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Short-term in vitro inhibition of glycogen synthase kinase 3 enhances insulin signaling in skeletal muscle type I of Zucker Diabetic Fatty rats - Evidence review

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1. ABSTRACT

The analyzed study evaluated whether selective inhibition of GSK-3 (glycogen synthase kinase 3, an enzyme that can reduce certain cellular responses to insulin) improved insulin signaling in isolated soleus muscle from Zucker Diabetic Fatty rats, an animal model of type 2 diabetes. After in vitro exposure to the inhibitor CT98014, the response to insulin was greater: glucose transport, insulin receptor phosphorylation, and phosphorylation of IRS-1 and AKT increased, while a phosphorylation of IRS-1 associated with reduced functionality decreased.In section 4.13 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, the research contributes to the narrative that excessive GSK-3b activity can attenuate insulin signaling downstream of the receptor. Independent studies in cells and in the muscle of insulin-resistant rats confirm the biological consistency of the GSK-3 → IRS-1 → PI3K/AKT chain. However, clinical evidence in humans remains limited, and it cannot be inferred that pharmacological inhibition of GSK-3 represents a proven treatment for diabetes.

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MISSION

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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.

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2. ANALYZED STUDY

The research question was whether high GSK-3 activity contributes to the defect in how skeletal muscle responds to insulin in experimental diabetes. The hypothesis was that selectively blocking this enzyme could make the transmission of the insulin signal more efficient toward the mechanisms that facilitate glucose entry into the cell.The relevant point for section 4.13 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi is the post-receptor part of the insulin cascade. After insulin binds to its receptor, IRS1 (insulin receptor substrate 1, an adapter protein that transmits the signal) helps activate PI3K and AKT, which are essential proteins for the metabolic response. GSK-3 can interfere with this sequence through phosphorylation on serine residues of IRS1—chemical modifications that, depending on the site affected, can reduce its efficiency.As this was an experiment on isolated rat muscle, the publication can document an acute biological effect in that model. It cannot establish on its own whether the same intervention produces clinical benefits in people with type 2 diabetes, nor can it prove that GSK-3 is the sole cause of insulin resistance.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The study used isolated soleus muscles, which are muscles predominantly composed of slow-twitch, oxidative fibers, taken from male Zucker Diabetic Fatty rats with overt diabetes. Insulin-sensitive lean Zucker rats were examined as a physiological control. The muscle was studied *in vitro*, meaning outside the organism, under controlled conditions.The muscle preparations were exposed to the compound CT98014, a selective GSK-3 inhibitor, at a concentration of 1 micromole per liter. Insulin was used at a maximum concentration of 5 mU/mL. Insulin-stimulated glucose transport and the phosphorylation of proteins belonging to the insulin signaling cascade were measured: the insulin receptor, IRS-1, AKT, and GSK-3b. The phosphorylation of IRS-1 at serine 307 was also evaluated, a modification associated in various experimental models with reduced efficiency of the insulin signal.The techniques included glucose transport assays and immunoblot analysis of phosphorylated proteins. The total number of animals, inclusion and exclusion criteria, breeding period, and any randomization are not reported in the available abstract. Information necessary to formally assess the risk of experimental bias is also not reported in the available material.

4. RESULTS

Before treatment, the muscle of Zucker Diabetic Fatty rats showed lower insulin-stimulated phosphorylation of GSK-3b at serine 9 compared to the muscle of lean rats: the reduction was 35% and was statistically significant. Since this phosphorylation normally contributes to inhibiting GSK-3b, the result is consistent with increased enzyme activity in the diabetic model.In the absence of insulin, the inhibitor produced no detectable effects on the measured parameters. In the presence of insulin, however, CT98014 increased glucose transport in the muscle by 28%. In simple terms, the treated muscle responded better to the hormone in transferring glucose from the external environment into the cells.The functional improvement was accompanied by a strengthening of the signaling cascade. Tyrosine phosphorylation of the insulin receptor increased by 52% and that of IRS-1 by 50%. Phosphorylation of AKT at serine 473, a signal linked to its activation, increased by 48%. Furthermore, the inhibitory phosphorylation of GSK-3b at serine 9 increased by 36%.Simultaneously, phosphorylation of IRS-1 at serine 307 was reduced by 26%. This is the direction of the result most relevant to the book's text: a modification of IRS-1 considered unfavorable to the insulin response decreased, while modifications associated with signal propagation increased. The study also observed a 31% reduction in the phosphorylation of JNK1/2, a family of kinases that can contribute to the inhibitory phosphorylation of IRS-1.The results therefore show that, in isolated diabetic muscle, acute inhibition of GSK-3 is associated with a coordinated increase in insulin signaling and glucose transport. However, they do not prove that every observed variation depends exclusively on the direct phosphorylation of IRS-1 by GSK-3: JNK and other pathway regulators may also contribute to the phenomenon.

5. INTERPRETATION

The general biological significance is that GSK-3 can act as a brake on the insulin response when its activity remains high. In the model studied, temporarily removing this brake made the sequence starting from the insulin receptor, passing through IRS-1 and AKT, and culminating in greater glucose transport in the muscle more effective.The segment of the chain most directly documented by the publication is: inhibition of GSK-3 reduction of IRS-1 phosphorylation at serine 307 and increase of IRS-1 phosphorylation on tyrosine greater activation of AKT increased glucose transport. The data on the insulin receptor is consistent with an improvement in the initial events of the cascade as well, but it does not identify with certainty the molecular mechanism that links GSK-3 to the increased phosphorylation of the receptor.This interpretation is consistent with the statement in the book regarding the increase in the phosphorylation of the insulin receptor, IRS-1, and AKT, and the reduction of the inhibitory phosphorylation of IRS-1. However, the model is animal-based, the treatment was performed on isolated tissue, and the effect was observed in the short term. It is therefore not possible to translate the result into a therapeutic conclusion for humans.

6. INDEPENDENT SUPPORTING EVIDENCE

Initial confirmation of the central narrative's key point comes from an independent cellular experiment. Eldar-Finkelman and Krebs showed that GSK-3 can phosphorylate IRS-1 and that this modification enables IRS-1 to hinder the tyrosine kinase activity of the insulin receptor. In cells expressing higher levels of GSK-3, insulin-induced phosphorylation of IRS-1 and the insulin receptor was reduced. The study therefore confirms the biological direction proposed in the book: higher GSK-3 activity is linked to lower efficiency of the insulin receptor–IRS-1 node [1]. The limitation is that the model consisted of genetically modified hamster ovary cells rather than intact human or animal muscle.A second study identified a possible molecular point of the brake exerted by GSK-3 with greater precision. In cells expressing the insulin receptor and IRS-1, phosphorylation of IRS-1 at serine 332 by GSK-3 attenuated the insulin signal. Mutations that prevented this phosphorylation increased insulin-induced tyrosine phosphorylation of IRS-1 and AKT activation. Furthermore, inhibition of GSK-3 with lithium reduced phosphorylation at serine 332 [2]. The site studied does not coincide with the serine 307 measured in the original study, but it reinforces the principle that certain GSK-3-dependent phosphorylations of IRS-1 can weaken the IRS-1–AKT pathway.The confirmation closest to the muscle model comes from prediabetic obese Zucker rats, in which a different selective GSK-3 inhibitor increased IRS-1 tyrosine phosphorylation, the association of IRS-1 with PI3K, and AKT phosphorylation. The increase in signaling was accompanied by greater insulin-stimulated glucose transport in the insulin-resistant muscle. The effect was not observed in the muscle of lean, insulin-sensitive rats and was not associated with an increase in insulin receptor phosphorylation [3]. This latter detail indicates that the improvement may occur primarily downstream of the receptor and that the contribution of the receptor itself may depend on the model, the metabolic state, and the muscle type.Taken together, these independent publications support a convergent chain of evidence. Cellular studies define the possible GSK-3–IRS-1 mechanism; the study on insulin-resistant rats confirms that inhibiting GSK-3 can increase signal transmission from IRS-1 to PI3K and AKT in muscle. The confirmation remains primarily mechanistic and preclinical, as clinical trials demonstrating the same circuit as an effective and safe therapeutic target in humans are still lacking.

7. CONFLICTING EVIDENCE

No directly conflicting evidence of sufficient relevance has been identified; this does not prove the absence of contrary results.However, there are significant contextual limitations. In an independent study on prediabetic Zucker rats, GSK-3 inhibition increased the phosphorylation of IRS-1, PI3K, and AKT without increasing insulin receptor phosphorylation [3]. This does not contradict the analyzed study, but it shows that the segment of the cascade most sensitive to GSK-3 inhibition may vary. Furthermore, GSK-3 has many cellular functions beyond the regulation of glucose metabolism; therefore, the effects of an inhibitor may depend on the dose, duration, tissue, and selectivity of the compound.The selected literature, therefore, does not allow us to claim that

GSK-3 inhibition always produces the same molecular profile in all tissues or that the increased signaling observed in rat muscle automatically translates into a clinical benefit in humans.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication subject to the Evidence Review from the independent research used to evaluate the same biological narrative.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Animal, isolated muscle in vitro	Soleus muscle of Zucker Diabetic Fatty rats and lean rats	GSK-3 inhibition increased glucose transport and insulin receptor–IRS-1–AKT signaling, reducing IRS-1 Ser307.	direct confirmation	moderate
[1]	Cellular and molecular	CHO cells with insulin receptor and IRS-1	Increased GSK-3 increased serine phosphorylation of IRS-1 and reduced insulin-stimulated phosphorylation of IRS-1 and the receptor.	direct confirmation	moderate
[2]	Cellular and molecular	Peptides, recombinant IRS-1, and CHO cells	GSK-3-dependent phosphorylation of IRS-1 at Ser332 attenuated signaling; preventing it increased tyrosine phosphorylation of IRS-1 and AKT.	direct confirmation	moderate
[3]	Animal, isolated muscle in vitro	Muscles of prediabetic obese Zucker rats and lean rats	GSK-3 inhibition increased IRS-1, PI3K, AKT, and glucose transport in insulin-resistant muscles, without increasing the receptor.	indirect confirmation	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The analyzed publication directly supports the section of the book regarding the recovery of insulin signaling following GSK-3 inhibition in diabetic rat muscle. The most significant result is the coordinated direction of the effects: a decrease in IRS-1 phosphorylation associated with reduced function, and an increase in insulin signaling through the receptor, IRS-1, and AKT.Independent evidence confirms that GSK-3 can negatively modulate IRS-1 and that reducing this activity can improve signaling toward PI3K and AKT in experimental models of insulin resistance. The narrative is therefore well-supported from a mechanistic standpoint. Clinical validation in humans remains necessary, especially to establish the efficacy, selectivity, and safety of potential pharmacological interventions targeting GSK-3.

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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