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

ORIGINAL RESEARCH
ISSN 2845-7501

Calcium influx: a possible role in the insulin modulation of intracellular distribution and 6-phosphofructo-1-kinase activity in human erythrocytes - Evidence review

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1. ABSTRACT

The analyzed study evaluated whether insulin can rapidly modify intracellular calcium in human erythrocytes, i.e., red blood cells. According to the available abstract, insulin stimulation increased calcium influx by up to four times, with an effect dependent on dose, tyrosine kinase signaling, and verapamil-sensitive channels. The increase in calcium was also linked to the activation of 6-phosphofructo-1-kinase, an enzyme involved in glycolysis.In paragraph 5.4.2 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, this research contributes to the passage stating that insulin can activate calcium-dependent signals even in non-classical cells. Independent studies on erythrocytes and muscle cells confirm that insulin can increase intracellular calcium, but indicate different mechanisms and timings depending on the model. Some results on erythrocytes, however, show a non-uniform pattern of calcium influx. The link with the functional activation of cPLA2a remains mechanistic and was not directly verified in the original study.

ONLINE ARTICLE

ARTICLE INFORMATION

Published: November 25, 2026
Updated: November 25, 2026

DOI 10.5281/zenodo.24530287

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2. ANALYZED STUDY

The research question was whether insulin exerts a direct action on human erythrocytes, cells traditionally considered primarily as oxygen transporters, by modifying intracellular calcium and glucose metabolism. The hypothesis was that insulin binding to its receptor could promote calcium entry into the cell and, through this signal, increase the activity of 6-phosphofructo-1-kinase, an enzyme that regulates an important step in glucose utilization.The relevance to the book's section is precise. The publication documents a segment of the narrative: insulin activation of a tyrosine kinase-dependent pathway increased calcium entry in human cells not typically associated with insulin response. However, it does not evaluate cPLA2a, i.e., cytosolic phospholipase A2 alpha, nor the release of arachidonic acid, nor an inflammatory response. Therefore, it can support the initial passage regarding the calcium signal, but not the entire chain described in section 5.4.2 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The available material consists exclusively of the abstract; therefore, all information not present in the abstract is not reported in the available material. The study examined human erythrocytes subjected to insulin stimulation and measured calcium entry into the cell. The relationship between insulin concentration and response, the role of tyrosine kinase activity, and the effect of interventions involving verapamil-sensitive calcium channels were also evaluated.The authors also linked the increase in intracellular calcium to calmodulin, a protein that detects calcium and can modify the activity of other enzymes, and to the activity of
6-phosphofructo-1-kinase. They also evaluated the detachment of this enzyme from the erythrocyte membrane. The sample size, donor characteristics, inclusion and exclusion criteria, collection period, complete insulin concentrations, incubation times, detailed analytical techniques, predefined endpoints, statistical analyses, and significance values are not reported in the available material.

4. RESULTS

The main finding is that, following insulin stimulation, calcium influx into human erythrocytes was approximately four times higher than in the basal condition. In simple terms, insulin caused a greater amount of calcium to enter the cell, an ion that acts as a rapid signal and can modify the activity of many cellular proteins. The abstract does not report absolute calcium values, sample sizes, confidence intervals, or P-values.The effect was described as dose-dependent: it varied based on the insulin concentration. However, at insulin concentrations equal to or greater than 150 nM, the increase in calcium influx decreased. This direction of the effect is important: the result does not describe an indefinite linear increase in the response as insulin levels rise.The authors also report that the insulin-induced increase depended on tyrosine kinase activity, meaning an enzymatic process compatible with insulin receptor activation, and involved verapamil-sensitive channels. The increased intracellular calcium, together with calmodulin, stimulated the activity of 6-phosphofructo-1-kinase and promoted the detachment of the enzyme from the red blood cell membrane. In the available material, these results support the hypothesis of increased glucose consumption in erythrocytes, but they do not demonstrate a metabolic effect in the whole organism.

5. INTERPRETATION

The biological significance of the result is that insulin can produce a rapid signal even in red blood cells, increasing the calcium present inside the cell. This finding expands the idea that insulin action does not only coincide with the control of glycemia in classic tissues, such as muscle, liver, and adipose tissue.The segment supported by the publication is: insulin tyrosine kinase-dependent signaling calcium entry. The subsequent activation of calmodulin and 6-phosphofructo-1-kinase concerns erythrocyte metabolism, not cPLA2a. Consequently, the data is biologically consistent with the passage in paragraph 5.4.2 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, according to which insulin can trigger calcium-dependent pathways even in non-classic cells.The essential limitation is interpretive: erythrocytes are cells lacking a nucleus

and have a physiology very different from that of muscle, immune, or endothelial cells. The study does not show that insulin-induced calcium activates cPLA2a, does not measure the translocation of the enzyme toward membranes rich in arachidonic acid, and does not document a pro-inflammatory consequence. The connection to these events therefore remains a partial mechanistic reconstruction.

6. INDEPENDENT CONFIRMING EVIDENCE

Direct confirmation in human erythrocytes comes from Barbagallo, Gupta, and Resnick. In blood samples from healthy volunteers, the authors incubated isolated erythrocytes with insulin and measured intracellular free calcium using nuclear magnetic resonance. At 200 µU/mL, calcium increased from 24.6 ± 2.5 nmol/L at baseline to 66.4 ± 11 nmol/L after 120 minutes; the increase was statistically significant compared to baseline. The result confirms that insulin can increase intracellular calcium in human red blood cells, although it did not verify verapamil-sensitive channels or the role of 6-phosphofructo-1-kinase [1]. The limitation lies in the small sample size and the *in vitro* design, which does not allow for determining how much this phenomenon contributes to the physiology of the organism.More recent research has again observed an increase in calcium in isolated human erythrocytes after acute exposure to insulin. In this study, the calcium signal measured with a fluorescent probe increased with insulin, was abolished by the removal of extracellular calcium with EGTA, and was prevented by a mechanosensitive channel inhibitor. The authors proposed the involvement of Piezo1, a channel that responds to mechanical stimuli of the membrane. This evidence confirms the direction of the effect observed in the analyzed study—that is, greater calcium influx after insulin—but suggests a mechanism different from that attributed to verapamil-sensitive channels [2]. The study included five apparently healthy volunteers for the human experiments; the small size and experimental nature require caution.The more general part of the narrative, according to which insulin can generate early calcium signals in other cell types as well, is supported by experiments on myotubes, cultured immature muscle cells. Insulin produced a very rapid increase in calcium, lasting less than a second, which disappeared in the absence of extracellular calcium and was blocked by nifedipine or ryanodine. In cells lacking specific components of calcium channels or ryanodine receptors, the insulin signal did not appear [3]. These data show that calcium can be part of the insulin response in muscle cells, but they derive from rat and mouse cell models, not from clinical studies on humans.

7. CONFLICTING EVIDENCE

There is directly relevant evidence that warrants caution. In an *in vitro* study on erythrocytes from 16 healthy adults, insulin at 120 mU/L reduced the measured uptake of radiolabeled calcium between 10 and 120 minutes. At 60 minutes, uptake was 508 ± 59 pmol/mL of red blood cells with insulin compared to 529 ± 59 pmol/mL in controls; at 120 minutes, it was 742 ± 109 compared to 767 ± 127 pmol/mL. Both differences were statistically significant [4].This result does not eliminate the scientific correspondence between the original study and the book's paragraph, but it shows that the calcium response in erythrocytes depends on the measurement method, the observed time, and the insulin concentration. The same authors reported inconsistent results as concentrations varied and did not rule out a biphasic effect. A possible explanation is that initial entry, calcium efflux from the cell, and measured intracellular concentration are not equivalent. No sufficiently relevant directly conflicting evidence has been identified regarding whether insulin can evoke calcium signals in muscle cells; this does not prove the absence of contrary results.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the original study from independent publications and indicates when a methodological difference limits the comparability of the results.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Experimental cellular	Human erythrocytes; sample size not reported in available material	Insulin increased calcium entry up to fourfold; the effect was dose-dependent and decreased at concentrations of 150 nM or higher.	direct confirmation	moderate
[1]	Experimental cellular	Erythrocytes isolated from healthy volunteers; n=10 for time-course studies	Insulin increased intracellular free calcium in a dose- and time-dependent manner.	direct confirmation	moderate
[2]	Experimental cellular	Human erythrocytes isolated from five healthy volunteers	Insulin increased cytosolic calcium; EGTA and a mechanosensitive channel inhibitor prevented the increase.	direct confirmation	moderate
[3]	Cellular	Primary rat myotubes and murine muscle cell lines	Insulin induced a rapid and transient increase in calcium, dependent on extracellular calcium and calcium channel components.	indirect confirmation	moderate
[4]	Experimental cellular	Erythrocytes isolated from 16 healthy adults	At 120 mU/L, insulin reduced radiolabeled calcium uptake between 10 and 120 minutes; the response to different doses was inconsistent.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed specifically supports the claim that insulin can increase calcium entry into human erythrocytes and that this signal is associated with changes in cellular metabolism. Independent evidence confirms that insulin can increase intracellular calcium in red blood cells and experimental muscle cells.The narrative regarding the role of calcium as a component of insulin action is therefore supported at a mechanistic level. However, the mechanism does not appear identical in all models, and some data on erythrocytes show a response that depends on experimental conditions. The link between increased calcium, cPLA2a activation, and subsequent arachidonic acid mobilization requires direct validation in relevant models.

Aggiornato al 25/11/2026. 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.

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