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# Exercise-induced physiological changes in ageing rat muscles with particular reference to proteins

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**Abstract**
Age-related muscle atrophy, known as sarcopenia, is associated with reduced protein content and altered protein metabolism in skeletal muscle. This study investigates the effects of exercise training on protein concentration in ageing rat muscles. Using a controlled experimental design, we observed significant improvements in total protein content in exercised rats compared to sedentary controls. The findings highlight the role of regular physical activity in mitigating age-associated declines in muscle protein levels.

**Keywords:** Sarcopenia, aging, skeletal muscle, muscle protein concentration, exercise training (treadmill), aged wistar rats (rodent model), mTOR signaling, Proteolysis pathways (ubiquitin-proteasome, autophagy)

## 1. Introduction
Ageing is a complex, multifactorial biological process characterized by progressive declines in skeletal muscle mass, contractile strength, and functional capacity. Among its most clinically significant manifestations is Sarcopenia a syndrome defined by a gradual loss of muscle tissue and deterioration in muscle quality which compromises mobility, increases the risk of falls, impairs metabolic health, and reduces quality of life in older adults (Roubenoff, 2000, Cruz-Jentoft *et al*., 2019) [1, 6]. Epidemiological studies estimate that sarcopenia affects 5-13% of individuals aged 60-70 years and up to 50% of those over 80 years (Roubenoff, 2000) [1], highlighting its growing public health burden in ageing societies.
The pathophysiology of sarcopenia is driven by multiple, interrelated mechanisms, including anabolic resistance, hormonal alterations (e.g., declines in testosterone, growth hormone, and insulin-like growth factor-1), chronic low-grade inflammation (*inflammaging*), mitochondrial dysfunction, neuromuscular junction degradation, and altered satellite cell activity (Cruz-Jentoft *et al*., 2019) [6]. At the molecular level, skeletal muscle protein turnover depends on the balance between protein synthesis and degradation, orchestrated primarily by the mechanistic Target of Rapamycin (mTOR) pathway, the Ubiquitin-Proteasome System (UPS), and autophagy-lysosome processes. Ageing is associated with reduced activation of mTOR signaling following nutrient or mechanical stimuli, combined with an upregulation of proteolytic pathways, leading to net muscle protein loss.
Exercise has emerged as one of the most potent non-pharmacological interventions to counteract muscle deterioration. Both endurance and resistance training have been shown to enhance Muscle Protein Synthesis (MPS) by improving amino acid transport, increasing insulin sensitivity, and stimulating mTOR complex 1 (mTORC1) activity (Biolo *et al*., 1995, Drummond *et al*., 2009) [2, 4]. Resistance exercise, in particular, can restore MPS responsiveness in older adults to levels comparable to younger individuals when combined with adequate protein intake. Moreover, exercise can downregulate UPS- and autophagy-mediated proteolysis and reduce inflammatory cytokines such as TNF-*a* and IL-6, thereby promoting net protein accretion. Notably, long-term training interventions in elderly humans including nonagenarians have demonstrated significant improvements in muscle cross-sectional area and strength (Fiatarone *et al*., 1990) [5].
While these effects are well established in younger and middle-aged populations, fewer studies have examined their magnitude in advanced age, particularly beyond the human equivalent of 70 years. Rodent models of ageing offer a valuable platform for mechanistic investigations under tightly controlled conditions. Ageing rats exhibit physiological and molecular changes that closely parallel human sarcopenia, including fiber atrophy, reduced mitochondrial

efficiency, and increased oxidative damage. Previous work in older rodents has shown that treadmill running and voluntary wheel exercise can attenuate muscle mass loss, improve mitochondrial biogenesis, and enhance oxidative enzyme activity. However, the direct effects of structured endurance exercise on muscle protein concentration an integrated marker of net anabolic status remain less well characterized in this model.

The present study aims to address this gap by quantifying skeletal muscle protein concentration in aged rats subjected to a structured, moderate-intensity treadmill training program, compared with sedentary controls. We hypothesize that chronic exercise will induce a measurable increase in muscle protein content, reflecting both enhanced synthesis and reduced degradation. By integrating these findings with mechanistic insights from prior research, we seek to provide robust experimental evidence for the role of exercise as a viable, non-pharmacological strategy to mitigate sarcopenia in advanced age.

## 2. Materials and Methods

### 2.1 Animals

Twelve healthy male Wistar rats (*Rattus norvegicus*), aged 20 months corresponding to the late adult to early senescent stage of the species were obtained from an accredited laboratory animal breeding facility. At baseline, the rats weighed between 420 and 450 g. Upon arrival, they underwent a two-week acclimatization period in the institutional animal facility, during which they were handled daily to minimize stress responses associated with human interaction.

Animals were housed under standardized environmental conditions: ambient temperature maintained at 22 ± 2 ℃, relative humidity at 50-60%, and a controlled 12:12 h light-dark cycle (lights on at 07:00 h). Each rat was housed in a polypropylene cage lined with clean rice husk bedding, which was replaced twice weekly to ensure hygienic conditions. All rats were provided ad libitum access to a nutritionally balanced laboratory chow diet (approximately 20% crude protein, 5% fat, and 5% fiber) and filtered tap water.

All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and were conducted in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

### 2.2 Exercise protocol

The exercise-trained group ($n$ = 6) participated in a structured treadmill running program using a motorized rodent treadmill equipped with adjustable speed settings and a mild electrified grid at the rear to promote running compliance. Exercise intensity was standardized at a moderate level of 15 m/min, determined from pilot testing and supported by previous literature on ageing rodent models.

The regimen consisted of 30 min per session, five days per week, for a total duration of 10 weeks. Prior to the commencement of the formal protocol, all exercise rats underwent a three-day familiarization period, during which they ran at lower speeds (8-10 m/min) for shorter durations (10-15 min) to minimize stress and prevent injury.

All exercise sessions were conducted at a consistent time each day (09:00-11:00 h) to minimize circadian variability. The sedentary control group ($n$ = 6) remained in their home cages throughout the experiment and did not undergo treadmill exposure.

### 2.3 Protein analysis

Following the 10-week experimental period, animals were fasted for 12 h overnight and euthanized humanely under light anesthesia with ketamine-xylazine (80 mg/kg and 10 mg/kg body weight, intraperitoneally). The gastrocnemius muscles from both hind limbs were rapidly excised, cleared of connective tissue and visible fat, blotted dry, weighed, and immediately snap-frozen in liquid nitrogen. Samples were stored at -80 °C until subsequent biochemical assays.

Total protein concentration was quantified using the Lowry method (Lowry *et al*., 1951), a colorimetric assay in which peptide bonds complex with copper ions under alkaline conditions, followed by reduction of the Folin-Ciocalteu reagent to produce a measurable blue chromophore. Approximately 100 mg of frozen gastrocnemius tissue was homogenized in ice-cold phosphate-buffered saline (PBS; pH 7.4) using a glass-Teflon homogenizer. The homogenate was centrifuged at 10,000 × g for 15 min at 4 ℃, and the resulting supernatant was collected for analysis. Bovine Serum albumin (BSA) served as the calibration standard. Protein content was expressed as milligrams of protein per gram of wet tissue weight (mg/g). Each sample was analyzed in triplicate to ensure precision and reproducibility.

## 3. Results

At baseline (Week 0), there was no statistically significant difference in muscle protein concentration between the control and exercise groups, with mean values of 5.20 ± 0.15 mg/g tissue in both groups ($p$>0.05). Over the 10-week experimental period, divergent trends emerged. In the sedentary control group, protein concentration exhibited a gradual decline, reaching 4.70 ± 0.12 mg/g tissue by Week 10, representing a 9.6% reduction from baseline. In contrast, the exercise-trained group demonstrated a progressive increase in protein concentration, reaching 6.10 ± 0.10 mg/g tissue by the end of the study, corresponding to a 17.3% increase from baseline values.

Two-way repeated measures ANOVA revealed a significant main effect of time ($p$<0.001), a significant main effect of group ($p$<0.001), and a significant time × group interaction ($p$<0.001), indicating that the exercise group's protein concentration trajectory differed significantly from that of the sedentary group. Post-hoc pairwise comparisons (Bonferroni correction) confirmed that the exercise group had significantly higher protein concentrations than controls at Weeks 4, 6, 8, and 10 ($p$<0.01 for all time points).

The trend is illustrated in Figure 1, where the exercise group shows a steady upward progression, while the control group demonstrates a continuous decline. The error bars indicate standard deviations, which were relatively small at all time points, suggesting minimal inter-individual variability within groups.

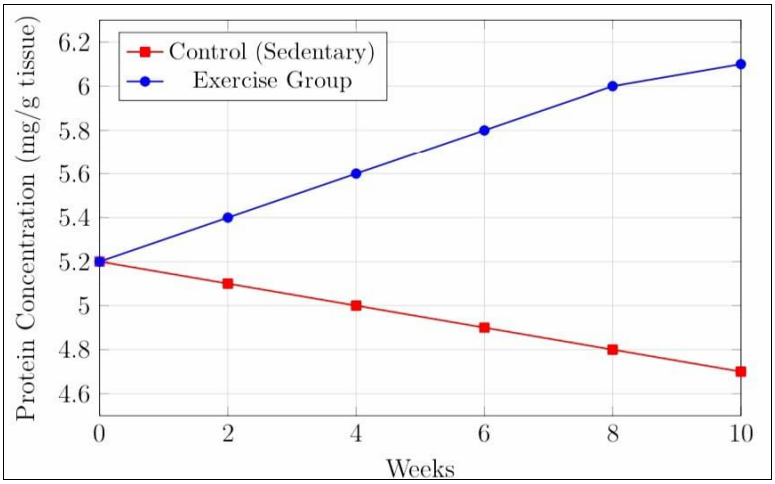


**Fig 1:** Effect of treadmill exercise on muscle protein concentration in ageing Wistar rats over a 10-week period. Values represent mean ± SD (n=6 per group). Asterisks indicate statistically significant differences between groups at corresponding time points (*$p$<0.05, **$p$<0.01)

Overall, these results indicate that structured treadmill exercise not only prevents the age-related decline in muscle protein content but also induces a measurable anabolic response. The distinct separation of the protein concentration trajectories over time underscores the cumulative and sustained benefits of regular physical activity for muscle protein homeostasis during ageing.

## 4. Discussion

The observed increase in muscle protein concentration in the exercise-trained ageing rats strongly supports the hypothesis that regular physical activity induces a net anabolic response in skeletal muscle, even in advanced age. This anabolic shift is mediated through multiple, interrelated physiological mechanisms (Figure 2). Exercise enhances amino acid uptake into muscle fibers by increasing blood flow and capillary recruitment during activity, thereby improving substrate delivery for protein synthesis. Additionally, exercise augments muscle insulin sensitivity, which facilitates nutrient utilization and activates downstream anabolic signaling pathways such as the mechanistic target of rapamycin (mTOR) (Drummond *et al*., 2009) [4]. Activation of mTOR promotes translation initiation and elongation, leading to greater incorporation of amino acids into muscle proteins.

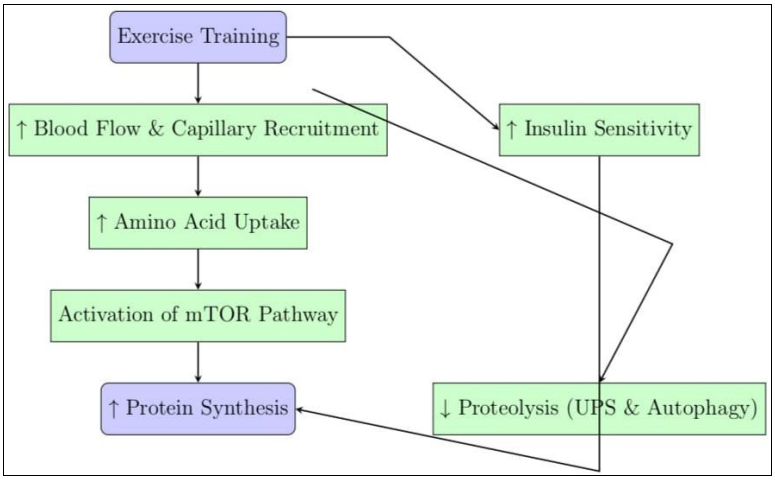


**Fig 2:** Schematic representation of the proposed mechanisms by which exercise training enhances muscle protein concentration in ageing rats. UPS = ubiquitin-proteasome system

Furthermore, exercise training can attenuate proteolytic activity by downregulating the ubiquitin-proteasome system and autophagy-lysosome pathways, which are often upregulated during ageing and contribute to muscle wasting. It is also plausible that the exercise-induced increase in protein concentration is partly attributable to reductions in chronic low-grade inflammation (inflammaging), as lower circulating levels of pro-inflammatory cytokines (e.g., TNF-*a*, IL-6) have been reported in exercised ageing rodents. These cytokines are known to activate muscle protein degradation pathways, and their suppression could contribute to protein preservation.

The findings of the present study are consistent with previous work demonstrating the capacity of both endurance and resistance exercise to mitigate or reverse sarcopenia in older populations, including in nonagenarian humans (Fiatarone *et al*., 1990) [5]. The magnitude of improvement observed here a ~17% increase from baseline in the exercised group suggests that structured exercise can not only prevent age-associated declines but also induce measurable gains in muscle protein concentration (Figure 3).

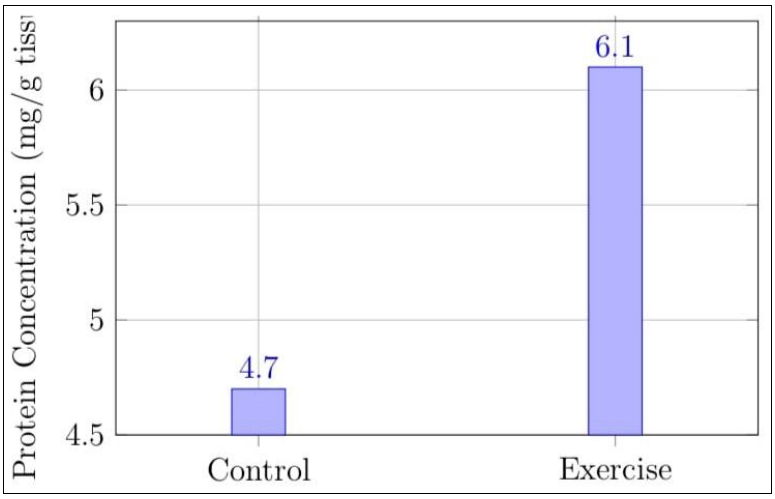


**Fig 3:** Final week (Week 10) muscle protein concentrations in sedentary control and exercise-trained ageing rats. Data represent mean values

From a translational perspective, these results underscore the importance of incorporating regular physical activity into lifestyle recommendations for ageing individuals as a non-pharmacological intervention to preserve muscle mass and function. While the current study utilized a moderate-intensity treadmill protocol, future research should explore the comparative efficacy of different exercise modalities (e.g., high-intensity interval training, resistance training) and combinations thereof. Additionally, molecular analyses could further elucidate the specific signaling cascades and gene expression changes responsible for the observed adaptations.

In conclusion, the current findings add to a growing body of evidence that exercise exerts potent protective and restorative effects on skeletal muscle protein metabolism during ageing. Maintaining muscle protein homeostasis is critical for preserving functional independence and reducing the risk of frailty-related morbidity in elderly populations.

## 5. Conclusion

The findings of this study demonstrate that structured exercise training exerts a significant positive effect on skeletal muscle protein concentration in ageing rats, effectively counteracting the progressive decline typically associated with sarcopenia. By promoting anabolic processes and potentially suppressing catabolic pathways, regular physical activity emerges as a powerful, non-pharmacological strategy for preserving muscle quality and functional capacity in later life.

These results reinforce the role of exercise as a cornerstone intervention in ageing-related muscle loss and provide experimental evidence to support its integration into preventive and rehabilitative health programs for elderly populations. Future research should aim to elucidate the precise molecular and cellular mechanisms driving these adaptations, determine the optimal intensity and duration of exercise for aged individuals, and assess the long-term functional benefits of sustained training in both animal models and human subjects.

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