

**INVESTIGATIONS ON GLUCOSE-STIMULATED
INSULIN SECRETION AND ITS POSSIBLE
ROLE IN PATHOLOGY OF VIRAL-INFECTION-
TRIGGERED TYPE 1 DIABETES.**

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**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES
INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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VIRAL-INFECTION-TRIGGERED TYPE 1 DIABETES**

by

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KUSUMA SCHOOL OF BIOLOGICAL SCIENCES

Submitted

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CERTIFICATE

This is to certify that the thesis entitled **“INVESTIGATIONS ON GLUCOSE-STIMULATED INSULIN SECRETION AND ITS POSSIBLE ROLE IN PATHOLOGY OF VIRAL-INFECTION-TRIGGERED TYPE 1 DIABETES”**, being submitted by **Firdos (Entry No: 2016BLZ8374)** to the **Indian Institute of Technology, Delhi**, for the award of the degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of “Indian Institute of Technology, Delhi”. The research reports and the results presented in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Type 1 Diabetes (T1D) disrupts the intricate equilibrium of glucose levels by specifically targeting and eliminating pancreatic β -cells responsible for insulin secretion, resulting in compromised control of glucose. This condition, commonly known as insulin-dependent diabetes, is characterized by low insulin levels. The intricate coordination of signaling pathways, transcription factors, and molecular mechanisms is crucial for the functioning of pancreatic β -cells, which respond to glucose stimulation by secreting insulin. The MIN6 cell line, derived from Mouse Insulinoma 6, proves to be a valuable model for uncovering the mechanistic complexities associated with the functions of pancreatic β -cells. Although a correlation is evident between elevated glucose consumption and heightened insulin secretion, it has been noted that intracellular ATP levels remain relatively constant once a specific extracellular glucose concentration is reached. This prompts inquiries regarding the cause-and-effect connection between glucose consumption (GC) and increased insulin secretion (eIS). Furthermore, our examination indicates that both total cellular protein levels and proteins in the culture "supernatant" remain consistent regardless of changes in extracellular glucose concentrations. This suggests that enhanced insulin secretion (eIS) might be associated with a trade-off involving the intracellular synthesis of other proteins, the secretion of secretory proteins, or both, potentially influenced by glucose consumption (GC) in cells. To acquire a more profound understanding, we conducted a transcriptome study on MIN6 cells exposed to hypoglycemic (HoG = 2.8 mM EG) and hyperglycemic (HyG = 25 mM EG) conditions. Our findings indicate that in hyperglycemic extracellular conditions, insulin secretion increases by approximately two-fold compared to hypoglycemic conditions. Interestingly, transcripts of secreted proteins and their isoforms decrease in hyperglycemic conditions, suggesting that enhanced insulin secretion in high glucose conditions is linked to a decrease in the transcription of other secreted proteins, alongside increased glucose consumption. Further exploration excavate into the direct involvement of intracellular glucose in insulin secretion, revealing significant differences between the amount of glucose transported into pancreatic β -cells and the amount phosphorylated inside these cells. The differential expression of genes associated with energy processes, particularly *Gapdh*, *Atp6v0a4*, and *Cox20*, provides valuable insights. Notably, upregulated genes in high glucose conditions, such as *Atp6v0a4*, *Cacnb4*, and *Kif11*, are linked to cellular secretion, adding complexity to the correlation. Our investigation extends to exploring the potential link between viral infections and their role in triggering T1D. Genetic

and environmental factors, including viral infections like enteroviruses, contribute significantly to T1D onset patterns. Mechanisms such as virus persistence, bystander activation, and molecular mimicry are implicated, but the precise pathogenesis remains elusive. Glucose-Stimulated Insulin Secretion (GSIS) underscores the importance of understanding intricate mechanisms like Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP)-potentiated GSIS in glycemic control and diabetes management. We have identified viral peptides in Chikungunya Virus (CHIKV) and Dengue virus (DV) proteomes that mimic PACAP. Our study suggests a potential impact on insulin secretion through PACAP-potentiated GSIS in pancreatic β -cells. The CHIKV peptide CV1, bearing high similarity to P38, may disrupt PACAP-potentiated insulin secretion, potentially contributing to diabetes-like conditions. Our research shows a molecular mechanism linking viral infection and T1D, indicating a temporal relationship and shedding light on the condition's nature. Interestingly, viral peptides can induce insensitivity to glucose and disturb PACAP-potentiated GSIS, expanding the scope of molecular mimic exploration. This research contributes to our understanding of a conserved cellular mechanism affecting insulin production, with implications for various cell types and critical relevance in T1D research.

एब्स्ट्रेक्ट

टाइप 1 डायबिटीज (टी1डी) डिस्क्रिप्स द इंट्रिकेट एक्विलिब्रियम् ऑफ ग्लूकोस लेवेल्स बाई स्पेसिफिकली टार्गेटिंग एंड एलिमिनेटिंग पैन्क्रिएटिक बी-सेल्स रेस्पॉन्सिबल फॉर इंसुलिन सीक्रेशन, रिजल्टिंग इन कॉम्प्रोमाइज्ड कंट्रोल ऑफ ग्लूकोस । दिस कंडीशन, कॉमन्ली नोउन अस इंसुलिन-डिपेंडेंट डायबिटीज, इज़ चैरैक्टराइज़्ड बाई लो इंसुलिन लेवेल्स। द इंट्रिकेट कोआर्डिनेशन ऑफ सिग्नलिंग पथवेस, ट्रांस्क्रिप्शन फैक्टर्स, एंड मोलेक्यूलर मेकेनिज्म्स इज़ क्रुशियल फॉर द फंक्शनिंग ऑफ पैन्क्रिएटिक बी-सेल्स, व्हिच रिस्पॉन्ड टू ग्लूकोस स्टिमुलेशन बाई सीक्रेटिंग इंसुलिन। द मिन 6 सेल लाइन, डराइव्ड फ्रम माउस इंसुलिनोमा 6, प्रूव्स टू बी ए वैल्युबल मॉडल फॉर अनकवरिंग द मेकेनिस्टिक कॉम्प्लेक्सिटीएस असोसिएटेड विद द फंक्शन्स ऑफ पैन्क्रिएटिक बी-सेल्स। अल्थोउघ् अ कॉर्रिलेशन इज़ एविडेंट बिटवीन एलेवेटेड ग्लूकोस कंसम्प्शन एंड हाइटेनेड इंसुलिन सीक्रेशन, इट हैज़ बीन् नोटेड दैट इंट्रासेल्लुलर एटीपी लेवेल्स रेमेन रेलेटिव्ली कॉन्स्टन्ट वन्स् अ स्पेसिफिक एक्सट्रासेल्लुलर ग्लूकोस कॉन्सेन्ट्रेशन इज़ रीछेड। दिस प्रॉम्प्स् इनक्रिएस रिगार्डिंग द कॉज-एंड-इफेक्ट कनेक्शन बिटवीन ग्लूकोस कंसम्प्शन (जीसी) एंड इन्क्रीस्ड इंसुलिन सीक्रेशन (ईआईएस)। फर्थरमोर, आवर् एक्जामिनेशन इंडिकेट्स दैट बोथ टोटल सेलुलर प्रोटीन लेवेल्स एंड प्रोटीन्स इन द कल्चर "सुपरनेटन्ट" रेमेन कंसिस्टेंट रेगार्डलेस्स् ऑफ़ चेंजेस् इन् एक्सट्रासेल्लुलर ग्लूकोस कंसंट्रेशन्स्। दिस सजेस्ट्स दैट एन्हांस्ड इंसुलिन सीक्रेशन (ईआईएस) माइट बी एसोसिएटेड विद ए ट्रेड-ऑफ़ इन्वॉल्विंग द इंट्रासेल्लुलर सिन्थेसिस ऑफ़ अदर प्रोटीन्स, द सीक्रेशन ऑफ़ सेक्रेटरी प्रोटीन्स, ओर बोथ, पोटेन्शियल्लय इन्फ्लुएंस्ड बाय ग्लूकोस कंसम्प्शन (जीसी) इन सेल्स्। टू एक्वायर् अ मोर प्रोफ़ाउन्ड अंडरस्टैंडिंग, बी कॉन्डक्टेड अ ट्रांस्क्रिप्टोमे स्टडी ऑन् मिन 6 सेल्स् एक्सपोज़्ड टू हाइपोग्लाइसेमिक (ह ओ ग =2.8 मिमिमोलार) एंड हाइपरग्लाइसेमिक (ह य जी =25 मिमिमोलार) कंडीशंसेस्। ऑवर् फाइंडिंग्स् इंडिकेट दैट इन हाइपरग्लाइसेमिक एक्सट्रासेल्लुलर कंडीशंसेस, इंसुलिन सीक्रेशन इन्क्रीसेज बाई अप्रोक्सिमेटली टू-फोल्ड कंफेयर्ड टू हाइपोग्लाइसेमिक कंडीशंसेस। इंट्रेस्टिंगली, ट्रांस्क्रिप्ट्स ऑफ़ सीक्रेटेड प्रोटीन्स एंड थेयर आईसोफ़ॉर्म्स् डिक्लीस् इन हाइपरग्लाइसेमिक कंडीशंसेस, सजेस्टिंग दैट एन्हांस्ड इंसुलिन सीक्रेशन इन हाइ ग्लूकोस कंडीशंसेस इज़ लिंकड टू अ डिक्लीस् इन द ट्रांस्क्रिप्शन ऑफ़ अदर सीक्रेटेड प्रोटीन्स, अलॉगसाइड इन्क्रीस्ड ग्लूकोस कंसम्प्शन। फर्थर् एक्स्प्लोरेशन एक्स्केवेटेस् इंटू द डायरेक्ट इन्वॉल्वमेंट ऑफ़ इंट्रासेल्लुलर ग्लूकोस इन इंसुलिन सीक्रेशन, रिवीलिंग सिग्निफिकेंट डिफरेंसेस् बिटवीन द अमाउंट ऑफ़ ग्लूकोस ट्रांसपोर्टेड इंटू पैन्क्रिएटिक बी-सेल्स एंड द अमाउंट फोस्फोरिलेटेड इनसाइड थीज़ सेल्स्। द डिफरेंशियल एक्सप्रेशन ऑफ़ जीन्स एसोसिएटेड विद एनर्जी प्रोसेसेस, पार्टिक्युलर्ली गपडेस, एटपी6वी0ए4, एंड कॉक्स20, प्रोवाइड्स वैल्युबल इनसाइट्स। नोटेबल्ली, उपरेगुलेटेड जीन्स इन हाई ग्लूकोस कंडीशंसेस, सच

एटपी6वी0ए4, कैक्रब4, एंड किफ11, आर लिंकड टू सेलुलर सीक्रेशन, अडिंग कॉम्प्लेक्सिटी टू द कॉरिलेशंस। आवर् इन्वेस्टिगेशन एक्सटेंड्स टू एक्सप्लोरिंग द पोटेन्शियल लिंक बिटवीन वायरल इन्फेक्शंस एंड देयर रोल इन् ट्रिगरिंग टी1डी। जेनेटिक् एंड एनवायरोनमेंटल् फैक्टर्स, इंकलूडिंग वायरल इन्फेक्शंस लाइक एंटरोवायरसेस्, कॉन्ट्रिब्यूट सिग्निफिकंटली टू टी1डी ऑन्सेट पैटर्न्स। मेकेनिज्म्स सच एस् वायरस पर्सिस्टन्स, बायस्टैंडर् ऐक्टिवेशन, एंड मोलेक्युलर् मिमिक्री आर इम्प्लिकेटेड, बट द प्रेसाइस पैथोजेनेसिस् रेमेंस् इलूसिव्। ग्लूकोस-स्टिमुलेटेड इंसुलिन सीक्रेशन (जीएसआईएस) अंडस्कॉर्सेस द इम्पोर्टेंस ऑफ़ अन्डरस्टैंडिंग इंट्रिकेट् मेकेनिज्म्स लाइक पिछूटरी एडेनालेट कायलेस्-एक्टिवेटिंग पोलिपेप्टाइड (पैकैप)-पोटेन्टिएटेड जीएसआईएस इन ग्लाइसेमिक कंट्रोल एंड डायबिटीज़ मैनेजमेंट। वी हैव आईडेंटिफाईड वायरल पेप्टाइड्स इन चिकुनगुनिया वायरस (चिकवी) एंड डेंगू वायरस (डीवी) प्रोटीयोम्स् दैट् मिमिक् पैकैप। ऑवर् स्टडी सजेस्ट्स ए पोटेन्शियल इम्पैक्ट् ऑन् इंसुलिन सीक्रेशन थ्रू पैकैप-पोटेन्टिएटेड जीएसआईएस इन पैनक्रिएटिक् बी-सेल्स। द चिकवी पेप्टाइड सीवी1, बेअरिंग हाइ सिमिलैरिटी टू पी38, मे डिस्क्रिप्ट् पैकैप-पोटेन्टिएटेड इंसुलिन सीक्रेशन, पोटेन्टियल्स कॉन्ट्रिब्यूटिंग टू डायबिटीज़-लाइक कंडीशंसेस। आवर् रिसर्च शोज ए मोलेक्युलर् मेकेनिज्म् लिंकिंग वायरल इन्फेक्शंस एंड टी1डी, इंडिकेटिंग ए टेम्पोरल् रिलेशनशिप एंड शेडिंग लाइट् ऑन् द कंडीशन'स नेचर्। इंटरेस्टिंगली, वायरल पेप्टाइड्स कैन् इंड्यूस् इंसेंसिटिविटी टू ग्लूकोस एंड डिस्टर्ब पैकैप-पोटेन्टिएटेड जीएसआईएस, एक्सपैंडिंग द स्कोप ऑफ़ मोलेक्युलर् मिमिक् एक्सप्लोरेशन्स। दिस् रिसर्च कॉन्ट्रिब्यूट्स टू आवर् अन्डरस्टैंडिंग् ऑफ़ ए कॉन्सर्व्ड सेलुलर् मेकेनिज्म् अफ़फेक्टिंग् इंसुलिन प्रोडक्शन, विथ् इम्प्लिकेशंस फॉर वेरिअस् सेल टाइप्स् एंड क्रिटिकल् रेलेवन्से इन टी1डी रिसर्च।

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List of Abbreviations

GSIS: Glucose-stimulated-insulin-secretion

T1D: Type 1 diabetes

T2D: Type 2 diabetes

EGC: Extracellular-glucose concentrations

MIN6: Mouse Insulinoma 6

eIS: Enhance Insulin Secretion

DM: Diabetes mellitus

ISGs: Insulin secretory granules

HoG: Hypoglycemic

HyG: Hyperglycemic

FPKM: Fragments Per Kilobase of transcript per Million mapped reads

TPM: Transcript per Million.

PACAP: Pituitary Adenylate Cyclase-Activating Polypeptide

ADT: Auto Dock Tools

DS: Discovery Studio 2021

DM: Diabetes mellitus

CV: Chikungunya virus

DV: Dengue virus

SeIT: Selenoprotein T

IGF1: Insulin growth factor