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Effect of the lysosomotropic agent chloroquine on mTORC1 activation and protein synthesis in human skeletal muscle - Evidence review

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1. ABSTRACT

The original study investigated whether chloroquine, a drug capable of altering lysosomal function, modified mTORC1 activation and muscle protein synthesis following the intake of essential amino acids. Fourteen healthy young adults were assigned to either chloroquine or a control group; the authors also conducted experiments on murine muscle cells. In humans, amino acids increased the phosphorylation of S6K1 and other mTORC1 pathway signals with no differences detected between groups, while muscle protein synthesis did not differ between chloroquine and control. In cells, however, chloroquine reduced the response to leucine and protein synthesis.In section 4.7 of “The Choice to Live in Health or Illness” by Dr. A. Colonnese and R. Panzironi, the study contributes to the clarification that S6K1 phosphorylation is a useful indicator of anabolic activation, but not a direct and sufficient quantitative measure of final protein synthesis. Independent studies in humans confirm both the necessary role of mTORC1 in the response to amino acids and the fact that variations in S6K1 may not translate proportionally into variations in protein synthesis. The evidence is therefore moderately supported and dependent on the experimental context.

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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 the lysosome, a cellular structure that recycles components and helps sense nutrients, was essential for amino acids to activate mTORC1 in human muscle. mTORC1 is a protein complex that promotes the initiation of new protein production. S6K1 is one of the proteins located downstream of mTORC1: its phosphorylation, which is the addition of a phosphate group that signals its activation, is often used as an index of the anabolic response.The relevance to paragraph 4.7 is specific. The publication measured both S6K1 phosphorylation and muscle protein synthesis in the same experiment. This allows for an assessment of whether the molecular signal and the final biological outcome necessarily proceed in an overlapping manner. However, the study was not designed to calculate an individual quantitative relationship between S6K1 phosphorylation and protein synthesis; it can therefore document the coexistence or lack of separation of the two responses under the tested conditions, but it cannot define an S6K1 value that predicts protein synthesis in every person or situation.

3. MATERIALS AND METHODS OF THE ORIGINAL STUDY

The authors conducted a randomized clinical trial in 14 healthy, recreationally active, non-smoking adults aged between 18 and 40 years. Ten participants were men and four were women. Seven individuals were assigned to the control group and seven to the chloroquine group. The treated group received 250 mg of chloroquine the evening before and 500 mg on the morning of the study, for a total of 750 mg; the control group did not receive a placebo. Participants consumed 10 g of essential amino acids after two vastus lateralis muscle biopsies. In the text, the authors report that one participant per group was excluded due to the inability to obtain the third biopsy, while the figure captions report seven participants per group: this discrepancy is not clarified in the available material.Muscle protein synthesis was measured using phenylalanine isotopic tracers and reported as the fractional synthesis rate or FSR, which is the percentage of muscle protein renewed in one hour. Muscle protein degradation and whole-body proteolysis were also analyzed. Using western blot, a technique that measures specific proteins or their phosphorylated forms, mTORC1, S6K1, 4E-BP1, and rpS6 were evaluated. Muscle samples were collected before the amino acid Intake and at 60 and 120 minutes afterward.In a second set of experiments, the authors used C2C12 myotubes, which are differentiated murine muscle cells in culture. They compared untreated cells, cells exposed to 1 mmol/L of leucine for 10 minutes, and cells pretreated with 2 mg/mL of chloroquine for one hour before leucine. Signaling was evaluated in eight replicates per condition. Cellular protein synthesis was assessed using the SUnSET method, based on the incorporation of puromycin into newly produced proteins, in six replicates per condition.

4. RESULTS

In human muscle, the availability of leucine and phenylalanine in the blood and muscle increased after the essential amino acid drink in both groups. No differences emerged between chloroquine and control for these concentrations. This indicates that any potential effect of the drug did not depend on a reduced appearance of the measured amino acids in the blood or muscle tissue.One hour after the amino acids, the phosphorylation of mTORC1, S6K1, 4E-BP1, and rpS6 increased compared to baseline in both the control group and the chloroquine group. After two hours, some signals remained statistically elevated compared to baseline only in the control group; in the chloroquine group, the increase for S6K1 showed a trend but did not reach the significance threshold declared by the authors, with P=0.07. However, no significant differences were reported between the groups for the total abundance of the analyzed proteins.Muscle protein synthesis did not differ between the groups at any time point, with P>0.05. After the amino acids, the FSR increased compared to rest in the chloroquine group, while in the control group there was a trend toward an increase with P=0.06. The study does not report the full numerical values of the FSR shown in the figure in the available text. Muscle protein degradation at rest also did not differ between the groups. Whole-body proteolysis decreased after the amino acids in the control group, but not in the chloroquine group; however, no significant difference was detected between the groups.In C2C12 cells, leucine increased the

phosphorylation of mTORC1 and S6K1 compared to the control. Pretreatment with chloroquine abolished this increase under the experimental conditions used. Chloroquine also reduced cellular protein synthesis both in a nutrient-rich environment and after nutrient deprivation. These results therefore show cellular inhibition, but do not demonstrate that the same intensity of lysosomal alteration was reached in human muscle with the administered dose.

5. INTERPRETATION

In simple terms, the study shows that a molecular signal and the final result of protein production are not the same thing. S6K1 participates in transmitting the mTORC1 signal to the cellular machinery that builds proteins, but the amount of phosphorylated S6K1 in a single sample does not automatically equate to the amount of protein built by the muscle over the entire observed period.The segment of the chain documented by the publication is: essential amino acids activation of mTORC1 and S6K1 muscle protein synthesis response. In humans, chloroquine did not statistically separate these steps: it did not significantly reduce either the S6K1 response at one hour or protein synthesis compared to the control. In murine cells, however, chloroquine reduced both the mTORC1-S6K1 response to leucine and protein synthesis. The contrast between the two models suggests that dose, drug distribution, cell type, and nutritional conditions can alter the interpretation of the mechanism.The result most relevant to the book's narrative is not that S6K1 is irrelevant. On the contrary, S6K1 was activated along with other anabolic signals after amino acids. The limitation is interpretive: this study does not prove that S6K1 phosphorylation constitutes a direct quantitative indicator of protein synthesis, because the pharmacological intervention in humans did not produce a significant dissociation between the two measures, and no individual correlation analysis was performed. The research therefore reinforces the need to consider S6K1 within a network of signals, rather than as the sole gauge of biological outcome.

6. INDEPENDENT CONFIRMATORY EVIDENCE

Initial confirmation of the pathway's role, distinct from the use of S6K1 as a simple quantitative indicator, comes from a randomized crossover study in eight young adults. After 10 g of essential amino acids, muscle protein synthesis increased by approximately 60% in the control group; the administration of rapamycin, an mTORC1 inhibitor, blocked the increase in protein synthesis and attenuated or blocked mTORC1 signaling. This result directly supports the fact that mTORC1 is necessary for the response to amino acids in human muscle. However, it does not establish that the intensity of S6K1 phosphorylation alone linearly measures the magnitude of protein synthesis [1].A metabolic experiment in humans tested amino acids and four levels of insulin while maintaining high amino acid availability. Tripling the amino acids with low insulin doubled muscle protein synthesis, with $P<0.01$. With the same amount of amino acids, increasing insulin instead increased p70S6K (i.e., S6K1) phosphorylation, but did not further increase muscle protein synthesis. This dissociation is particularly relevant to the book: S6K1 activation may accompany an anabolic environment without, by itself, providing a quantitative reading of final protein synthesis [2].Another randomized study in 21 young men and women compared 10 g of essential amino acids alone or combined with 30 g of sucrose or alanine. All groups showed an increase in S6K1 at 60 minutes. Some mTORC1 signals were more prolonged with carbohydrates or alanine, but the areas under the curve for protein synthesis, protein degradation, and net protein balance did not differ between the three groups. The longer duration of some signals did not, therefore, translate into a greater overall anabolic effect over the course of three hours [3].Overall, this research confirms a more precise chain than the popular science formulation: mTORC1 and S6K1 are a relevant part of the response to amino acids, but protein synthesis also depends on substrate availability, the duration of the stimulus, other protein translation factors, and the metabolic context. The studies are conducted primarily in healthy young adults and observe acute responses; they do not allow for direct inferences regarding long-term effects on muscle mass, strength, or health.

7. CONFLICTING EVIDENCE

The evidence does not suggest that S6K1 is a valueless marker. In a study of 16 healthy young people and 16 healthy older adults, strength training increased the phosphorylation of mTOR, S6K1, and other translation proteins in the young group, along with muscle protein synthesis. In the young participants, at 24 hours, the phosphorylation of mTOR and S6K1 was positively correlated with protein synthesis. This shows that, under certain conditions, S6K1 can be associated with the synthetic response [4].In the same study, however, the older adults showed neither a significant increase in mTORC1-S6K1 signaling nor an increase in protein synthesis after exercise; furthermore, in the older adults, the authors did not observe a correlation between phosphorylation and protein synthesis. The evidence is therefore contextual, not truly contradictory to the statement in the book: it confirms that the relationship can exist, but it is not automatically constant across ages, stimuli, and physiological conditions. No directly conflicting evidence sufficiently relevant to the topic was identified; this does not prove the absence of contrary results.

8. QUADRO COMPARATIVO E SINTESI DELLE EVIDENZE

The table distinguishes the study under review from the independent publications used to evaluate the passage in the book.

Quadro comparativo

Reference	Study type	Population or model	Main result	Direction of evidence	Strength of evidence
Original study	Randomized human study and cellular study	14 healthy young adults; C2C12 murine myotubes	Amino acids increased S6K1 in both human groups with no difference in protein synthesis between chloroquine and control; in cells, chloroquine reduced signaling and synthesis.	biologically consistent evidence	moderate
[1]	Crossover RCT	8 healthy young adults	Rapamycin blocked the approximately 60% increase in protein synthesis induced by 10 g of essential amino acids.	direct confirmation	moderate
[2]	Experimental metabolic study	Healthy young men	With constant amino acids, the increase in S6K1 phosphorylation induced by insulin did not further increase muscle protein synthesis.	direct confirmation	moderate

[3]	Parallel-group RCT	21 young men and women	More prolonged mTORC1 signals in some groups did not increase the overall anabolic effect compared to essential amino acids alone.	direct confirmation	moderate
[4]	Comparative experimental study	16 healthy young and 16 healthy older adults after resistance exercise	In young adults, S6K1 and protein synthesis increased and were correlated; in older adults, both responses were attenuated and the correlation did not emerge.	discordant evidence	moderate

9. SINTESI EDITORIALE E CONCLUSIONI CONCLUSIONS

The analyzed study appropriately supports the idea that S6K1 is part of the anabolic response regulated by mTORC1, but it does not allow for the use of its phosphorylation as an independent quantitative measure of muscle protein synthesis. Chloroquine inhibited signaling and synthesis in cells, but did not produce the same detectable effect in human muscle at the dose used. Independent evidence shows that mTORC1 is necessary for the response to amino acids and, at the same time, that the magnitude of molecular signals does not always proportionally determine the magnitude of the functional outcome. The book passage is therefore moderately supported and always requires an interpretation dependent on the biological context.

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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