ROLE OF EXTRACELLULAR ATP AND IRISIN AS EXERCISE MIMICKING MEDIATORS IN NEURONAL INSULIN SIGNALLING

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by

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Dedicated to Anish…

This is the second hardest thing I've had to do

CERTIFICATE

This is to certify that the thesis entitled "Role of extracellular ATP and Irisin as exercise mimicking mediators in neuronal insulin signalling" being submitted by Ms. Ishitha Reddy Gaddam to Indian Institute of Technology-Delhi for the award of the degree of "Doctor of Philosophy" is a record of bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with rules and regulations of Indian Institute of Technology-Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/ Diploma.



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Place: New Delhi

DECLARATION

I hereby declare that:

1. The work presented in this thesis is original and has been carried out by me as a student of IIT Delhi.

2. To the best of my knowledge, the work presented in this thesis has not been submitted, either in whole or in part, to any other institution for the award of any degree or diploma.

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5. I take full responsibility for all data presented in this thesis and confirm that no data has been collected without appropriate permission. Where required, ethical approval has been obtained for the research, and all relevant approvals and documentation have been included as part of the thesis submission.

6. All sources of information, including data, ideas, and text, have been duly acknowledged and appropriately cited in the thesis.

7. The use of artificial intelligence (AI) tools (if any), including large language models, has been appropriately disclosed. All analysis, interpretations, and conclusions presented in this thesis are my own. Alternatively, I declare that no AI tools, including large language models, have been used in the preparation of this thesis.

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Ishitha Reddy

ABSTRACT

Neuronal insulin signaling is crucial for regulating glucose metabolism, neuronal survival, and cognitive functions in the brain, with disruptions contributing to insulin resistance and potentially leading to Type 2 diabetes (T2D) and Alzheimer's disease (AD). Despite exercise's well-documented benefits for body and brain health, the specific brain signaling mechanisms remain unclear, and *in vitro* studies are limited. We investigated alternative pathways to maintain glucose homeostasis beyond traditional insulin signaling. Using an ATP-mediated neuronal *in vitro* exercise model, we observed elevated levels of markers upregulated by exercise, increased glucose uptake, and activation of insulin signaling molecules. Under insulin-resistance conditions, ATP surpassed insulin, acting as an exercise-like bypass mechanism. Similarly, Irisin, an exerkine released during exercise, has been implicated in muscle–brain communication and metabolic regulation. We examined its effects on hippocampal HT22 cells under both insulin-sensitive and insulin-resistant conditions. Acute Irisin treatment significantly upregulated exercise-related markers, and phosphoryated FAK, as part of its established integrin-FAK signaling axis. Additionally, Irisin also stimulated neuronal insulin signaling, promoting Akt, As160, GSK3 phosphorylation and GLUT4 translocation to enhance glucose uptake. However, under chronic hyperinsulinemia-induced insulin resistance, Irisin's effects were impaired. These findings suggest that while ATP can bypass insulin resistance, Irisin's neurotrophic and insulin signaling effects require intact insulin signaling. Together, our results suggest a possible role for ATP and Irisin in the context of T2D and AD.

Maintaining glucose homeostasis in neurons is critical for brain function, including energy metabolism, synaptic plasticity, and cognitive processes. Impairment of neuronal insulin signaling can lead to insulin resistance, a condition strongly associated with metabolic disorders such as type 2 diabetes and neurodegenerative diseases including Alzheimer's disease. While exercise has been shown to improve neuronal insulin sensitivity, the precise molecular mediators responsible for these effects remain poorly understood. Identifying alternative pathways that can maintain glucose homeostasis in neurons, particularly under insulin-resistant conditions, is of considerable interest.

While looking for such alternative pathways, extracellular ATP (eATP) emerged as a potential mediator. ATP is released during exercise and acts through purinergic receptors to regulate neuronal metabolism, calcium signaling, and mitochondrial function. In peripheral tissues, ATP has been shown to influence glucose uptake and insulin sensitivity, but its role in neurons, particularly in the context of insulin signaling and resistance, has not been systematically studied. Exploring ATP-mediated purinergic signaling in hippocampal neurons may provide insights into how exercise-like effects can be mimicked and whether ATP can bypass impaired insulin signaling to maintain glucose homeostasis.

Irisin, a myokine released from skeletal muscle during exercise, represents another candidate for mediating exercise-induced effects in neurons. Irisin has been shown to promote glucose metabolism in peripheral tissues, but its involvement in neuronal insulin signaling is not well characterized. Specifically, it is unclear whether Irisin can overcome insulin resistance in neurons. Investigating Irisin's action in hippocampal neurons could help understand its potential as a neurotrophic and insulin-sensitizing stimulant, further linking the muscle–brain axis to neuronal metabolic health.

Therefore, in the present study, attempts were made:

1. To investigate the role of ATP in regulating exercise-associated signaling and insulin pathways in hippocampal neurons.
2. To explore the role of Irisin in mediating exercise-like effects on neuronal insulin signaling.
3. To assess the potential of ATP and Irisin to alleviate insulin resistance in mouse hippocampal neurons.

Through these objectives, the study aims to provide a mechanistic understanding of alternative and complementary pathways for maintaining neuronal glucose homeostasis and to define the roles of ATP and Irisin within the exercise-mediated brain-muscle axis.

As such, the present work is divided into four chapters:

Chapter 1: Introduction - This chapter introduces key concepts of exercise, outlines systemic physiological responses, and details the intricacies of the exercise responsome. The section also examines neuronal exercise signalling and the role of exercise in modulating neuronal insulin resistance, concluding with a discussion of current research and potential strategies to mitigate metabolic disorders.

Chapter 2: Purinergic signalling by ATP induces exercise-like effects and ameliorates insulin resistance in HT22 mouse hippocampal cells - This chapter showcases the experimental findings, wherein extracellular ATP (eATP) mediated purinergic signalling has been explored as an exercise- mimicking bypass mechanism in neuronal cells. It examines the effects of eATP on exercise-associated neuronal and mitochondrial markers, its interaction with insulin signalling, and its ability to improve glucose uptake and alleviate neuronal insulin resistance, highlighting its potential relevance to metabolic and neurodegenerative disorders.

Chapter 3: Irisin promotes insulin signaling in HT22 hippocampal cells but is attenuated by hyperinsulinemia-induced insulin resistance - This chapter demonstrates the effects of Irisin on neuronal exercise markers and insulin pathway activation in hippocampal neurons. It highlights the selective nature of Irisin action, demonstrating it mediation of the activation of insulin signalling molecules, glucose uptake and GLUT4 translocation under insulin-sensitive conditions and their attenuation in insulin-resistant states.

Chapter 4: Summary - provides an overview of the present study, highlighting its significance and potential future implications.

सारांश

मस्तिष्क में न्यूरोनल इंसुलिन सिग्नलिंग ग्लूकोज़ चयापचय, न्यूरोनल जीवितता तथा संज्ञानात्मक कार्यों के नियमन के लिए अत्यंत महत्वपूर्ण है। इसमें व्यवधान आने से इंसुलिन प्रतिरोध उत्पन्न हो सकता है, जो संभावित रूप से टाइप 2 मधुमेह (T2D) और अल्ज़ाइमर रोग (AD) का कारण बन सकता है। यद्यपि व्यायाम के शरीर और मस्तिष्क स्वास्थ्य पर लाभ सुविदित हैं, फिर भी मस्तिष्क में कार्यरत विशिष्ट सिग्नलिंग तंत्र अभी स्पष्ट नहीं हैं, और इन विट्रो अध्ययन सीमित हैं।

हमने पारंपरिक इंसुलिन सिग्नलिंग से परे ग्लूकोज़ होमियोस्टेसिस बनाए रखने वाले वैकल्पिक मार्गों की जांच की। ATP-मध्यस्थित न्यूरोनल इन विट्रो व्यायाम मॉडल का उपयोग करते हुए, हमने व्यायाम से बढ़ने वाले मार्करों के उच्च स्तर, ग्लूकोज़ अवशोषण में वृद्धि तथा इंसुलिन सिग्नलिंग अणुओं की सक्रियता देखी। इंसुलिन-प्रतिरोध की स्थिति में ATP ने इंसुलिन से बेहतर कार्य करते हुए व्यायाम-सदृश बायपास तंत्र के रूप में कार्य किया।

इसी प्रकार, आइरिसिन (Irisin), जो व्यायाम के दौरान मुक्त होने वाला एक एक्सरकाइन है, मांसपेशी–मस्तिष्क संचार तथा चयापचय नियमन में महत्वपूर्ण माना गया है। हमने इंसुलिन-संवेदनशील तथा इंसुलिन-प्रतिरोधी दोनों स्थितियों में हिप्पोकैम्पल HT22 कोशिकाओं पर इसके प्रभावों का अध्ययन किया। तीव्र आइरिसिन उपचार से व्यायाम-संबंधित मार्करों में उल्लेखनीय वृद्धि हुई तथा इसके स्थापित इंटीग्रिन-FAK सिग्नलिंग अक्ष के अंतर्गत फॉस्फोरिलेटेड FAK में वृद्धि देखी गई। इसके अतिरिक्त, आइरिसिन ने न्यूरोनल इंसुलिन सिग्नलिंग को भी सक्रिय किया, जिससे Akt, As160, GSK3 के फॉस्फोरिलेशन तथा GLUT4 के स्थानांतरण को प्रोत्साहन मिला और परिणामस्वरूप ग्लूकोज़ अवशोषण बढ़ा।

हालांकि, दीर्घकालिक हाइपरइंसुलिनेमिया-प्रेरित इंसुलिन प्रतिरोध की स्थिति में आइरिसिन के प्रभाव कमज़ोर हो गए। ये निष्कर्ष दर्शाते हैं कि जहाँ ATP इंसुलिन प्रतिरोध को बायपास कर सकता है, वहीं

आइरिसिन के न्यूरोप्रोटेक्टिव और इंसुलिन सिग्नलिंग संबंधी प्रभावों के लिए अखंड इंसुलिन सिग्नलिंग आवश्यक है।

समग्र रूप से, हमारे परिणाम T2D और AD के संदर्भ में ATP और आइरिसिन की संभावित भूमिका का संकेत देते हैं।

न्यूरॉनों में ग्लूकोज़ होमियोस्टेसिस बनाए रखना मस्तिष्क के कार्यों, जैसे ऊर्जा चयापचय, सिनेप्टिक प्लास्टिसिटी और संज्ञानात्मक प्रक्रियाओं—के लिए अत्यंत आवश्यक है। न्यूरोनल इंसुलिन सिग्नलिंग में बाधा आने से इंसुलिन प्रतिरोध उत्पन्न हो सकता है, जो टाइप 2 मधुमेह तथा अल्ज़ाइमर रोग जैसे चयापचय एवं न्यूरोडीजेनेरेटिव विकारों से दृढ़ता से संबंधित है। यद्यपि व्यायाम न्यूरोनल इंसुलिन संवेदनशीलता में सुधार करता है, परंतु इसके लिए उत्तरदायी सटीक आणविक मध्यस्थ अभी स्पष्ट नहीं हैं। विशेष रूप से इंसुलिन-प्रतिरोध की स्थिति में, न्यूरॉनों में ग्लूकोज़ होमियोस्टेसिस बनाए रखने वाले वैकल्पिक मार्गों की पहचान अत्यंत महत्वपूर्ण है।

ऐसे वैकल्पिक मार्गों की खोज के दौरान, बाह्यकोशिकीय ATP (eATP) एक संभावित मध्यस्थ के रूप में उभरा। व्यायाम के दौरान ATP मुक्त होता है और प्यूरिनर्जिक रिसेप्टर्स के माध्यम से न्यूरोनल चयापचय, कैल्शियम सिग्नलिंग तथा माइटोकॉन्ड्रियल क्रिया को नियंत्रित करता है। परिधीय ऊतकों में ATP को ग्लूकोज़ अवशोषण और इंसुलिन संवेदनशीलता को प्रभावित करते हुए दिखाया गया है, किंतु न्यूरॉनों में, विशेषकर इंसुलिन सिग्नलिंग और प्रतिरोध के संदर्भ में, इसकी भूमिका का व्यवस्थित अध्ययन नहीं किया गया है। हिप्पोकैम्पल न्यूरॉनों में ATP-मध्यस्थित प्यूरिनर्जिक सिग्नलिंग की जांच यह समझने में सहायता कर सकती है कि व्यायाम-सदृश प्रभावों की नकल कैसे की जा सकती है और क्या ATP क्षतिग्रस्त इंसुलिन सिग्नलिंग को बायपास कर ग्लूकोज़ होमियोस्टेसिस बनाए रख सकता है।

आइरिसिन, जो व्यायाम के दौरान कंकालीय मांसपेशियों से मुक्त होने वाला एक मायोकाइन है, न्यूरॉनों में व्यायाम-प्रेरित प्रभावों का एक अन्य संभावित मध्यस्थ है। यद्यपि आइरिसिन को परिधीय ऊतकों में ग्लूकोज़ चयापचय को बढ़ावा देने वाला पाया गया है, परंतु न्यूरोनल इंसुलिन सिग्नलिंग में इसकी भूमिका स्पष्ट रूप से परिभाषित नहीं है। विशेष रूप से, यह स्पष्ट नहीं है कि क्या आइरिसिन न्यूरॉनों में इंसुलिन प्रतिरोध को दूर कर सकता है। हिप्पोकैम्पल न्यूरॉनों में आइरिसिन की क्रिया का अध्ययन इसके न्यूरोप्रोटेक्टिव तथा इंसुलिन-संवेदनशीलता बढ़ाने वाले प्रभावों को समझने में सहायक हो सकता है, जिससे मांसपेशी–मस्तिष्क अक्ष और न्यूरोनल चयापचय स्वास्थ्य के बीच संबंध और स्पष्ट हो सके।

अतः वर्तमान अध्ययन में निम्नलिखित प्रयास किए गए:

1. हिप्पोकैम्पल न्यूरॉनों में व्यायाम-संबंधित सिग्नलिंग तथा इंसुलिन मार्गों के नियमन में ATP की भूमिका की जांच करना।
2. न्यूरोनल इंसुलिन सिग्नलिंग पर व्यायाम-सदृश प्रभावों के मध्यस्थ के रूप में आइरिसिन की भूमिका का अध्ययन करना।
3. माउस हिप्पोकैम्पल न्यूरॉनों में इंसुलिन प्रतिरोध को कम करने में ATP और आइरिसिन की संभावित क्षमता का आकलन करना।

इन उद्देश्यों के माध्यम से, यह अध्ययन न्यूरोनल ग्लूकोज़ होमियोस्टेसिस बनाए रखने वाले वैकल्पिक एवं पूरक मार्गों की यांत्रिक समझ प्रदान करने तथा व्यायाम-मध्यस्थित मस्तिष्क-मांसपेशी अक्ष में ATP और आइरिसिन की भूमिकाओं को परिभाषित करने का प्रयास करता है।

यह शोधकार्य चार अध्यायों में विभाजित है:

अध्याय 1: परिचय - इस अध्याय में व्यायाम की प्रमुख अवधारणाओं, प्रणालीगत शारीरिक प्रतिक्रियाओं तथा व्यायाम रिस्पॉन्सोम की जटिलताओं का परिचय दिया गया है। साथ ही न्यूरोनल व्यायाम सिग्नलिंग और न्यूरोनल इंसुलिन प्रतिरोध के नियमन में व्यायाम की भूमिका का विश्लेषण करते हुए वर्तमान शोध एवं चयापचय विकारों को कम करने की संभावित रणनीतियों पर चर्चा की गई है।

अध्याय 2: ATP द्वारा प्यूरिनर्जिक सिग्नलिंग HT22 माउस हिप्पोकैम्पल कोशिकाओं में व्यायाम-सदृश प्रभाव उत्पन्न करती है और इंसुलिन प्रतिरोध में सुधार करती है - इस अध्याय में प्रायोगिक निष्कर्ष प्रस्तुत किए गए हैं, जिनमें बाह्यकोशिकीय ATP (eATP) को न्यूरोनल कोशिकाओं में व्यायाम-सदृश बायपास तंत्र के रूप में अध्ययन किया गया है। इसमें eATP के व्यायाम-संबंधित न्यूरोनल एवं माइटोकॉन्ड्रियल मार्करों पर प्रभाव, इंसुलिन सिग्नलिंग के साथ इसकी अंतःक्रिया, तथा ग्लूकोज़ अवशोषण बढ़ाने और न्यूरोनल इंसुलिन प्रतिरोध कम करने की इसकी क्षमता का विश्लेषण किया गया है।

अध्याय 3: आइरिसिन HT22 हिप्पोकैम्पल कोशिकाओं में इंसुलिन सिग्नलिंग को प्रोत्साहित करता है, किंतु हाइपरइंसुलिनेमिया-प्रेरित इंसुलिन प्रतिरोध से इसका प्रभाव कम हो जाता है - इस अध्याय में हिप्पोकैम्पल न्यूरॉनों में व्यायाम मार्करों और इंसुलिन मार्ग सक्रियता पर आइरिसिन के प्रभावों का वर्णन किया गया है। इसमें दर्शाया गया है कि इंसुलिन-संवेदनशील परिस्थितियों में आइरिसिन इंसुलिन सिग्नलिंग अणुओं की सक्रियता, ग्लूकोज़ अवशोषण तथा GLUT4 स्थानांतरण को बढ़ावा देता है, जबकि इंसुलिन-प्रतिरोधी अवस्थाओं में ये प्रभाव कम हो जाते हैं।

अध्याय 4: सारांश - इस अध्याय में वर्तमान अध्ययन का समग्र अवलोकन प्रस्तुत किया गया है, जिसमें इसके महत्व तथा संभावित भविष्यगत प्रभावों को रेखांकित किया गया है।

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ABBREVIATIONS

Aβ	Amyloid beta
AICAR	Amino imidazole carboxamide ribonucleotide
AMPK	AMP-activated protein kinase
AS160	AKT substrate of 160 kDa
BAT	Brown adipose tissue
BDNF	Brain derived neurotrophic factor
CREB	cAMP-responsive element-binding protein
CS	Citrate synthase
DMEM	Dulbecco's Minimum essential media
eATP	Extracellular adenosine triphosphate
EPS	Electrical pulse stimulation
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
FGF21	Fibroblast growth factor 21
FNDC5	Fibronectin type III domain-containing 5
FoxOs	Forkhead box O proteins
GABA	γ-aminobutyric acid
GLUT3	Glucose transporter type 3
GLUT4	Glucose transporter type 4
GSK3	Glycogen synthase kinase 3
GSK3α	Glycogen synthase kinase 3 alpha

GSK3β	Glycogen synthase kinase 3 beta
HFD	High fat diet
IGF-1	Insulin growth factor 1
InsR	Insulin receptor
IR	Insulin resistance
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
METRNL	Meteorin-like protein
mBDNF	Mature BDNF
NAD+	Nicotinamide adenine dinucleotide
NeuroD	Neuronal differentiation
Neurog2	Neurogenin 2
PBS	Phosphate buffered saline
PDK1	3-Phosphoinositide-dependent protein kinase 1
PGC1α	Peroxisome proliferator-activated receptor gamma co-activator 1 alpha
PKCα	Protein kinase C alpha
PLC-γ	Phospholipase C gamma
PP1γ	Protein phosphatase 1 catalytic subunit gamma
PPADS	Pyridoxalphosphate-6-azophenyl-2 4-disulfonic acid
SIRT1	Sirtuin 1
SIM	Structured Illumination Microscopy

SPARC	Secreted protein acidic and rich in cysteine
T2D	Type 2 diabetes
T3D	Type 3 diabetes
TrkB	Tropomyosin receptor kinase B
VEGF	Vascular endothelial growth factor
WAT	White adipose tissue