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Trilobatin and improvement of insulin resistance via the IRS-AKT-GLUT4 pathway in C2C12 myotubes and ob/ob mice

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1. ABSTRACT

The analyzed study evaluated whether trilobatin, a natural substance isolated from a plant, could attenuate insulin resistance in muscle. The authors used C2C12 myotubes exposed to palmitate, a cellular model of fatty acid excess, and ob/ob mice, a murine model of genetic obesity. Trilobatin increased the uptake of a fluorescent glucose analog in the cells and, in the mice, reduced fasting glycemia and insulin, while also improving glucose tolerance.The relevant point for "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi is the recovery of insulin signaling in the muscle: phosphorylation of IRS1 and AKT, increased presence of GLUT4 at the membrane, and greater glucose entry. However, the study did not measure AS160 and does not demonstrate therapeutic efficacy in humans. Independent evidence confirms that, in human and animal muscle, alterations in AKT, AS160, and GLUT4 translocation are associated with a reduced insulin response. Furthermore, research on stevioside supports a similar narrative for the IR/IRS1/AKT/GLUT4 axis in diabetic rats. Significant limitations regarding the model, duration, and clinical transferability remain.

ONLINE ARTICLE

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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.

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 trilobatin could counteract skeletal muscle insulin resistance and whether this effect was accompanied by the recovery of the IRS1-AKT-GLUT4 pathway. In simple terms, the authors verified whether a natural compound could help muscle cells bring GLUT4 proteins back to their surface, which are the transporters that allow glucose to enter the cell.The hypothesis was biologically plausible because insulin normally activates a sequence of intracellular signals that moves GLUT4 from the inside of the cell to the membrane. However, the publication subject to the Evidence Review evaluates cells and animals, not people with diabetes or insulin resistance. It can therefore document experimental effects and a possible mechanism, but it cannot establish that trilobatin prevents or treats diabetes in humans.In paragraph 4.4 of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, the study is particularly relevant to the IRS1 AKT GLUT4 segment of the insulin chain. It does not directly document the AKT AS160 step, as AS160 was not measured. Furthermore, the study did not directly evaluate the recovery of insulin receptor expression or oxidative stress.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors used two experimental models. The first included C2C12 myotubes, murine cells differentiated in the laboratory to assume skeletal muscle characteristics. Insulin resistance was induced by exposing the cells to 0.2 mM palmitate for 24 hours. The cells were then treated for 24 hours with 0.1, 1, or 10 µM trilobatin and stimulated with 100 nM insulin. In some trials, 10 µM LY294002 was added, an inhibitor of PI3K, a protein located upstream of AKT in the insulin pathway.Glucose uptake was estimated using 2-NBDG, a fluorescent glucose analog. Phosphorylation—a chemical modification that can regulate protein activity—of IRS1 and AKT was measured by western blot. The distribution of GLUT4 between the cytoplasm and the membrane was evaluated by separating the cellular fractions and measuring the proteins present.The second model included 20 male ob/ob mice aged 8-10 weeks, randomly assigned to a control or trilobatin group, with 10 animals per group. Ten C57BL/6 mice of the same age were used as a non-obese control. Trilobatin was administered intragastrically at a dose of 10 mg/kg per day for four weeks. Body weight, food and water consumption, fasting glycemia and insulin, HOMA-IR index, glucose tolerance test, and proteins in the gastrocnemius muscle were measured. For some muscle western blots, three animals per group were randomly selected. Data were expressed as mean ± standard deviation and analyzed using t-tests or analysis of variance; the significance threshold was $P < 0.05$.

4. RESULTS

In the cellular model, palmitate significantly reduced insulin-induced 2-NBDG uptake. Trilobatin counteracted this reduction in a dose-dependent manner, with $P < 0.01$ compared to cells treated with palmitate and insulin. In practical terms, cells exposed to the compound regained a greater capacity to incorporate the glucose analog.Trilobatin also increased the proportion of GLUT4 detected in the cell membrane and reduced it in the cytoplasm compared to palmitate alone. This shift is consistent with increased GLUT4 translocation to the cell surface. When cells were pretreated with LY294002, the increase in glucose uptake and the change in GLUT4 distribution induced by trilobatin were blocked. This finding indicates that PI3K activity is necessary for these effects in the experimental model.The authors reported changes in the phosphorylation of IRS1 and AKT, particularly at the Thr308 site of AKT, while they did not observe a significant effect on the Ser473 site of AKT. The textual description of the results regarding IRS1 Ser307 is not fully consistent across different sections of the article; therefore, it is not possible to reliably attribute a unique direction of the effect on that specific site based on the available material.In ob/ob mice, four weeks of trilobatin did not significantly change body weight, weight variation, or food or water intake. However, it reduced fasting glycemia and insulin and improved the glucose tolerance test; the authors report $P < 0.05$ for the comparison with untreated ob/ob mice. In the gastrocnemius muscle, treatment was associated with a recovery of total GLUT4 expression and its presence in the membrane and cytoplasm fractions, as well as changes in IRS1 and

AKT consistent with improved insulin signaling.

5. INTERPRETATION

The primary significance of the results is that trilobatin partially corrected a central defect of muscle insulin resistance in the models used: glucose uptake into cells exposed to palmitate was reduced, and GLUT4 reached the membrane less effectively; after treatment, both aspects improved. The blockade achieved with the PI3K inhibitor makes it plausible that part of the effect depends on PI3K-AKT signaling.The technical detail is that the publication primarily documents the IRS1/PI3K/AKT pathway GLUT4 translocation glucose uptake. The phosphorylation of AKT represents an activation signal, while the presence of GLUT4 in the membrane represents the physical step that makes glucose more accessible to the cell. The effect of LY294002 adds an element of experimental causality to the cellular system, but a pharmacological inhibitor does not prove that trilobatin acts exclusively on that target.Regarding the narrative of the book, the point supported is therefore the recovery of the IRS1-AKT-GLUT4 axis in an insulin-resistant muscle model. The entire AKT-AS160-GLUT4 axis is not directly demonstrated: AS160, also known as TBC1D4, was not measured. Furthermore, no clinical trials have been conducted, nor are there data available on the safety, bioavailability, effective dose, or clinical benefit of trilobatin in humans.

6. INDEPENDENT CONFIRMATORY EVIDENCE

Independent confirmation of the final part of the paragraph's narrative comes from a study in diabetic rats fed a high-fat, high-sucrose diet. After 45 days, 20 mg/kg per day of stevioside improved glucose and insulin tolerance, reduced elevated glycemia and insulin levels, and normalized several indicators of oxidative stress in the gastrocnemius muscle. In the same tissue, the authors observed the recovery of IR/IRS1/AKT signaling components and an increase in GLUT4, with an increase in glucose uptake and oxidation. This study does not evaluate trilobatin, but it confirms in a distinct animal model the idea expressed in the book that an experimental intervention can improve muscular insulin response along with IRS1-AKT-GLUT4 signaling [1].The link between excess lipids, insulin signaling, and reduced glucose entry has also been observed in isolated human muscle. In rectus abdominis muscle samples from obese individuals, four hours of exposure to 2 mM palmitate reduced insulin-stimulated glucose uptake by 55% compared to the control. The reduction was accompanied by lower phosphorylation of AKT and AS160. This result is particularly relevant because it places AS160, which was not measured in the original study, between AKT and the control of GLUT4 availability in human muscle. However, this is an ex vivo experiment, not a treatment administered to living people [2].A further study on humans examined vastus lateralis muscle biopsies during a euglycemic-hyperinsulinemic clamp, a test that keeps glycemia constant while insulin is infused to measure insulin sensitivity. 8 lean subjects, 9 with obesity and normal glucose tolerance, and 10 with type 2 diabetes were studied. In participants with type 2 diabetes, insulin induced lower phosphorylation of AS160 at the Thr642 site and lower phosphorylation of AKT Thr308 compared to lean subjects. The study therefore shows, in humans, a biological consistency between AKT attenuation, AS160 defect, and lower muscular insulin response, without, however, demonstrating that these alterations are the sole cause of the disease [3].

7. CONFLICTING EVIDENCE

An important element of complexity emerges from an experiment on isolated rat soleus muscle exposed to palmitate. In the first six hours, palmitate reduced insulin-stimulated glucose transport by 33%, AKT phosphorylation by 55%, and GLUT4 translocation by 40%, in accordance with the pathway considered in the original study. However, after 12 and 18 hours, glucose transport decreased further, by 66% and 89% respectively, without further reductions in AKT or GLUT4 translocation. The authors therefore proposed a second phase, in which the intrinsic activity of GLUT4 already present on the membrane would decrease [4].This evidence does not contradict the fact that IRS1-AKT-GLUT4 is a relevant axis, but it indicates that the amount of GLUT4 at the membrane does not necessarily explain all insulin resistance, especially during prolonged exposure to lipids. The mechanism may depend on the duration of exposure, the experimental model, and additional alterations in transporter function. No directly conflicting evidence

sufficiently relevant to the effect of trilobatin itself has been identified; this does not prove the absence of contrary results.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the publication subject to the review from the independent research used to confirm or define the narrative of the paragraph.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Cellular and animal	C2C12 myotubes treated with palmitate; ob/ob mice	Trilobatin increased glucose uptake and GLUT4 translocation; in mice, it reduced fasting glycemia and insulin.	direct confirmation	moderate
[1]	Animal	Wistar rats with diet-induced diabetes (high-fat and sucrose)	Stevioside improved glucose and insulin tolerance, IR/IRS1/AKT signaling, and GLUT4 availability in muscle.	indirect confirmation	moderate
[2]	Human ex vivo	Rectus abdominis muscle isolated from lean and obese subjects	In muscle from obese subjects, palmitate reduced insulin-induced glucose uptake and AKT and AS160 phosphorylation.	direct confirmation	moderate
[3]	Human pathophysiological	8 lean, 9 obese, and 10 type 2 diabetes subjects undergoing a clamp	In type 2 diabetes, the insulin response of AKT and AS160 Thr642 was reduced in skeletal muscle.	biologically consistent evidence	moderate
[4]	Animal ex vivo	Isolated rat soleus muscle exposed to palmitate	The late reduction in glucose transport continued even without further reductions in GLUT4 translocation or the signals studied.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed convincingly supports, in the cell and mouse models used, that trilobatin may be associated with increased glucose uptake and a recovery of IRS1-AKT-GLUT4 signaling in muscle. Its placement in the book's section is therefore scientifically consistent with the segment of the insulin cascade actually measured.The overall narrative is confirmed primarily at a mechanistic level. Independent studies support the role of AKT, AS160, and GLUT4 in insulin-resistant human muscle, and an animal study on stevioside describes a convergent molecular framework. However, it has not been demonstrated that trilobatin produces clinical benefits in people, nor that all defects of insulin resistance depend solely on GLUT4 translocation.

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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