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ORIGINAL RESEARCH

Hyperbaric oxygen treatment increases intestinal stem cell proliferation via the mTORC1/S6K1 signaling pathway in Mus musculus - Evidence review

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1. ABSTRACT

The analyzed study evaluated whether hyperbaric oxygen therapy, i.e., the administration of pure oxygen at high pressure, could modify the behavior of intestinal stem cells in mice. After twenty sessions, the authors observed an increase in proliferating cells in the intestinal crypts, along with changes consistent with greater involvement of the mTORC1/S6K1 pathway. No differences emerged in the activity of two enzymes used as indirect indicators of oxidative metabolism.In the narrative of “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, the publication contributes to the passage stating that the mTORC1-S6K1 axis can promote proliferation through biosynthetic programs, rather than through a simple increase in energy production. Independent studies in mouse models confirm that the activation of mTORC1 and S6K1 in intestinal stem cells can increase protein synthesis and regenerative activity. However, other research shows that the effect of the pathway varies between active stem cells, reserve stem cells, and crypt support cells. The confirmation is therefore primarily mechanistic and does not demonstrate a clinical effect of hyperbaric oxygen therapy in healthy humans.

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MISSION

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2. ANALYZED STUDY

The research question was whether hyperbaric oxygen therapy could increase the proliferation of intestinal stem cells and whether this effect was associated with the mTORC1/S6K1 pathway. mTORC1 is a protein complex that integrates information on nutrients, growth factors, and environmental conditions; S6K1 is a downstream target protein that contributes to increasing the production of proteins necessary for cell growth.The relevance to paragraph 4.7 lies in the fact that the study examines, in a rapidly renewing tissue, the link between mTORC1/S6K1 pathway signals and cell proliferation. In the narrative of "The Choice to Live in Health or Illness" by Dr. A. Colonnese and R. Panzironi, the publication is used to argue that the selective activation of this axis can promote a proliferative response without it being explained simply by an increase in energy production.The study can show experimental associations in the duodenum of young male mice subjected to a specific exposure protocol. It cannot establish that the same effect occurs in the human intestine, that it improves an intestinal disease, or that increased proliferation is always beneficial. Furthermore, the expression of pathway genes and the phosphorylation of S6K1 are important biological indicators, but they do not by themselves constitute a complete measure of protein synthesis or functional tissue regeneration.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors stated that they used 46 three-month-old male BALB/c mice. However, the experimental groups described in the text include a total of 40 animals: four groups of five animals to compare control and hyperbaric oxygen therapy for 10 or 20 days, plus four groups of five animals for the rapamycin experiment. This numerical discrepancy is not clarified in the available material.Each session involved 100% oxygen, a pressure of 2 atmospheres absolute, and a total duration of 90 minutes: 15 minutes of compression, 60 minutes at the set pressure, and 15 minutes of decompression. The animals received either 10 or 20 daily sessions. In the pharmacological groups, rapamycin, a drug that inhibits mTORC1, was administered intraperitoneally at 2 mg/kg every other day for 20 days; the comparison group received DMSO, the drug vehicle.One hour after the last session, the animals received bromodeoxyuridine, or BrdU, a molecule that incorporates into the DNA of dividing cells. In the duodenum, the authors measured BrdU-positive cells per crypt and their co-localization with Olfm4, a marker for intestinal stem cells. They also performed immunohistochemistry and immunofluorescence, quantitative PCR to measure gene expression, western blot to detect phosphorylated S6K1, and enzymatic assays for citrate synthase and cytochrome c oxidase.Statistical analyses included permutation tests and Kolmogorov-Smirnov tests to compare the distribution of proliferating cells. For some experiments, sample sizes of three, four, or five animals per group are reported. No long-term follow-up data, direct measurements of global protein synthesis, functional tests of intestinal regeneration after injury, or direct measurements of cellular oxygen consumption are reported.

4. RESULTS

The main result was an increase in proliferation in the duodenal crypts after 20 days of treatment, but not after 10 days. Crypts are small structures at the base of the intestinal mucosa where stem cells and progenitor cells reside. The number of BrdU-positive cells per crypt was higher in the group treated for 20 days compared to the control, with significance reported by the authors as $P<0.05$ and five animals per group.The co-localization of BrdU with Olfm4 indicated that a portion of the cells entering division consisted of intestinal stem cells. The authors also observed an increase in the total number of cells in the crypt and in BrdU-positive cells not identified by Olfm4. Therefore, the treatment may have also involved progenitor cells or other stem cell populations, but this attribution was not directly demonstrated.Regarding the mechanism, after 20 sessions, the levels of messenger RNA for Mtor, Skar, Eef2, and ribosomal protein S6 increased; the reported P values were 0.02, 0.02, 0.01, and less than 0.05, respectively, with five animals per group. In simple terms, these results are consistent with greater activation of a program that promotes protein production and cell growth. Conversely, no significant differences emerged for Eif4e, associated with another branch of mTORC1, nor for Foxo1, used by the authors as an indicator of the mTORC2/SGK1 branch.In the

comparison with rapamycin, the drug reduced the phosphorylation of S6K1, i.e., the activated form of the protein. The combination of rapamycin and hyperbaric oxygen therapy showed the presence of phosphorylated S6K1, with no significant differences compared to the groups with DMSO. However, the association between hyperbaric treatment and activation of the mTORC1/S6K1 axis is based primarily on gene expression and this pharmacological experiment, not on a complete quantification of the phosphorylation of all nodes of the pathway in animals treated with oxygen alone.Finally, no significant differences were detected in the activity of citrate synthase or cytochrome c oxidase, two enzymes linked to oxidative metabolism, in samples of three animals per group. This indicates that the treatment did not modify these two indicators in the analyzed duodenal tissue. However, it does not demonstrate the absence of any change in energy metabolism, because cellular energy production can also be regulated by other enzymes, substrates, and processes that were not measured.

5. INTERPRETATION

For the non-specialist reader, the key finding is that in the mouse model, the increased oxygen availability was not accompanied by a measurable increase in two energy-related enzymes, while it was accompanied by greater proliferation of crypt cells and signals consistent with the involvement of mTORC1/S6K1. This makes it biologically plausible to interpret that the proliferative response depends primarily on cell-building programs, including protein synthesis, rather than a simple acceleration of energy production.The chain of events documented by the publication subject to the Evidence Review is: hyperbaric exposure → modulation of the mTORC1/S6K1 axis → increased proliferation in intestinal crypts. The absence of changes in citrate synthase and cytochrome c oxidase adds an element consistent with the statement in the book, but does not allow for the conclusion that energy plays no role in the cellular response.The strength of the inference is limited by the animal model, the small sample sizes, and the fact that an increase in messenger RNA does not necessarily equate to an increase in protein activity. Furthermore, mTORC1 and S6K1 have effects that depend on cell type, duration of activation, nutrient availability, and the presence of tissue damage. The study therefore supports a specific mechanistic step in the narrative, not a general rule that activating mTORC1/S6K1 always promotes intestinal health.

6. INDEPENDENT SUPPORTING EVIDENCE

Direct confirmation of the mTORC1/S6K1 → protein synthesis → intestinal stem cell increase pathway comes from an independent experiment on caloric restriction in mice. Igarashi and Guarente observed that, in intestinal stem cells, SIRT1 promoted the deacetylation of S6K1 and increased its phosphorylation by mTORC1. This increase was associated with greater protein synthesis and an increase in the number of intestinal stem cells. Rapamycin abolished the expansion observed in that model [1]. The study does not concern hyperbaric oxygen therapy, but it confirms the central biological link of the paragraph: S6K1 activated downstream of mTORC1 can translate into a biosynthetic and proliferative program.A second mouse study showed that refeeding after a short Fasting period increased the proliferation of Lgr5-positive intestinal stem cells and their regenerative capacity. The authors attributed this effect to a strong induction of mTORC1, which increased protein synthesis through polyamine metabolism; inhibition of mTORC1, polyamine production, or protein synthesis eliminated the regenerative effect [2]. This evidence is independent and reinforces the sequence mTORC1 → increased protein synthesis → greater stem cell activity. The relevant limitation is that the model was refeeding after Fasting, not exposure to hyperbaric oxygen; furthermore, the same proliferation was associated with an increase in tumorigenicity in predisposed mice.Regarding the more upstream event, i.e., the possibility that hyperbaric oxygen therapy is associated with greater proliferative activity in the intestinal mucosa, a small prospective series in patients with refractory ulcerative colitis reported, after hyperbaric treatment, a significant increase in the labeling index for PCNA in CD44-positive cells of the colonic mucosa, along with clinical and histological improvement [3]. PCNA is an indicator of cells engaged in the cell cycle and CD44 can identify crypt populations, but it is not equivalent to the Olfm4 marker used in the mouse study. Furthermore, the

series lacked a control group and did not measure mTORC1 or S6K1. Therefore, it offers only indirect and preliminary confirmation of the relationship between hyperbaric treatment and proliferative activity in human mucosa.

7. CONFLICTING EVIDENCE

Important contextual evidence comes from a study on reserve intestinal stem cells, which are less active cells that can contribute to regeneration following injury. In this mouse model, caloric restriction reduced mTORC1 in reserve stem cells and protected them from DNA damage; conversely, stimulation of mTORC1 increased their sensitivity to radiation and impaired regeneration after injury [4].This finding does not deny that mTORC1/S6K1 activation can support proliferation and protein synthesis in active stem cells. However, it shows that the biological effect is not uniform: it can vary according to the cell population, the timing of the response, and the presence of stress or injury. In particular, what promotes rapid expansion in active stem cells may not protect a reserve population exposed to genomic damage.No directly conflicting evidence sufficiently relevant to test the same hyperbaric oxygen therapy protocol, the same duodenal region, and the same mTORC1/S6K1 axis in an independent model 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 shows that the convergence primarily concerns the biological mechanism, not a general clinical effect of hyperbaric oxygen therapy.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Experimental animal	Male BALB/c mice, duodenum	Twenty hyperbaric sessions increased crypt proliferation and signals consistent with mTORC1/S6K1, with no changes in citrate synthase and cytochrome c oxidase.	direct confirmation	moderate
[1]	Animal, intestinal cells and organoids	Mouse intestinal stem cells and Paneth cells	mTORC1 and S6K1 increased protein synthesis and the number of intestinal stem cells during caloric restriction.	direct confirmation	moderate
[2]	Animal and organoids	Mouse Lgr5-positive intestinal stem cells after fasting and refeeding	mTORC1 induction increased protein synthesis, proliferation, and regenerative capacity; inhibition of the pathway abolished the effect.	direct confirmation	moderate

[3]	Prospective observational without control	Patients with refractory ulcerative colitis	After hyperbaric oxygen therapy, proliferative signals in CD44-positive cells of the colonic mucosa increased.	indirect confirmation	preliminary
[4]	Animal, genetic and pharmacological	Mouse reserve intestinal stem cells	Reduction of mTORC1 protected reserve cells from damage; its stimulation increased vulnerability to radiation.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI

CONCLUSIONS

The study analyzed provides experimental evidence in mice showing that twenty sessions of hyperbaric oxygen therapy increase proliferation in intestinal crypts and modify indicators of the mTORC1/S6K1 axis. The result is consistent with the idea that S6K1 participates in biosynthetic programs capable of supporting cell growth and division.Independent research convincingly confirms, in mouse models, that mTORC1 and S6K1 can increase protein synthesis and the activity of intestinal stem cells. However, the significance of this pathway depends on the cell population and the biological situation: its activation is not automatically favorable during tissue damage or in every regenerative context.The book's narrative is therefore supported at a mechanistic level. Independent validation of the effect of hyperbaric oxygen therapy on the mTORC1/S6K1 pathway in the human intestine remains necessary, through controlled studies that measure both proliferation and clinically relevant outcomes.

Aggiornato al 17/11/2026. 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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