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# Insulin regulates AKT/GSK-3b signaling, Tau phosphorylation, and redox homeostasis in SH-SY5Y neuroblastoma cells - Evidence review

**Dott. A. Conte**[1]

1 Nutritional Biologist, Italy

## 1. ABSTRACT

The analyzed study evaluated whether insulin modifies a molecular sequence linked to cell survival in human SH-SY5Y neuroblastoma cells: AKT, GSK-3b, Tau, and markers of oxidative stress. After exposure to insulin, the cells showed an increase in AKT phosphorylation and inhibitory GSK-3b phosphorylation, a reduction in Tau phosphorylation, and changes consistent with lower lipid peroxidation and a stronger antioxidant response.The reference is relevant to paragraph 4.12 of “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi because it documents, in a cellular model, the AKT → GSK-3b inactivation → reduction of Tau phosphorylation segment, along with changes in redox homeostasis and indicators of cell death. Independent studies confirm this chain in cultured neurons and link GSK-3b activity to the regulation of NRF2, a factor that supports antioxidant defenses. However, other experiments show that the Tau response to insulin can vary over time. The most prudent conclusion is that the mechanism is supported at the cellular level, without direct demonstration of neuroprotection in humans.

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 insulin can modify a signaling network in SH-SY5Y cells that includes AKT, GSK-3b, the Tau protein, and defense systems against oxidative stress. AKT is a protein that transmits signals for growth, metabolism, and survival. GSK-3b is an enzyme that, when it remains active, can promote the phosphorylation of various cellular targets, including Tau. Phosphorylation is the addition of small chemical groups to a protein and can change its function, location, or interactions.The relevance to section 4.12 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi is specific: the publication examines whether insulin stimulation increases AKT and the inhibitory phosphorylation of GSK-3b, associating this with a reduction in phosphorylated Tau. The study also evaluates markers related to cell survival and redox balance, that is, the control of potentially harmful oxidizing molecules.Since the model consists of neuroblastoma tumor cells cultured in the laboratory, the research can document molecular and cellular responses under those conditions. It cannot demonstrate that insulin prevents neurological decline, modifies the course of Alzheimer's disease, or produces neuroprotection in the human body.

## 3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors used human SH-SY5Y cells, a neuroblastoma cell line frequently used to study biological signals relevant to neurons, though not equivalent to mature human neurons. The cells were maintained in culture medium and, prior to treatment, exposed for four hours to a lower serum concentration to reduce basal growth signals. Recombinant human insulin was then administered at a concentration of 10–8 molar, equal to 10 nanomolar.Rapid signaling events were evaluated after 30 minutes, while variables such as metabolic activity, number of viable cells, lactate dehydrogenase release, caspase-3 activity, and certain antioxidant response proteins were analyzed after 24 hours. Caspase-3 is an enzyme involved in apoptosis, or programmed cell death. The release of lactate dehydrogenase into the culture medium is, conversely, an indicator of loss of cell membrane integrity.Metabolic activity was measured using the MTT assay, which detects the ability of cells to reduce a colored substance but does not directly measure proliferation. The number of viable cells was evaluated using Trypan Blue exclusion. The proteins p-AKT, p-GSK-3b Ser9, p-Tau Ser396, NRF2, Cu/Zn-SOD, and Mn-SOD were measured via western blot, a technique that allows for the estimation of the relative quantity of specific proteins. Malondialdehyde, or MDA, was used as an indicator of lipid peroxidation, which is oxidative damage to lipids.Data were expressed as mean ± standard deviation of at least four independent biological replicates per group. The authors used the two-tailed unpaired Student's t-test and considered a P-value of less than 0.05 to be significant. The exact number of replicates for each individual experiment is not reported in the available material. There are also temporal inconsistencies: for NRF2 and phosphorylated Tau, the results text reports 30 minutes, while the methods section indicates 24 hours; for MDA, the results report one hour, while the methods indicate 24 hours.

## 4. RESULTS

In simple terms, insulin modified signals in SH-SY5Y cells that are consistent with higher metabolic activity and lower indicators of cellular damage or death. After 24 hours, the reduction of the MTT reagent increased by 31.25% compared to the control. This data suggests an increase in the activity of NADPH-dependent cellular oxidoreductases, rather than direct evidence of increased proliferation. The number of viable cells rose to 119.15% of control values.Indicators of cell death decreased. Caspase-3 activity was reduced by 31.26%, while lactate dehydrogenase release went from 0.85 ± 0.04 to 0.70 ± 0.07 nmol·min–1, a reduction of 19.2%. In the context of cell culture, these results indicate lower activation of an apoptosis marker and lower cytotoxicity; they do not directly demonstrate neuroprotection.The result most relevant to the book's section concerns the AKT-GSK-3b-Tau sequence. After insulin treatment, AKT phosphorylation increased by approximately 45% in the detailed analysis of the results; the abstract reports an increase of 34.2%. GSK-3b phosphorylation at the Ser9 residue increased by approximately 32%; the abstract indicates an increase of 19.9%. Phosphorylation at Ser9 is generally interpreted as the functional inactivation of

GSK-3b. In parallel, Tau phosphorylated at the Ser396 residue decreased by approximately 50%; the abstract reports a reduction of 53.39%.Regarding redox balance, insulin was associated with a decrease in MDA from 1.075 to 0.925 nmol/mL, equal to a reduction of 13.95%. NRF2 increased by approximately 30–40% in the detailed analyses, while the abstract reports an increase of 18.77%. Cu/Zn-SOD and Mn-SOD proteins, two enzymes that help neutralize reactive oxygen species in different cellular compartments, increased by approximately 50–60% and 80–100%, respectively, in the described figures. Exact P-values and effect sizes with confidence intervals are not reported in the available material.

## 5. INTERPRETATION

The clearest biological significance is that, under the experimental conditions adopted, insulin shifted the cellular signaling network in a direction compatible with lower GSK-3b activity and lower Tau phosphorylation. In simplified terms, AKT can act as a signal that brakes GSK-3b; when this brake increases, one of the mechanisms that can contribute to Tau phosphorylation decreases.The segment directly documented by the publication is: insulin increase in p-AKT increase in p-GSK-3b Ser9, indicative of GSK-3b inactivation reduction in p-Tau Ser396. The data also show an increase in the amount of NRF2 and superoxide dismutases, with a decrease in MDA. However, the authors did not measure either the translocation of NRF2 into the nucleus or the transcriptional activation of its target genes. Therefore, the increase in NRF2 protein does not equate to a complete demonstration of the functional activation of the NRF2 pathway.The decrease in caspase-3 and lactate dehydrogenase is consistent with fewer signs of cell death and injury, but it does not allow for the determination of which molecular step caused them. Furthermore, the cells were in basal conditions, without an insult that would reproduce a neurodegenerative disease. The publication therefore supports a cellular regulatory mechanism, not the efficacy of a treatment in humans.

## 6. INDEPENDENT SUPPORTING EVIDENCE

Particularly direct confirmation of the central part of the narrative comes from cultured human NT2N neurons. Hong and Lee showed that GSK-3 phosphorylates Tau and reduces its affinity for microtubules, intracellular structures that contribute to the architecture and transport within the cell. In the same model, insulin and insulin-like growth factor 1 reduced Tau phosphorylation and increased Tau binding to microtubules. The authors also linked this effect to the inhibition of GSK-3 through the PI3K/AKT pathway. This research directly confirms, in another human neuronal model, the insulin/PI3K/AKT GSK-3 inhibition reduction of Tau phosphorylation pathway [1]. The limitation is that these are still cultured cells, not patients or functioning human brain tissue.The component related to cell survival is supported by an experimental study on Rat-1 fibroblasts and PC12 cells, a line with neuronal characteristics. The artificial increase of the catalytically active form of GSK-3 induced apoptosis, while a dominant-negative form of GSK-3 prevented the apoptosis caused by PI3K inhibition. This independent result positions GSK-3 as an important downstream effector of the PI3K/AKT pathway in the control of cell death [2]. The connection with the analyzed study is biologically consistent, but it does not prove that the lower caspase-3 activity observed in SH-SY5Y cells depends exclusively on GSK-3b.For the redox branch, Rojo and colleagues studied N2A neuroblasts and cerebellar granular neurons subjected to oxidative stress. They observed that the activation of GSK-3b promoted the exclusion of NRF2 from the nucleus and reduced its transcriptional activity, while the inhibition of GSK-3b promoted the nuclear accumulation of NRF2 and increased the expression of heme oxygenase-1, a protein involved in the antioxidant response. The expression of a constitutively active form of GSK-3b also increased the sensitivity of neuroblasts to oxidative damage and cell death [3]. This evidence indirectly confirms the GSK-3b NRF2 defense against oxidative stress pathway, but it does not prove that insulin activates NRF2 with an identical mechanism in the cells of the analyzed study.

## 7. CONFLICTING EVIDENCE

The direction of the effect of insulin on Tau is not necessarily uniform across every time interval and every cell model. In SH-SY5Y cells, Lesort and colleagues observed a very rapid and transient increase in Tau phosphorylation following exposure

to insulin, associated with a transient activation of GSK-3b. After 60 minutes, however, Tau phosphorylation decreased along with GSK-3b activity [4].This result does not automatically refute the analyzed study, which found lower phosphorylated Tau after more prolonged exposure. However, it shows that the response can depend significantly on the observation time and the sequence of activated signals. Consequently, it is incorrect to describe the effect of insulin on Tau as invariably reductive: in cells, it can be biphasic, with an initial increase followed by a reduction. No further sufficiently relevant conflicting evidence has been identified; 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 the level at which each contributes to the chain described in the book's paragraph.

### Quadro comparativo

| Reference | Study type | Population or model | Main result | Direction of evidence | Strength of evidence |
|---|---|---|---|---|---|
| Original study | Experimental cellular | Human SH-SY5Y neuroblastoma cells | Insulin increased p-AKT and p-GSK-3b Ser9, reduced p-Tau Ser396, and modified redox and cell death markers. | biologically consistent evidence | preliminary |
| [1] | Experimental cellular | Cultured human NT2N neurons | Insulin and IGF-1 reduced Tau phosphorylation via PI3K/AKT and GSK-3 inhibition. | direct confirmation | moderate |
| [2] | Experimental cellular | Rat-1 fibroblasts and PC12 cells | Active GSK-3 induced apoptosis; dominant-negative GSK-3 protected against apoptosis following PI3K inhibition. | indirect confirmation | moderate |
| [3], [4] | Experimental cellular | Cultured neuroblasts and neurons; SH-SY5Y cells | GSK-3b reduced the NRF2 response to oxidative stress; in SH-SY5Y, insulin produced an initial and transient increase in phosphorylated Tau before reduction. | biologically consistent evidence; discordant evidence | moderate |

## 9. SINTESI EDITORIALE E CONCLUSIONI

### CONCLUSIONS

The study analyzed shows that insulin, in SH-SY5Y cells, is associated with increased AKT phosphorylation, increased inhibitory phosphorylation of GSK-3b, and decreased Tau phosphorylation. It also observes a reduction in indicators of cellular damage and lipid peroxidation, with an increase in proteins involved in the antioxidant response.The central point of the narrative is well-supported at a mechanistic level by independent research: the PI3K/AKT-GSK-3 axis can regulate Tau and influence cell survival; GSK-3b can also modulate the NRF2 response to oxidative stress. Validation in mature neurons, disease models, and human studies remains necessary. Furthermore, the response to Tau may change based on the duration of insulin exposure.

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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