# Beta Cell Stress triggers formation of Hybrid Insulin Peptide through granular pH modulation


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## Introduction

- Hybrid insulin peptides (HIPs) are antigenic targets of autoreactive CD4 T cells in type 1 diabetes **(Figure 1)**
- Presence of HIPs in human and murine islets has been validated using mass spectrometry (MS)[1]
- Several diabetes-triggering CD4 T cells have been isolated from the non-obese diabetic (NOD) animal model of type 1 diabetes (T1D)
- HIP reactive T cells have been identified in residual pancreatic islets of T1D organ donors
- Significantly elevated levels of HIP-reactive T cells have been detected in the peripheral blood of recent onset T1D patients
- Disease relevant HIPs: Validated by MS and have a known corresponding HIP-reactive T cell in humans or NOD mice **(Figure 2)**
- The protease cathepsin D (CatD) was shown to be responsible for HIP formation in insulin secretory granules[2] **(Figure 3)**
- HIP are reliably detectable in mouse islets, however their detection in human islets has been sporadic
- Murine CatD forms HIP11 from C-peptide precursors at a pH below 6.0, well with the reported pH range (pH 5-6) of insulin granules [3,4]
- Human CatD does not form HIPs above pH 5.2 revealing species-specific differences when comparing mice with humans **(Figure 4)**
- Glucose was reported to cause a drop in the pH of insulin granules[5]
- We propose a mechanism by which HIP formation could be upregulated within glucose stressed islets **(Figure 5)**

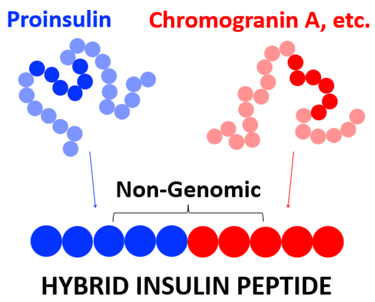


**Figure 1. Formation of a HIP produces an amino acid sequence that is not recognized as "self"[1]**

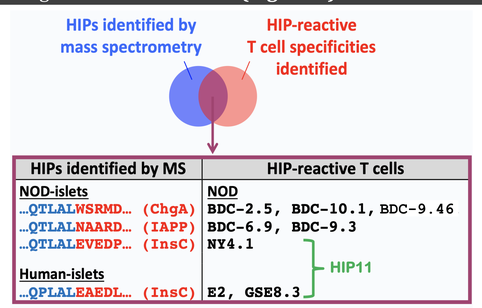


**Figure 2. Disease relevant HIPs validated by mass spectrometry and recognized by T cells linked to T1D pathogenesis**

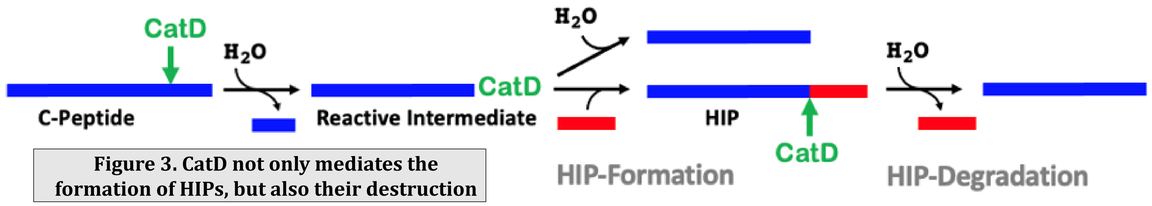


**Figure 3. CatD not only mediates the formation of HIPs, but also their destruction**

## Hypothesis

***Glucose stress of human islets will upregulate HIP formation in human islets***

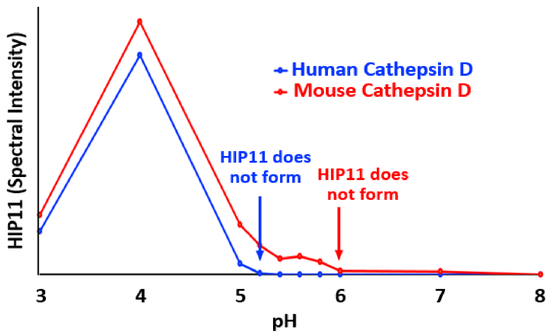


**Figure 4. Unlike murine CatD, human CatD does not form HIPs above pH 5.0: C-peptide was co-incubated with a fixed concentration of CatD at various pH levels for 2 hours. Reactions were stopped by boiling and neutralization, and the formation of HIP11 (a product of two linked C-peptide fragments) was monitored by LC-MS/MS. HIP11 spectral intensities, representative for its abundance, are plotted against the reaction pH. Murine CatD generates HIP11 at pH values below 6.0, while human CatD requires treatments below pH 5.2 for HIP formation, highlighting a key difference between the two species.**

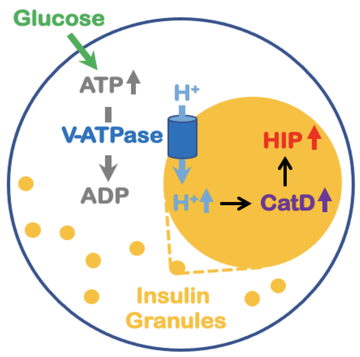


**Figure 5. Elevated glucose levels triggering protease-mediated HIP-formation in beta-cells: Elevated glucose levels lead to an increased Glucose uptake by beta-cells. This leads to a rise in cellular ATP-levels, which in return boosts activity of the vesicular proton pump (V-ATPase). Because of elevated proton (H+) influx, the granular pH drops thereby enhancing CatD-activity and formation of HIPs.**

## Methods and Results

- Human islets were cultured in the presence of various glucose concentrations
- Islets were lysed and islet peptide content was analyzed by LC-MS/MS.
- A previously unidentified HIP was detected at elevated (285mg/dL), but not higher or lower glucose levels
- The identity of the new HIP (neoHIP) was validated using the P-VIS method **(Figure 6)**
- The new HIP was also detected in human islets treated with 100uM C381, which enhances the activity of V-ATPase **(Figure 7)** and islets treated with IL-1β **(Figure 8)**

## Results

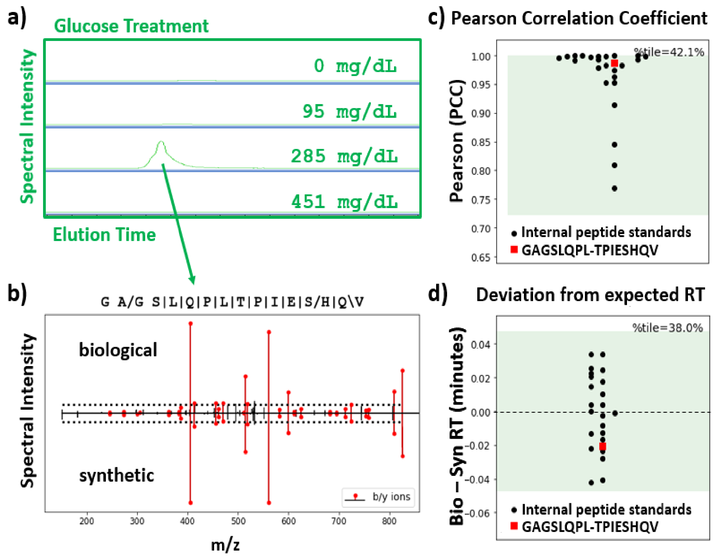


**Figure 6. Glucose-treatment induces HIP-formation in human islets: (a) Extracted ion chromatograms (EICs) reveal the formation of a previously unknown HIP (neoHIP, m/z = 545.29) in islets cultured with 285 mg/dL glucose. (b) Peptide validation using internal peptide standards (P-VIS)[6] reveals high similarity between fragmentation spectra of the biological neoHIP and its synthetic counterpart. (c) Statistical confidence in spectral match: The Pearson correlation coefficient (PCC) for neoHIP (red square) falls within the high confidence interval (green area) established by internal peptide standards (IPS) that were spiked into the biological sample and the synthetic peptide sample. (d) Retention time (RT) validation: The co-elution profile of neoHIP (red square) falls within the high confidence interval derived from IPS.**

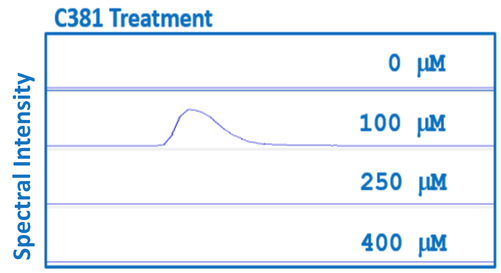


**Fig. 7. C381 treatment induces HIP formation in human islets: Human islets were treated for 24 hours in the presence and absence of C381. Following treatment, islets were lysed, and peptides were isolated by size exclusion chromatography and analyzed by LC-MS/MS. Extracted ion chromatograms for neoHIP (m/z = 545.29) reveal its formation specifically in islet samples treated in presence of 100mM C381.**

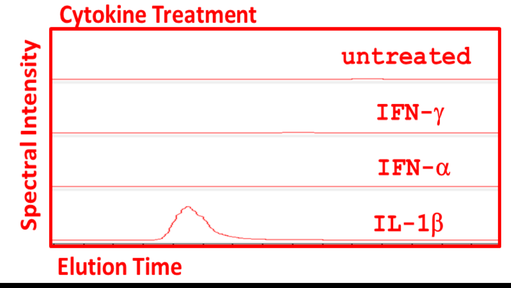


**Fig. 8. IL-1β treatment induces HIP formation in human islets: Human islets were treated for 24 hours with or without IFN-γ, IFN-α, or IL-1β. Following treatment, islets were lysed, and peptides were isolated by size exclusion chromatography and analyzed by LC-MS/MS. Extracted ion chromatograms for neoHIP (m/z = 545.29) reveal the formation of neoHIP specifically in islet samples treated with IL-1β, while no detectable levels of neoHIP were observed in untreated islets or those treated with IFN-γ or IFN-α.**

## Conclusion and Future Directions

- There appears to be an optimal glucose concentration required for upregulating HIP formation
- The results of this study implicate pH as a primary driver of HIP formation, however the range of pH-sensitivity is not yet known
- Future work will focus on the role of neoHIP as a marker for beta cell stress
- To validate the role of pH in HIP formation, we plan to block the activity of vATPase in treated human islets using the proton pump inhibitor Bafilomycin A1
- Stress-induced HIP formation could provide an explanation for the highly variable disease progression observed in autoantibody positive at-risk individuals. challenging the current linear progression model

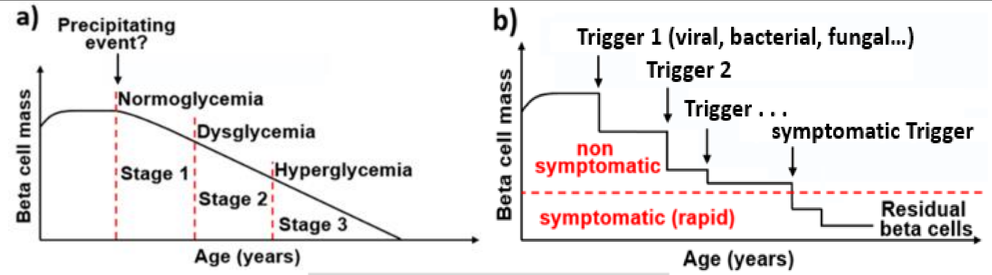


**Figure 9. Models for disease progression in T1D: (a) Following Eisenbarth's model[7], an undefined triggering event initiates beta-cell destruction, gradually advancing until symptomatic stage 3 T1D manifests. This gradual process is highly variable and can take many years. (b) Multiple triggering events, such as those induced by cytokines, induce temporary acidification of insulin granules, thereby activating CatD and other granular proteases to mediate HIP-formation. Beta-cells containing HIPs undergo destruction, until clearance of pathogen and cytokines has occurred. This will deplete beta cells of HIPs thereby rendering them inaccessible to disease-driving T cells of the adaptive immune system.**

## References


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