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Essential role of protein kinase B (PKB) in insulin-induced inactivation of glycogen synthase kinase 3. Characterization of a dominant-negative PKB mutant - Review of evidence

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1. ABSTRACT

The analyzed publication evaluated whether AKT, also known as protein kinase B or PKB, is the necessary link between the insulin signal and the inactivation of GSK-3, a normally active kinase that can restrain certain cellular processes. The authors used a dominant-negative PKB mutant, i.e., a modified version of the protein capable of hindering the activation of normal PKB. In the available material, consisting only of the abstract, the number of experiments, the precise type of cells used, and complete quantitative data are not reported.When PKB was blocked, insulin no longer inactivated GSK-3; furthermore, PKB and GSK-3 were detected in the same molecular complex. The study therefore contributes to the passage in "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi regarding the role of AKT in the inhibitory phosphorylation of GSK-3b. Independent research confirms the insulin–PI3K–AKT–GSK-3 axis in biochemical, cellular, and insulin-sensitive tissue models. However, in specific pathological contexts, GSK-3b can also be regulated by circuits other than AKT. The confirmation is therefore solid on a mechanistic level, but it does not equate to proving that AKT is the only possible regulator of GSK-3b in every tissue or disease.

ONLINE ARTICLE

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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 protein kinase B, now commonly called AKT, was truly necessary to turn off GSK-3 after an insulin stimulus. GSK-3, short for glycogen synthase kinase 3, is an enzyme that adds phosphate groups to other proteins and, in many cells, is active even under basal conditions. Insulin activates a sequence of intracellular signals in which PI3K (phosphoinositide 3-kinase, an enzyme that produces membrane signaling lipids) contributes to the activation of AKT.The publication is relevant to section 4.12 because it addresses the central segment of the chain described in the book: insulin activation of PI3K and AKT, followed by the inactivation of GSK-3b. In particular, it supports the claim that AKT and GSK-3b can be found in the same molecular complex. However, it should be noted that
co-immunoprecipitation, a technique that isolates proteins present in the same cell extract together, documents an association in a complex but does not by itself prove a direct, exclusive, and stable contact between two purified proteins.The study can therefore demonstrate, in the cell model examined, that PKB activity is necessary for the insulin-mediated inactivation of GSK-3 and that the two proteins are associated in an experimentally detectable complex. It cannot prove that this mechanism is identical in all human tissues, nor that AKT is the only kinase capable of modifying GSK-3b in every biological context.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The only material available is the abstract of the publication. The authors constructed a variant of PKB called PKB-CAAX, described as a dominant-negative mutant. In simple terms, this is an altered version of the protein that interferes with the normal activation of PKB induced by growth factors, despite being expressed at levels similar to unmodified PKB.The mutant was expressed in cells and used to evaluate the effect of blocking PKB on insulin-induced GSK-3 regulation. The authors also used co-immunoprecipitation to verify whether PKB and GSK-3 could be recovered in the same protein complex. An oncogenic form of PKB, called gagPKB, was also analyzed to observe the subcellular distribution of GSK-3.The detailed experimental design, the type and number of cell lines, the number of replicates, insulin concentrations, duration of exposures, complete controls, statistical criteria, and significance values are not reported in the available material. Furthermore, no complete numerical data on the extent of GSK-3 inhibition or the intensity of the association between the proteins are provided.

4. RESULTS

The key finding is that PKB-CAAX efficiently inhibited PKB activation triggered by growth factors and, simultaneously, prevented the insulin-induced regulation of GSK-3. In simple terms, when the authors blocked the ability of AKT/PKB to activate, insulin lost the ability to turn off GSK-3 in their cell model. The authors therefore concluded that PKB activation was necessary for insulin-induced GSK-3 inactivation.The study also showed that PKB and GSK-3 co-immunoprecipitated. This means that, in cell extracts, the two proteins were recovered in the same complex. This finding is consistent with a functional proximity that could make the inhibitory phosphorylation of GSK-3 efficient. However, the abstract does not report quantitative measurements of the association, and co-immunoprecipitation alone does not establish whether the interaction is direct or mediated by other proteins.Finally, the transforming form gagPKB shifted GSK-3 from the cytosolic fraction—that is, the fluid part inside the cell—to the membrane fraction. This observation indicates that an altered and persistently active PKB can modify the cellular localization of GSK-3. The physiological significance of this shift cannot be defined with certainty from the available data, nor can it be automatically transferred to normal cells exposed to insulin.

5. INTERPRETATION

The primary biological significance is that AKT can function as a link between the signal received from insulin and the reduction of GSK-3 activity. When GSK-3b is phosphorylated at the Ser9 residue—that is, at a specific amino acid of the protein—its activity decreases. In many cells, this helps remove a brake placed by GSK-3 on processes such as the activation of glycogen synthase, an enzyme involved in the storage of glucose in the form of glycogen.Compared to the

book's paragraph, the specifically documented segment is: insulin activation of PKB/AKT inactivation of GSK-3, with the association of PKB and GSK-3 in the same molecular complex. The study does not directly demonstrate all the broader consequences attributed in the paragraph to the AKT–GSK-3b axis, such as neuronal survival, muscle mass control, platelet function, or effects in neurodegenerative and tumor diseases.The authors' conclusion that PKB is necessary and sufficient in their experimental system is consistent with the design based on a dominant-negative mutant. The main limitation is that the full text is not available in the provided material. It is therefore not possible to directly verify the statistical robustness, reproducibility across different models, or the precision of individual experiments. Furthermore, the book's statement that the complex makes the process "extremely efficient" represents a plausible interpretation, but the efficiency of phosphate transfer is not quantified in the available abstract.

6. INDEPENDENT SUPPORTING EVIDENCE

Initial independent confirmation was provided by Cross and colleagues in a previous study, conducted using biochemical and cellular approaches. The authors ruled out that two candidate kinases, p90RSK and p70S6K, could explain the insulin-induced inhibition of GSK-3 under the conditions examined. They subsequently identified PKB/AKT as the insulin-activated kinase capable of inactivating GSK-3, while PI3K inhibitors blocked both PKB activation and GSK-3 inhibition. This research confirms the narrative thread of the PI3K AKT reduced GSK-3 activity pathway. It is a direct confirmation of the mechanism in experimental models, but it is not a clinical study and does not demonstrate the absolute uniqueness of AKT in all cell types [1].A second independent study extended the observation to two tissues particularly relevant to the insulin response: skeletal muscle and adipose tissue. Following insulin stimulation, PKBa activity increased more than tenfold, and GSK-3 activity decreased by 50 ± 10%. Rapamycin did not prevent either PKB activation or the decrease in GSK-3 within the first five minutes. In practical terms, the data show that the temporal relationship of increasing AKT and decreasing GSK-3 is also observable in metabolically important tissues and does not depend, under those acute conditions, on the pathway blocked by rapamycin. The limitation is that the temporal and pharmacological association strengthens the model, but does not by itself equate to a demonstration of direct physical interaction between AKT and GSK-3 [2].Taken together, these studies are independent of the publication analyzed and converge on the fact that insulin can activate AKT and reduce GSK-3 activity through a PI3K-dependent pathway. They therefore support the mechanistic core of the paragraph: AKT is an important regulator of GSK-3 inactivation in insulin signaling.

7. CONFLICTING EVIDENCE

No directly conflicting evidence sufficiently relevant to the fact that AKT can inactivate GSK-3 following insulin stimulation has been identified; this does not prove the absence of contrary results. However, there is data that limits the generalization of the idea that AKT is always the only relevant pathway.In a model of tuberous sclerosis, a genetic condition associated with impaired mTORC1 regulation, Pal and colleagues observed that insulin could promote the phosphorylation and inactivation of GSK-3b through ERK1/2, a different family of kinases, without a measurable reduction in AKT or mTORC1 activity. Inhibition of ERK1/2 restored GSK-3b activity and reduced protein synthesis in the experimental model. This result does not negate the role of AKT demonstrated by the original study; instead, it shows that in cells with TSC2 alterations and in other experimental contexts examined by the authors, the regulation of GSK-3b can depend on alternative circuits. The limitation is significant: these are primarily cellular and animal models of disease, not the normal insulin response in humans [3].

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the study subject to the Evidence Review from independent publications that confirm or define the scope of the book's narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and molecular	Cell model not specified in the available abstract	Blocking PKB prevented insulin-induced GSK-3 inactivation; PKB and GSK-3 co-immunoprecipitat	direct confirmation	moderate
[1]	Cellular and biochemical	Experimental models with cells and muscle	PI3K and PKB/AKT were found to be necessary for insulin-mediated phosphorylation and inhibition of GSK-3 under the studied conditions.	direct confirmation	moderate
[2]	Animal and ex vivo tissue	Skeletal muscle and adipocytes	Insulin increased PKBa more than tenfold and reduced GSK-3 activity by 50 ± 10%.	direct confirmation	moderate
[3]	Cellular and animal	Tsc2−/− cells, HEK cells, and tuberous sclerosis model	In a pathological context, ERK1/2 contributed to the inhibitory phosphorylation of GSK-3b independently of AKT.	discordant evidence	preliminary

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The analyzed study specifically supports the finding that AKT/PKB is an essential mediator of insulin-induced GSK-3 inactivation in the experimental model examined. The association of PKB and GSK-3 within the same molecular complex is consistent with close functional regulation, but it does not, by itself, prove a direct link between purified proteins.Independent evidence confirms that the PI3K–AKT–GSK-3 axis operates in experimental models and in tissues that are metabolically sensitive to insulin. The book's narrative is therefore well-supported at a mechanistic level. It remains necessary to avoid overgeneralization: other signaling pathways may contribute to the regulation of GSK-3b in specific cellular or pathological contexts.

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.

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