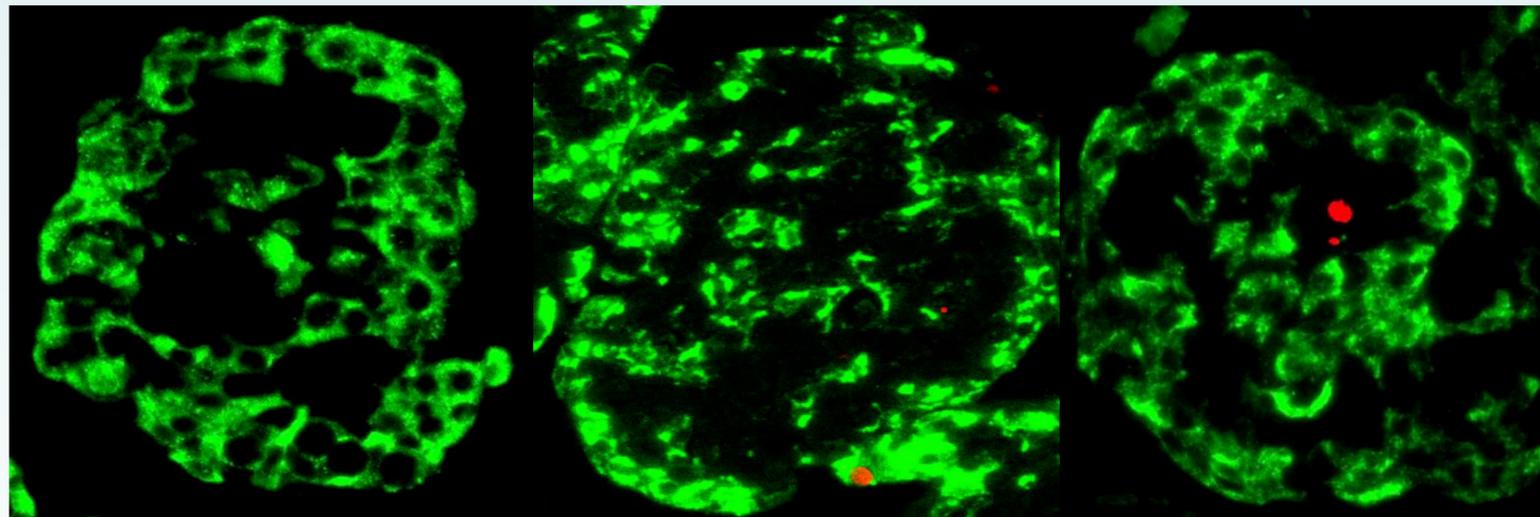
## Introduction

Amyloids are fibrillar protein aggregates that are rich in  $\beta$ -sheet structures and are involved in the pathology of more than 30 different human disorders, including T2D.<sup>2</sup> IAPP is a 37-amino acid peptide and is co-secreted with insulin from the pancreatic  $\beta$ -cells.<sup>3</sup> Like insulin, it is secreted in response to  $\beta$ -cell secretagogues, including glucose.<sup>2</sup> T2D is often onset in adulthood and is characterized by the inability of the peripheral tissues to respond to insulin and ultimately for  $\beta$ -cells to produce it.<sup>3</sup> T2D is an example of a local amyloidosis disease, in which islet amyloid polypeptide aggregates locally in  $\beta$ -cells.<sup>2</sup> The aggregation plays an important role in the decline of  $\beta$ -cell mass and function, and its formation and  $\beta$ -cell cytotoxicity occurs through a variety of mechanisms.<sup>4</sup> T1D involves the inability to produce insulin due to the absence of pancreatic  $\beta$ -cells and is onset in the early years.<sup>2</sup> In T1D, where the  $\beta$ -cells are unable to produce insulin, islet transplantation is a potential curative therapy for the loss of  $\beta$ -cell mass.<sup>1</sup> In a recent case study, liver material from four deceased islet-bearing recipients demonstrated amyloid depositions in the grafted pancreatic islets, suggesting the participation of IAPP in their decline.<sup>5</sup> Exenatide has been shown to increase the secretion of insulin while decreasing the secretion of glucagon, and its beneficial effects were evaluated in this study.

## Methodology

The study included three groups of islets: day 0 (isolated islets before culture) group, day 7 untreated (isolated islets were cultured without treatment) group, and day 7 treated (isolated islets were cultured with 10nmol/L exenatide (Byetta)) group. Immunolabelling for insulin and TUNEL was performed in order to visualize the pancreatic  $\beta$ -cells and to observe their death. Guinea pig anti-insulin was used as a primary antibody and goat anti-guinea pig was used as a secondary antibody. Roche In Situ Death Detection Kit TMR red was used in order to visualize the cell death of  $\beta$ -cells and was noted as TUNEL+ cells. Quantification of the  $\beta$ -cells was used as a means of determining the effectivity of the treatment. A blind method was used and the codes to the micrographs were released following the conclusion of quantification. A single-factor ANOVA was used to perform a statistical analysis of the treated and untreated cohorts, with a level of significance of 5% (0.05).

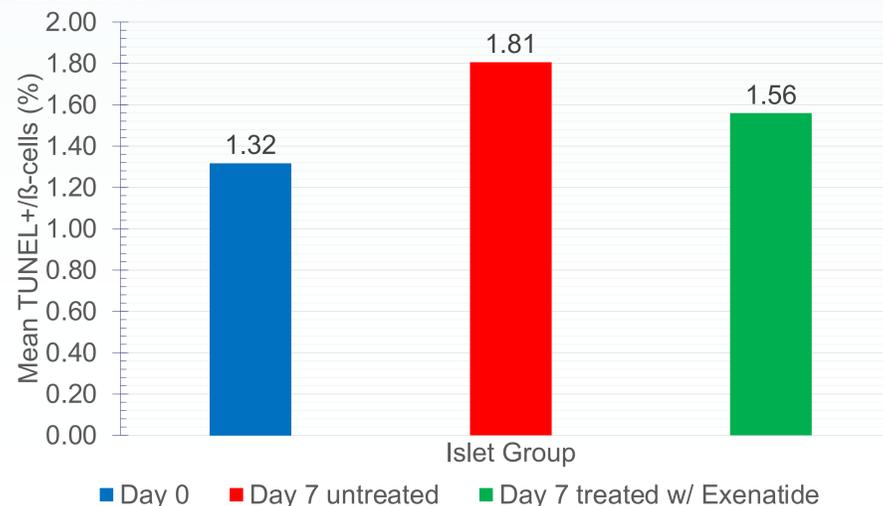
## Results



**Figure 1** (above). A micrograph of an islet that is representative of the day 0 cohort. Individual  $\beta$ -cells (green) can be easily detected and appear rich in colour, with observable separation and nucleuses.

**Figure 2** (above). A micrograph of an islet that is representative of the 7 days cultured without treatment cohort. The presence of more TUNEL+  $\beta$ -cells (red) can be seen which speaks on the decreasing health of the  $\beta$ -cells in culture.

**Figure 3** (above). A micrograph of an islet that is representative of the 7 days cultured with treatment cohort. The presence of less TUNEL+ (red)  $\beta$ -cells, speaks on the better health of the  $\beta$ -cells in culture.



**Figure 4** (left). A graph summarizing the mean percentages of TUNEL+  $\beta$ -cells to  $\beta$ -cells from all three cohorts. The mean percentage is lower in the treated cohort further suggesting that the exenatide treatment is effective, however further statistical analysis is required in order to make a better conclusion

## Conclusion

A level of significance of 5% (0.05) was used and the null hypothesis was that there is no significant difference between the two cohorts. A p-value of 0.7 was calculated, for there being a difference between the two cohorts. Despite consistently lowering the number of TUNEL+  $\beta$ -cells in the exenatide treated group (which can be observed in Figure 4), this difference did not reach a level of significance likely due to the small sample size in this summer project. This is not a complete project and further studies are still in progress, in order to increase the sample size of islets. Lastly, alternative GLP-1 agonists should also be considered for research, as this study indicates that they may be capable of enhancing the function of  $\beta$ -cells which may prove to be beneficial in *ex vivo* treatment of islets. These findings may prove to be of significance, especially for treatment of islets prior to transplantation.

## References

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