LIFE Science Hub
JOURNAL OF SCIENCE
EXPANDING KNOWLEDGE ABOUT NUTRITION AND HEALTH

ORIGINAL RESEARCH
ISSN 2845-7501

Insulin induces ROS-activated and IP_3-dependent Ca^{2+} release, both converging on GLUT4 translocation - A review of the evidence

D.ssa D. Iodice[1]

1 Biologist, Italy

1. ABSTRACT

The analyzed study examined whether insulin, in addition to the classic pathway leading to AKT activation, utilizes intracellular calcium-based signals to promote the transfer of GLUT4 to the surface of muscle cells. Using muscle cells and pharmacological and molecular tools, the authors observed that insulin induced two calcium release circuits: one associated with the controlled production of reactive oxygen species and the ryanodine receptor, and the other dependent on IP_3.In section 5.4.2 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, the publication contributes to the narrative that calcium represents a complementary signal to insulin action in striated muscle. Independent studies in isolated muscles and mouse models support the idea that modifying calcium entry or mobilization can alter insulin-stimulated glucose uptake. However, the majority of the evidence is experimental, and a chronic increase in resting calcium may instead be associated with reduced insulin action. The most prudent conclusion is that rapid, localized, and regulated calcium signals participate in muscle insulin action, without demonstrating that every increase in calcium is beneficial.

ONLINE ARTICLE

ARTICLE INFORMATION

Published: November 23, 2026
Updated: November 23, 2026

DOI 10.5281/zenodo.24470919

MISSION

LIFE Science Hub – Journal of Science was founded with the aim of making scientific knowledge accessible without giving up methodological rigour, quality of sources and authoritativeness of content. The journal promotes the dissemination of knowledge in the health, nutrition, medical and longevity sciences, building a bridge between the world of research and society.

Through articles, reviews, in-depth analyses and expert contributions, the Journal champions evidence-based medicine and science, fostering a correct interpretation of scientific data and countering misinformation. Every piece of content is developed with the aim of translating the complexity of research into understandable, useful and applicable information, while keeping intact the principles of accuracy, transparency and scientific independence.

Free from political, industrial and commercial influence, the journal stands as an authoritative and independent editorial space, dedicated to promoting scientific culture, professional development and the growth of health awareness among the public.

2. ANALYZED STUDY

The research question was whether intracellular calcium directly participates in how insulin promotes glucose entry into muscle cells. In simple terms, the authors verified whether insulin activates only its best-known metabolic pathway or if it also uses brief calcium signals to bring more GLUT4 to the cell membrane. GLUT4 is the transporter that allows glucose to enter the muscle fiber.The hypothesis evaluated was that insulin activates two convergent calcium mobilization pathways. The first involved low amounts of reactive oxygen species or ROS (oxidizing molecules that, at low concentrations, can act as cellular signals) and the type 1 ryanodine receptor, RyR1. The second involved IP_3 (inositol 1,4,5-trisphosphate, a small signaling molecule) and its intracellular receptor.The relevance to the book's paragraph is therefore specific: the publication documents that the calcium signal is not necessarily a secondary effect of insulin, but can contribute to the regulation of GLUT4 trafficking in muscle cells. However, the study cannot demonstrate that this circuit is indispensable in every human muscle, nor that the activation of cPLA2a or the release of arachidonic acid described later in the chapter derives directly from it.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The available material consists only of the abstract; for this reason, information not explicitly reported cannot be reconstructed. The authors studied skeletal muscle cells expressing GLUT4myc, an experimental form of GLUT4 equipped with a small external tag that allows for the measurement of how much protein reaches the cell surface.To evaluate the role of ROS, the authors used antioxidants, the inhibitory peptide gp91-ds-tat, and the silencing of p47phox, a protein necessary for the activity of NOX2, an enzyme that produces ROS. Calcium release was explored using agonists or inhibitors of intracellular channels, including Xestospongin B, an inhibitor of IP_3 receptors. The authors also evaluated hydrogen peroxide production, calcium mobilization, IP_3 production, calcium transfer to mitochondria, and GLUT4myc translocation.The number of cultures, the number of biological replicates, the complete concentrations of reagents, exclusion criteria, detailed statistical endpoints, and P-values are not reported in the available material. It is therefore not possible to fully assess the precision, effect size, and statistical robustness of the individual experiments.

4. RESULTS

The main finding is that hydrogen peroxide increased the presence of GLUT4myc on the cell surface and that this effect was additive to that of insulin. In practical terms, a controlled amount of oxidizing signal appeared to promote the movement of the glucose transporter toward the point of the cell where it can facilitate glucose entry.The antioxidants N-acetyl-L-cysteine and Trolox, the inhibition of NOX2 via gp91-ds-tat, and the silencing of p47phox reduced insulin-induced GLUT4myc translocation. Furthermore, gp91-ds-tat suppressed insulin-induced hydrogen peroxide production. These results are consistent with the involvement of ROS produced by NOX2 in the insulin signaling observed in the cell model.A ryanodine receptor agonist increased GLUT4myc translocation. Insulin also stimulated calcium release through RyR1 by promoting S-glutathionylation of the channel, a reversible chemical modification associated with oxidative stress. In parallel, insulin activated the PI3K-phospholipase C-IP_3 pathway, with calcium release from intracellular stores and calcium transfer to the mitochondria.The IP_3 receptor inhibitor, Xestospongin B, reduced both insulin-induced IP_3 production and GLUT4myc translocation. The data as a whole therefore suggest that the RyR1 and IP_3 pathways contribute to the cellular response to insulin. The available material does not report absolute values, percentages, confidence intervals, or P-values; it is therefore not possible to quantify the magnitude of the individual effects.

5. INTERPRETATION

For the non-specialist reader, the biological message is that insulin does not act merely as a switch that activates a single cascade. In muscle cells, it can also generate small and transient calcium variations, which are useful for coordinating the movement of GLUT4 toward the membrane. The more GLUT4 reaches the surface, the greater the possibility for glucose to enter the cell.The segment of the chain documented by the

analyzed study is: insulin controlled production of ROS and production of IP_3 intracellular calcium release through RyR1 and IP_3 receptors increased GLUT4 translocation. This interpretation is supported by the inhibition and activation experiments described in the abstract, but it remains limited to an experimental cellular system.The publication does not prove that calcium is always essential for insulin action in all muscle regions, nor does it demonstrate a clinical effect on glycemia, diabetes, or inflammation in humans. Furthermore, it does not demonstrate the subsequent step proposed in the chapter, namely the functional activation of cPLA2a. Its contribution to the paragraph is more circumscribed: it supports the existence of calcium signals that accompany the canonical insulin pathway in the control of muscle metabolism.

6. INDEPENDENT CONFIRMING EVIDENCE

Initial independent confirmation comes from a study on isolated muscle fibers and whole mouse muscles. Exposure to insulin increased calcium entry into individual muscle cells. Maneuvers that reduced this entry also reduced the uptake of 2-deoxy-glucose, an analog used to measure glucose transport; conversely, those that increased calcium entry increased insulin-stimulated glucose uptake. The effect was observed in both normal muscles and ob/ob mice with insulin resistance. The authors did not detect an inhibition of insulin-induced AKT or ERK activation, suggesting a relatively late role for calcium, which is compatible with GLUT4 trafficking [1]. This evidence directly confirms the passage in the book stating that calcium can contribute to insulin action in muscle, although it remains an animal experiment.A second study by the same independent research group identified a possible calcium entry point through TRPC3, a cell membrane channel. In adult muscle cells from normal and ob/ob mice, silencing TRPC3 reduced diacylglycerol analog-induced calcium entry by approximately 70% and reduced insulin-mediated glucose uptake. Furthermore, TRPC3 and GLUT4 were found to be localized in close proximity within the transverse tubules, which are invaginations of the muscle fiber membrane [2]. The work supports an upstream part of the narrative, namely that calcium signals localized near the membrane can modulate insulin-induced glucose transport. However, it does not prove that TRPC3 is the only channel involved.Further independent validation was obtained in mouse skeletal muscle, where insulin and muscle contraction showed the use of different calcium signals. The study attributed a role to IP_3 and NAADP, a second messenger that mobilizes calcium from intracellular stores, in insulin-induced GLUT4 translocation and glucose uptake. In mice fed a high-fat diet, the defect selectively concerned insulin-induced calcium signaling and was associated with reduced NAADP formation [3]. This finding is consistent with the idea that calcium is not just a signal for contraction, but can also participate in a specific insulin pathway. However, it remains an animal model and does not define whether the ROS-RyR1-IP_3 mechanism observed in the original study is reproduced identically in humans.

7. CONFLICTING EVIDENCE

Available evidence indicates that not every increase in intracellular calcium improves insulin action. In a mouse model of susceptibility to malignant hyperthermia and in db/db mice, a chronic increase in resting intracellular calcium was associated with a reduction in insulin-stimulated muscle glucose uptake. In both models, treatment with dantrolene reduced intracellular calcium and increased glucose uptake in the impaired muscles, without increasing it in control muscles [4].This result does not directly refute the analyzed study, as it compares a persistent and pathological condition of elevated calcium with short, localized, and regulated signals generated by insulin. However, it necessitates an important distinction: the potential favorable contribution of calcium to insulin action depends on the intensity, duration, cellular location, and pathophysiological context. No directly conflicting evidence sufficiently relevant to the specific dual ROS-RyR1 and IP_3 pathway described in the original study has been identified; this does not prove the absence of contrary results.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication subject to the review from independent evidence. Most of the data concern cells or mouse models, not clinical studies on humans.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and molecular	Skeletal muscle cells with GLUT4myc	Insulin activates ROS-RyR1 and IP$_3$-calcium pathways that increase GLUT4 translocation.	biologically consistent evidence	moderate
[1]	Animal and ex vivo	Isolated fibers and muscles from normal and ob/ob mice	Reducing calcium entry decreased insulin-induced glucose uptake; increasing it enhanced it.	direct confirmation	moderate
[2]	Animal, cellular and molecular	Adult muscle cells from normal and ob/ob mice	Silencing TRPC3 reduced calcium entry and insulin-mediated glucose uptake.	indirect confirmation	moderate
[3]	Animal and physiological	Skeletal muscle and high-fat diet mice	Calcium signals associated with IP$_3$ and NAADP participated in insulin-induced GLUT4 translocation.	indirect confirmation	moderate
[4]	Animal, in vivo and in vitro	RYR1-p.R163C and db/db mice	Chronically elevated intracellular calcium was associated with lower insulin-induced glucose uptake.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed credibly supports the idea that insulin can generate intracellular calcium signals in muscle cells through ROS-RyR1 and IP$_3$ pathways. These signals are associated with an increase in GLUT4 translocation, a relevant step for glucose entry into the cell.Independent evidence confirms that regulated calcium modulation can contribute to insulin-induced glucose transport in muscle. However, this confirmation is primarily experimental, obtained in cells and animals. It does not directly demonstrate the entire chain proposed in the chapter, including the activation of cPLA2a. The role of calcium therefore appears well-supported at a mechanistic level, but requires validation in humans and a clear distinction between short physiological signals and chronic pathological increases.

Aggiornato al 24/11/2026. Questo contenuto riflette una sintesi divulgativa delle evidenze scientifiche al momento della pubblicazione e non sostituisce il parere medico.

10. TRANSPARENCY AND SCIENTIFIC RESPONSIBILITY

Artificial Intelligence was used solely as a tool for editorial and documentary support. All content, interpretations, bibliographic citations and conclusions have been verified, reviewed and approved by the author, who assumes full scientific responsibility for them.

The manuscript was also subjected to independent peer review prior to publication.

To ensure the greatest transparency, the complete history of the article, including the dates of submission, revision, acceptance and publication, as well as information on the peer review process, is available on the dedicated Article History page.

11. RIFERIMENTI BIBLIOGRAFICI

[1] **The Role of Ca2+ Influx for Insulin-Mediated Glucose Uptake in Skeletal Muscle**
Lanner et al., 2006
Diabetes
DOI: https://doi.org/10.2337/db05-1613

[2] **Knockdown of TRPC3 with siRNA coupled to carbon nanotubes results in decreased insulin mediated glucose uptake in adult skeletal muscle cells**
Lanner et al., 2009
The FASEB Journal
DOI: https://doi.org/10.1096/fj.08-116814

[3] **Exercise Ameliorates Insulin Resistance via Ca2+ Signals Distinct From Those of Insulin for GLUT4 Translocation in Skeletal Muscles**
Park et al., 2015
Diabetes
DOI: https://doi.org/10.2337/db14-0939

[4] **Chronic Elevation of Skeletal Muscle [Ca2+]i Impairs Glucose Uptake. An in Vivo and in Vitro Study**
Uryash et al., 2022
Frontiers in Physiology
DOI: https://doi.org/10.3389/fphys.2022.872624