DOI: 10.32999/ksu2524-0838/2025-37-5

УДК 612.172.1

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DOES LEUCINE-ENRICHED PROTEIN REALLY AFFECT PROTEIN LEVELS IN THE MUSCLES OF OLD MICE?

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Sarcopenia, characterised by the progressive loss of skeletal muscle mass, strength, and functionality, represents a significant health concern in the aging population. One of the promising approaches to preventing sarcopenia is the use of the amino acid leucine, which activates the mTOR signalling pathway and promotes muscle protein synthesis. Reduced muscle strength and coordination increase the risk of falls, which can lead to severe injuries, such as hip or vertebral fractures. Such injuries often require prolonged recovery and may result in disability.

Sarcopenia is often accompanied by an increase in fat mass against the background of muscle loss. This reduces the basal metabolic rate, contributing to the development of obesity and metabolic diseases such as type 2 diabetes. Loss of muscle mass lowers overall physical endurance, complicating the function of the heart and blood vessels, which increases the risk of cardiovascular diseases and related complications.

The study was conducted on aged mice (25 months old), divided into three groups: a control group, a group receiving pure leucine supplementation, and a group receiving leucine-enriched whey protein. At the end of the experiment, the levels of leucine, glucose, and insulin in the blood, as well as protein synthesis in muscle tissue, were assessed.

The results showed that the administration of pure leucine significantly increased its concentration in the blood (8-fold) and muscle tissue (3.8-fold) but did not promote muscle protein synthesis. In contrast, administering leucine in a whey protein mixture showed a less pronounced increase in leucine levels but effectively enhanced protein synthesis (by 1.2-fold) and reduced glucose levels. Insulin levels increased in both experimental groups, but this effect was less pronounced in the leucine mixture group.

These findings underscore the potential efficacy of using leucine in combination with whey protein as an effective strategy for maintaining muscle mass and function during ageing. The obtained data have practical significance for the development of dietary and pharmacological strategies in combating sarcopenia.

Keywords: *insulin, leucine, sarcopenia, muscle aging, whey protein.*

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ЧИ ДІЙСНО ЗБАГАЧЕНИЙ ЛЕЙЦИНОМ БІЛОК ВПЛИВАЄ НА РІВЕНЬ ПРОТЕЇНУ У М'ЯЗАХ СТАРИХ МИШЕЙ?

У статті розглянуто ефекти збагаченого лейцином сироваткового протеїну на м'язову активність у літніх мишей. Саркопенія, що характеризується прогресуючою втратою м'язової маси, сили та функціональності, є значущою проблемою для здоров'я людей похилого віку. Одним із перспективних підходів до запобігання саркопенії є використання амінокислоти лейцину, яка активує сигнальний шлях mTOR і сприяє синтезу білка в м'язах. Через зменшення м'язової сили та координації зростає ризик падінь, що може призвести до серйозних травм, зокрема переломів стегна чи хребців. Такі травми часто вимагають тривалого відновлення та можуть призвести до інвалідності.

Саркопенія часто супроводжується збільшенням жирової маси на фоні втрати м'язів. Це знижує рівень базального метаболізму, що сприяє розвитку ожиріння та метаболічних захворювань, таких як цукровий діабет 2 типу. Втрата м'язової маси знижує загальну витривалість організму, що ускладнює роботу серця та судин. Це підвищує ризик серцево-судинних захворювань та ускладнень.

Дослідження виконували на старих мишах (віком 25 місяців), яких поділили на три групи: контрольну, групу з введенням чистого лейцину та групу зі збагаченим лейцином сироватковим протеїном. Наприкінці експерименту визначали рівень лейцину, глюкози та інсуліну в крові, а також синтез білка в м'язовій тканині.

Результати показали, що введення чистого лейцину значно підвищувало його концентрацію в крові (у 8 разів) і м'язовій тканині (у 3,8 рази), але не сприяло синтезу білка в м'язах. Натомість введення лейцину в суміші із сироватковим протеїном демонструвало менш виражене підвищення рівня лейцину, проте ефективно посилювало синтез білка (в 1,2 рази) та знижувало рівень глюкози. Рівень інсуліну підвищувався в обох експериментальних групах, однак цей ефект був менш вираженим у групі із сумішшю лейцину.

Висновки дослідження підкреслюють доцільність використання лейцину у поєднанні із сироватковим протеїном як ефективної стратегії для підтримання м'язової маси та функції в умовах старіння. Отримані дані мають практичне значення для розробки харчових та фармакологічних стратегій у боротьбі з саркопенією.

Ключові слова: *інсулін, лейцин, саркопенія, старіння м'язів, сироватковий протеїн.*

Aging is a natural biological process characterized by progressive declines in physiological and functional capacities, including the musculoskeletal system. One of the hallmark features of aging is sarcopenia, the loss of skeletal muscle mass, strength, and function. Sarcopenia significantly impacts the quality of life, reducing mobility, increasing the risk of falls, and contributing to chronic health conditions such as frailty and metabolic disorders [4, 14]. Despite its prevalence and clinical implications, the underlying mechanisms of muscle aging remain incompletely understood, necessitating further research into preventative and therapeutic strategies [7].

At the cellular level, muscle aging is driven by a complex interplay of factors, including impaired protein synthesis, mitochondrial dysfunction, chronic low-grade inflammation, and dysregulated signaling pathways such as the mechanistic

target of rapamycin (mTOR) and ubiquitin-proteasome systems. These changes result in an imbalance between anabolic and catabolic processes, contributing to the net loss of muscle protein. Furthermore, aged muscles exhibit reduced regenerative capacity due to the diminished functionality of satellite cells, the resident stem cells responsible for muscle repair and growth [2, 14].

Dietary interventions, particularly those targeting protein intake, have emerged as promising approaches to mitigate muscle aging. Among these, the amino acid leucine has garnered attention for its potent anabolic properties. Leucine activates the mTOR signaling pathway, a key regulator of muscle protein synthesis, making it a focal point in strategies to combat sarcopenia [11, 13]. Additionally, whey protein, a rich source of leucine and other essential amino acids, is well-documented for its efficacy in promoting muscle health [1]. However, the specific effects of leucine-enriched whey protein in the context of aging remain underexplored [9].

This study investigates the impact of leucine-enriched whey protein supplementation on muscle function in aged models. By elucidating the potential benefits of targeted nutritional strategies, this research aims to contribute to the development of effective interventions for preserving muscle health and functional independence in aging populations.

MATERIALS AND METHODS

The study was conducted on male BALB/c laboratory mice aged 25 months, corresponding to the human aging period of approximately 70–75 years. The animals were kept under standard vivarium conditions with free access to food and water. The research adhered to Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty."

The mice were divided into three groups:

Control Group: received no supplements and served as the baseline group.

Leucine Group: administered daily leucine (Sigma, 0.75 g/kg) via oral gavage.

Leucine-Enriched Whey Protein Group: administered 0.5 ml of leucine-enriched whey protein (Vansiton, equivalent to 0.75 g/kg of leucine) daily via oral gavage. The leucine and leucine-enriched protein interventions were carried out for 10 days.

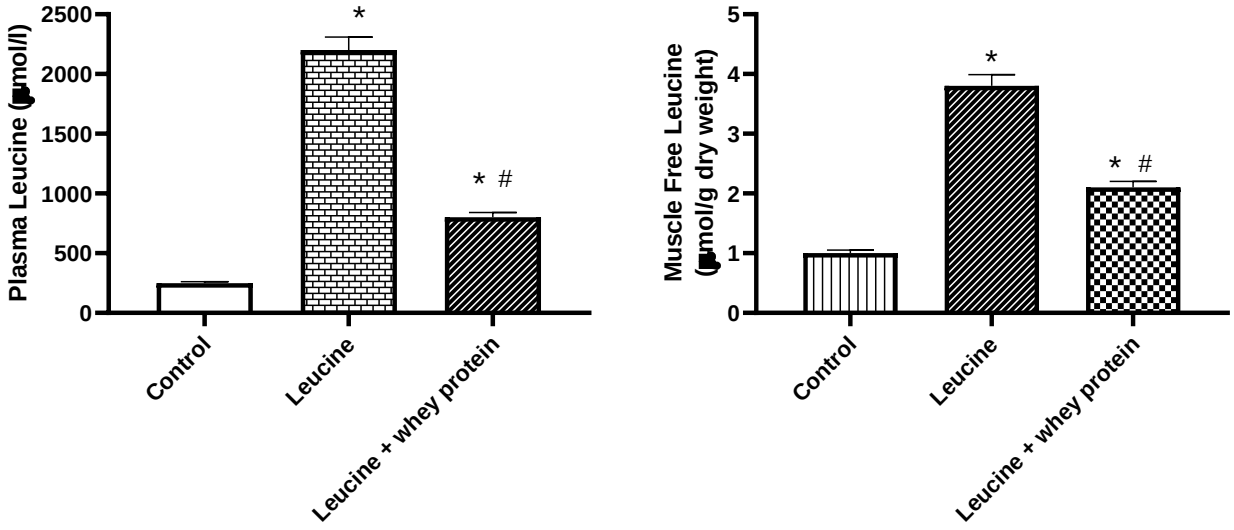
Blood and Tissue Sampling. Blood samples were collected at the beginning and end of the experiment and stored frozen for further analysis. Plasma leucine levels, glucose, and serum insulin concentrations were measured using enzyme-linked immunosorbent assay (ELISA).

Muscle Protein Synthesis Measurement. At the end of the experiment, muscle protein synthesis in the anterior thigh muscle was assessed using the SUnSET method [8]. This method involves immunodetection of puromycin-labeled peptides, providing a reliable measure of protein synthesis.

Statistical Analysis. Data analysis was performed using the Statistica 10 software. Statistical significance of differences was determined using the Mann–Whitney and Wilcoxon tests. Changes were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The leucine level in the blood differed significantly across the experimental groups. Compared to the control group, the animals receiving leucine exhibited a markedly higher concentration of leucine in their blood serum (Figure 1-A). Specifically, the blood leucine level increased 8-fold in the group administered pure leucine. In contrast, the group receiving the leucine-enriched whey protein mixture showed a 3-fold increase in blood leucine levels.



A B

Fig. 1. Leucine Levels in Blood (A) and Muscle Tissue (B) in Mice Receiving Leucine and Leucine-Enriched Whey Protein

Notes: *P < 0,05 compared to control values, #P < 0,05 compared to the group receiving leucine

The results of leucine levels in muscle tissue turned out to be interesting. In the group of animals that received leucine, its content in the muscles increased by 3.8 times (compared to the control). In the group that received a mixture of leucine and whey protein, it increased by 2 times (Fig. 1-B).

Insulin plays an important role in the development of sarcopenia—age-related reduction in muscle mass and strength. Insulin stimulates protein synthesis in muscles and suppresses their breakdown. Under healthy conditions, it provides an anabolic effect that helps maintain muscle mass. However, with age, insulin resistance may develop—a condition in which tissues become less sensitive to insulin. This reduces its ability to stimulate protein synthesis, leading to the loss of muscle mass [7].

After the administration of leucine, insulin levels increased 3.5 times (Fig. 2-A). In the group that received leucine with whey protein, the levels increased 2 times (Fig. 2-B).

In response, the pancreas begins to produce more insulin to compensate for its reduced effectiveness, leading to increased insulin levels in the blood (hyperinsulinemia). The rise in insulin levels is also an adaptive reaction of the body, which, with prolonged exposure, can have negative consequences.

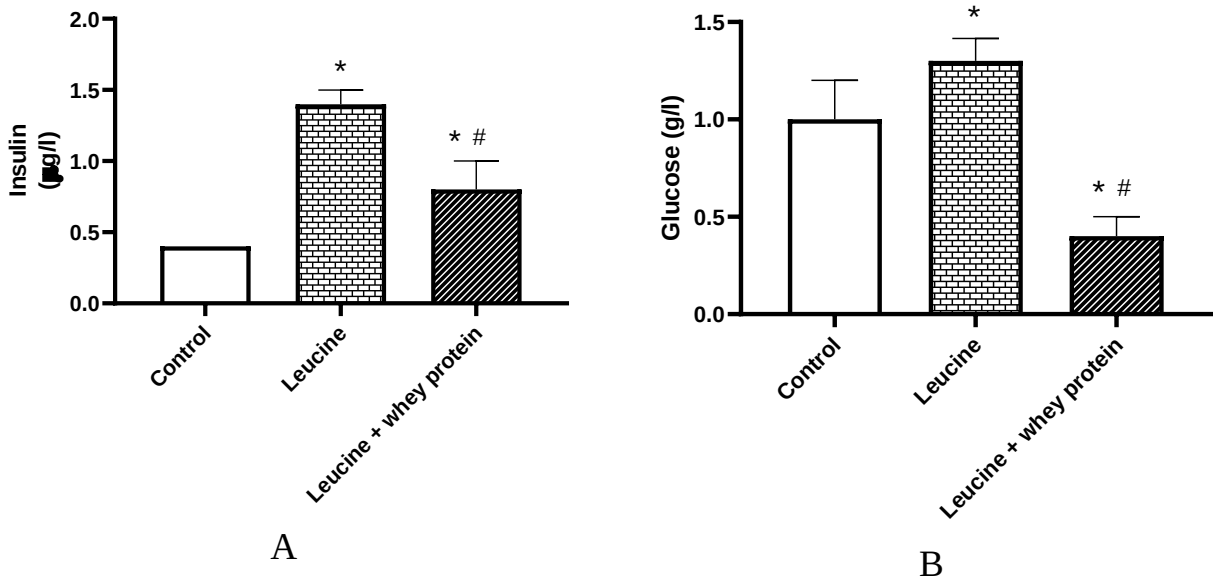


Fig. 2. Blood insulin levels (A) and glucose levels (B) in mice that received leucine and leucine in combination with whey protein.

Notes: *P < 0,05 compared to control values, #P < 0,05 compared to the group receiving leucine

In older mice that received leucine in combination with whey protein, protein synthesis in muscle tissue was enhanced by 1.2 times. Interestingly, in the group that received leucine alone, the activity of protein synthesis significantly decreased (by 2.2 times) (Fig. 3).

Leucine is an essential amino acid that plays a key role in maintaining muscle mass and function, especially in older age. With aging, the sensitivity of muscle tissue to protein synthesis stimulators declines, but leucine helps activate signaling pathways such as mTOR, which promote muscle protein synthesis. This is important for preventing sarcopenia (loss of muscle mass and strength). It is recommended to include leucine-rich foods (such as meat, fish, eggs, and dairy products) or use supplements to counteract age-related changes [3].

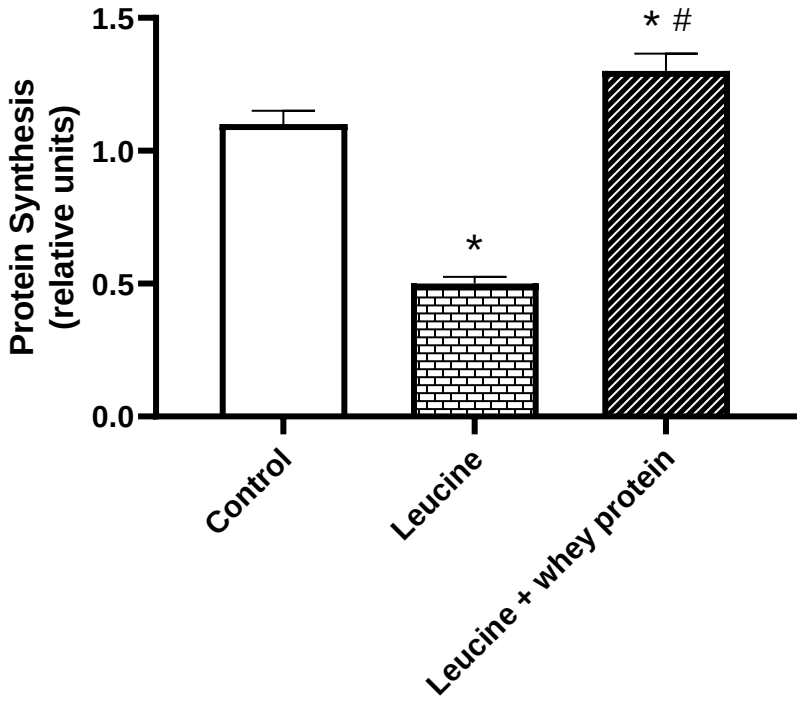


Fig. 3. Protein synthesis in the muscles of mice that received leucine and leucine in combination with whey protein

Notes: *P < 0,05 compared to control values, #P < 0,05 compared to the group receiving leucine

Sarcopenia is characterized by the progressive loss of muscle mass, strength, and functionality that occurs with age. It is a significant concern for older adults, as it affects their quality of life and increases the risk of falls, fractures, disability, and mortality. The decline in anabolic hormones (testosterone, estrogen, growth hormone) and the increase in insulin resistance contribute to the progressive reduction of protein content in muscle fibers. Among the therapeutic strategies for sarcopenia is the use of the amino acid leucine. Leucine is an essential amino acid critical for stimulating protein synthesis in muscles [6].

The use of leucine in its pure form, as shown in the study, led to an increase in the levels of this amino acid in the blood and muscles. However, it did not enhance protein synthesis in the muscles. At the same time, elevated levels of insulin and glucose in the blood were observed.

Consumption of leucine in combination with whey protein demonstrated a reduction in glucose levels, which we associate with enhanced protein synthesis in the muscles. The obtained results align with findings from other researchers who studied the effect of the amino acid leucine on muscle recovery processes [5, 12].

Thus, the conducted study yielded results indicating that the intake of the amino acid leucine combined with whey protein is the most effective way to enhance protein synthesis in older mice.

CONCLUSIONS

Administration of pure leucine to elderly laboratory mice resulted in an increase in its levels in blood plasma. When leucine was administered in combination with whey protein, plasma leucine levels increased by the tenth day but not significantly compared to the administration of pure leucine. The level of leucine in muscle tissue at the end of the experiment was also high in the group of animals that received pure leucine. Leucine combined with whey protein also contributed to an increase in its levels in muscle tissue.

The levels of insulin and glucose in the blood were dependent on the amount of leucine. After consuming pure leucine, both insulin and glucose levels increased. However, when leucine was consumed in combination with whey protein, glucose levels did not increase compared to the control.

Notably, administration of leucine alone did not significantly enhance protein synthesis in muscles. It is generally assumed that leucine intake should promote muscle protein recovery. However, it was found that leucine combined with whey protein significantly enhanced protein synthesis in the muscles of elderly mice, which should be considered during rehabilitation strategies.

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Стаття надійшла до редакції / The article was received 22.03.2025