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Constitutive activity of glycogen synthase kinase-3β: positive regulation of steady-state levels of insulin receptor substrates 1 and 2 in adrenal chromaffin cells - Evidence review

Dott. A. Conte[1]

1 Nutritional Biologist, Italy

1. ABSTRACT

The analyzed study evaluated whether the activity of GSK-3β, a protein that regulates numerous cellular signals, contributes to maintaining the quantity of IRS-1 and IRS-2, two proteins essential for transmitting the insulin signal within the cell. In cultured bovine chromaffin cells, pharmacological inhibition of GSK-3β or its insulin-induced inactivation reduced the protein levels of IRS-1 and IRS-2. Experiments with protein degradation inhibitors indicated a prevalent role for the proteasome, the cellular system that eliminates proteins that are no longer needed or are damaged.The work contributes to the narrative of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi because it documents the quantitative side of the relationship between GSK-3β and IRS proteins: the kinase can reduce their functional efficiency under certain conditions, but in the specific cellular model examined, it also helps to prevent their excessive decrease. Independent studies confirm that increased GSK-3 activity can hinder insulin signaling and that reducing it improves certain post-receptor steps in animal models. However, the dual function regarding IRS levels remains demonstrated primarily in experimental systems and does not constitute direct evidence in humans.

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2. ANALYZED STUDY

The research question was whether the basal activity of GSK-3b—that is, its activity present even in the absence of immediate stimulation—regulates the available quantity of IRS-1 and IRS-2 in bovine adrenal chromaffin cells. IRS-1 and IRS-2 are adapter proteins: they collect signals from the insulin receptor and transmit them to intracellular pathways that regulate metabolism, cell growth, and survival.The authors' hypothesis was that GSK-3b does not act solely as a brake on certain steps of insulin signaling, but also contributes to the stability of IRS proteins. This distinction is relevant to section 4.13 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi: the book describes an apparent paradox in which GSK-3b can reduce the functional efficiency of IRS-1 but, under specific conditions, contribute to maintaining the cellular quantity of IRS-1 and IRS-2.The study can demonstrate molecular variations in cultured cells and suggest the mechanisms involved. However, it cannot establish that the same balance occurs identically in muscle, liver, adipose tissue, or in people with insulin resistance. Furthermore, it cannot demonstrate that pharmacologically modifying GSK-3b represents an effective or safe treatment in humans.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors used chromaffin cells derived from the bovine adrenal medulla and maintained in culture. The number of biological preparations and the selection criteria for the material are not reported in the available material, which consists only of the abstract.To reduce GSK-3 activity, lithium chloride was used at concentrations between 1 and 20 millimolar for 12 hours, and SB216763, described by the authors as a selective GSK-3 inhibitor, was used at concentrations between 0.1 and 30 micromolar for 12 hours. Exposure to insulin, at 100 nanomolar for 24 hours, was also evaluated. Phosphorylation of serine 9 of GSK-3b was used as an indicator of its inactivation: greater phosphorylation at that site is generally associated with lower kinase activity.To clarify how IRS-1 and IRS-2 levels decreased, the authors used proteasome inhibitors—the system that selectively degrades proteins marked for elimination—as well as inhibitors of calpain and lysosomes, other cellular degradation pathways. They also measured the messenger RNA levels of IRS-1 and IRS-2, the molecule that carries the information necessary to produce a protein. The laboratory techniques and detailed statistical analyses are not reported in the available material.

4. RESULTS

In simple terms, pharmacologically reducing GSK-3b activity decreased the amount of IRS-1 and IRS-2 in the cells. With lithium chloride for 12 hours, the inhibitory phosphorylation of GSK-3b at serine 9 increased by approximately 44%. In the same interval, IRS-1 decreased by about 38% and IRS-2 by about 62%, with a concentration-dependent effect.The result was also observed with SB216763. After 12 hours, IRS-1 decreased by about 38% and IRS-2 by about 48%; simultaneously, beta-catenin, a protein normally degraded under the control of GSK-3 as well, increased by about 47%. This latter finding is consistent with effective GSK-3 inhibition, but it does not completely rule out the possibility that the drugs had additional cellular effects.Insulin, administered for 24 hours, increased the inhibitory phosphorylation of GSK-3b by approximately 104%. In parallel, IRS-1 decreased by about 41% and IRS-2 by about 72%. After washing the cells previously exposed to insulin for 12 hours, both GSK-3b phosphorylation and IRS protein levels returned to the levels of untreated cells within 24 hours. This trend indicates reversibility in the studied model, not permanent cellular damage.Proteasome inhibitors almost completely prevented the decrease of IRS-1 and countered that of IRS-2 induced by lithium or SB216763, by approximately 100% and 69%, respectively. Conversely, calpain and lysosome inhibitors did not prevent the reduction of IRS proteins. For IRS-2, but not for IRS-1, lithium and SB216763 also reduced the relative messenger RNA. The observed picture therefore suggests that GSK-3b inhibition reduces IRS-1 primarily by promoting its proteasomal degradation and reduces IRS-2 both through protein degradation and through lower messenger RNA availability.

5. INTERPRETATION

The main significance of this work is that the relationship between GSK-3b and IRS is not unidirectional. When GSK-3b phosphorylates specific sites on IRS-1, it can hinder insulin signal transmission. However, in the bovine chromaffin cells examined in this study, the presence of basal GSK-3b activity also contributes to maintaining IRS-1 and IRS-2 levels, preventing them from decreasing via the proteasome.The segment documented directly by the publication subject to the Evidence Review is therefore: inactivation of GSK-3b reduction of cellular levels of IRS-1 and IRS-2 predominant involvement of proteasomal degradation; for IRS-2, a reduction in messenger RNA expression is also added. This is the experimental basis for the passage in the book regarding the maintenance of the quantitative balance of IRS proteins.This data does not necessarily contradict studies that attribute a negative role to GSK-3 in insulin sensitivity. A protein can, in fact, have different functions depending on the target, the tissue, the duration of the stimulus, and the metabolic state of the cell. However, the study does not directly measure glucose transport, insulin resistance, or clinical outcomes; therefore, it does not prove that the reduction of IRS induced by GSK-3b inhibitors causes metabolic disease in the organism.

6. INDEPENDENT CONFIRMING EVIDENCE

Initial confirmation of the functional aspect of this narrative comes from an in vitro experiment using Chinese hamster ovary cells expressing the insulin receptor and IRS-1. Phosphorylation of IRS-1 by GSK-3 reduced the insulin-induced phosphorylation of both IRS-1 and the insulin receptor. In simple terms, when GSK-3 modifies IRS-1 at specific sites, the adapter transmits the signal less effectively and can also hinder the upstream receptor. This research confirms the branch of the chain where GSK-3 limits the functional efficiency of IRS-1, but it does not evaluate the quantitative stability of IRS-1 and IRS-2 observed in the original study [1].A second study examined isolated muscles from obese Zucker rats, a model of prediabetic insulin resistance. Selective inhibition of GSK-3 increased insulin-stimulated glucose transport by 24% in the epitrochlearis muscle and by 20% in the soleus. In the soleus, it also increased IRS-1 phosphorylation by 45%, the association of IRS-1 with the p85 subunit of PI3K by 72%, and AKT phosphorylation by 30%. The insulin receptor, however, remained unchanged. These results support the passage in the book suggesting that GSK-3 hyperactivity may primarily compromise events downstream of the receptor, though the model is animal-based and drug exposure was brief [2].Confirmation on a genetic level comes from mice with reduced GSK-3b availability. In mice with only one functional copy of the insulin receptor, the genetic reduction of GSK-3b improved whole-body glucose disposal and reduced insulin resistance. In mice lacking IRS-2, characterized by GSK-3b activity in pancreatic islets approximately four times higher than normal, the reduction of GSK-3b preserved beta cells and prevented the onset of diabetes. This finding is consistent with the pathophysiological relevance of the IRS–AKT–GSK-3b axis, but it does not directly demonstrate that GSK-3b maintains IRS protein levels in human cells [3].

7. CONFLICTING EVIDENCE

No directly conflicting evidence of sufficient relevance has been identified; this does not prove the absence of contrary results. The main caution stems from context-dependency: in the original study, the inhibition of GSK-3b reduced IRS-1 and IRS-2 in bovine chromaffin cells, whereas in studies on insulin-resistant muscle, the reduction of GSK-3 activity improved downstream IRS-1 signaling and glucose transport [2].This difference does not equate to a clear experimental contradiction. The models involve different cells and tissues, use different exposure times, and measure different outcomes: the abundance of IRS proteins in the first case, and insulin response capacity in the second. The available literature therefore indicates that GSK-3b cannot be described solely as a negative regulator or solely as a stability factor for IRS proteins; both functions can emerge under different biological conditions.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the original study, which clarifies the quantitative maintenance of IRS-1 and IRS-2 in a specific cellular model, from independent publications that confirm other segments of the same biological narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Experimental cellular	Cultured bovine adrenal chromaffin cells	GSK-3b inhibition reduced IRS-1 and IRS-2; the proteasome mediated much of the protein reduction.	direct confirmation	preliminary
[1]	Cellular and biochemical	Recombinant proteins and Chinese hamster ovary cells with IRS-1 and insulin receptor	Phosphorylation of IRS-1 by GSK-3 reduced the tyrosine kinase response of the insulin receptor.	direct confirmation	moderate
[2]	Ex vivo animal	Soleus and epitrochlearis muscles of prediabetic obese Zucker rats	GSK-3 inhibition increased IRS-1–PI3K–AKT signaling and insulin-stimulated glucose transport.	indirect confirmation	moderate
[3]	Genetic animal	Mouse models of insulin resistance with reduced GSK-3b expression	Genetic reduction of GSK-3b improved glycemic control and prevented diabetes in the IRS-2 deficient model.	biologically consistent evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed shows that, in cultured bovine chromaffin cells, basal GSK-3b activity helps maintain the levels of IRS-1 and IRS-2. When the kinase is inhibited, both proteins decrease; proteasomal degradation accounts for much of this effect, and IRS-2 also decreases at the messenger RNA level.The book's narrative is supported at a mechanistic level: GSK-3b can reduce the efficiency of IRS-1 in certain contexts, but it can also participate in controlling its quantity and that of IRS-2. Independent studies reinforce the role of GSK-3 as a brake on post-receptor insulin signaling in cell and animal models. Validation in human metabolic tissues and clinical studies remains necessary before attributing a direct therapeutic significance to this mechanism.

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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