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Original Article

Combined and Isolated Effects of High-Intensity Interval Training and Melatonin Supplementation on Skeletal Muscle Mass in Military Cadets

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ABSTRACT

Background: High-intensity interval training (HIIT) is a time-efficient strategy for improving physical readiness; however, the supportive role of melatonin in exercise-induced muscular adaptations remains unclear. This study examined isolated and combined effects of four weeks of HIIT and melatonin supplementation on skeletal muscle mass in military cadets.

Methods: In a quasi-experimental pretest-posttest study, 31 physically active male military cadets were allocated into Melatonin (n=10), HIIT (n=10), and HIIT+Melatonin (n=11) groups. Running-based HIIT group was three times weekly for four weeks, consisted of 30-second sprint intervals at 85-95% maximum heart rate followed by 60-second active recovery periods. Supplementation group received 5 mg melatonin nightly. Skeletal muscle mass was assessed using multi-frequency bioelectrical impedance analysis.

Results: Skeletal muscle mass did not significantly change in the Melatonin group. Significant increases were observed in both the HIIT group (p=0.001) and the HIIT+Melatonin group (p<0.001). ANCOVA provided baseline-adjusted estimates of group differences (p=0.001); however, these findings were interpreted cautiously because some model assumptions were not fully satisfied. Bonferroni-adjusted comparisons indicated a significant adjusted difference between HIIT+Melatonin and Melatonin groups, while the adjusted difference between HIIT+Melatonin and HIIT did not remain statistically significant.

Conclusion: A four-week HIIT intervention was associated with increased skeletal muscle mass in military cadets. Melatonin supplementation showed no significant anabolic effect. Although the combined intervention produced the largest numerical increase, these findings should be interpreted cautiously because of the small sample size, baseline group differences, absence of a true control group, lack of placebo supplementation, and absence of participant blinding.

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Introduction

Maintaining a high level of physical readiness is essential for military personnel, as operational performance largely depends on muscular fitness, endurance capacity, and optimal body composition. Among these components, skeletal muscle mass represent key determinant of physical capability and mission effectiveness in military environments (1). Therefore, identifying efficient and time-effective strategies to enhance muscle mass has become a priority within applied military exercise physiology. High-intensity interval training (HIIT) has emerged as a practical training approach capable of eliciting substantial physiological adaptations within limited training time. This training model, characterized by repeated bouts of near-maximal effort interspersed with brief recovery periods, has been shown to improve cardiorespiratory fitness, metabolic function, and neuromuscular performance (2, 3). Evidence from meta-analytic research further indicates that even low-volume HIIT protocols can meaningfully enhance physical fitness in adult populations (4). At the molecular level, HIIT may stimulate anabolic signaling pathways, facilitating muscle protein synthesis and skeletal muscle remodeling (5). Owing to its time efficiency and functional relevance, HIIT has been recommended as a suitable conditioning strategy for military settings (1).

Alongside exercise stimuli, sleep-related hormonal regulation has been increasingly recognized as an important factor influencing recovery and muscular adaptation. Melatonin, a pineal-derived hormone primarily involved in circadian rhythm regulation, also exhibits antioxidant and anti-inflammatory properties that may contribute to post-exercise recovery (6, 7). It has been described as a multifunctional biological signal involved in neuroendocrine modulation and cellular protection (8). Although melatonin is commonly used to improve sleep quality, its potential indirect contribution to muscle adaptation--possibly via improved recovery and reduced oxidative stress--remains incompletely understood (9, 10). Moreover, evidence regarding the direct influence of melatonin supplementation on skeletal muscle mass is still inconsistent.

Importantly, the combined influence of structured HIIT and melatonin supplementation has not been sufficiently investigated, particularly in military cadet populations exposed to high physical and psychological demands. Most previous investigations have examined these interventions in isolation, leaving uncertainty regarding their potential interactive effects on body composition. Therefore, the present study aimed to evaluate the effects of four weeks of HIIT performed with and without melatonin supplementation on skeletal muscle mass in military cadets.

Materials and Methods

This quasi-experimental study employed a pre-test/post-test design to examine the isolated and combined effects of HIIT and melatonin supplementation on skeletal muscle mass in military cadets. Ethical approval was obtained from the Ethics Committee of Imam Ali Military University, Tehran, Iran. All participants provided written informed consent prior to participation, in accordance with the Declaration of Helsinki. Totally, 31 physically active male military cadets (18-25 years) from Imam Ali Military University were recruited and allocated into three groups of Melatonin (n=10), HIIT (n=10), and HIIT+Melatonin (n=11).

Inclusion criteria were being male military cadets aged 18-25 years in absence of cardiovascular, metabolic, musculoskeletal, or sleep-related disorders, and willingness to participate in the study. Exclusion criteria were injury during the intervention, absence from more than two training sessions, failure to comply with melatonin supplementation, use of medications affecting sleep or metabolism, or participation in additional structured exercise programs during the study period. Participants assigned to the HIIT and HIIT+Melatonin groups completed supervised HIIT sessions three times per week for four weeks. Each session consisted of an 8-minute warm-up, followed by repeated 30-second running bouts at 85-95% of maximum heart rate interspersed with 60-second active recovery periods at 50-60% of maximum heart rate. The number of intervals progressively increased throughout the intervention. Each session concluded with a 5-minute cool-down period (Table 1).

Participants in the Melatonin and HIIT+Melatonin groups received 5 mg of melatonin (Parsineh Dayan Teb, Iran) nightly at 21:00 for four consecutive weeks. Compliance with supplementation was monitored throughout the intervention period. Previous studies have suggested that melatonin may contribute to circadian rhythm regulation, sleep quality, and recovery-related physiological processes (6-8). No placebo supplementation was used during the study due to logistical limitations associated with conducting research in a military environment.

Skeletal muscle mass was assessed using a validated bioelectrical impedance analyzer (InBody 230, Biospace Co., Seoul, South Korea). All measurements were performed in the morning

Table 1: HIIT training protocol of participants.

Week	Sprint duration	Recovery duration	Recovery type	Intensity	Number of intervals
Week 1	30 s	60 s	Active recovery	85-95% HRmax	6
Week 2	30 s	60 s	Active recovery	85-95% HRmax	7
Week 3	30 s	60 s	Active recovery	85-95% HRmax	8
Week 4	30 s	60 s	Active recovery	85-95% HRmax	8-10

HRmax: Maximum heart rate that was estimated using the equation HRmax=220-age. HIIT: High-intensity interval training.

Table 2: Baseline characteristics of participants included in the study.

Variable	Melatonin (n=10)	HIIT (n=10)	HIIT+Melatonin (n=11)	*P* value
Age (years)	22.60±1.71	23.40±1.71	23.27±1.49	0.507
Height (cm)	182.10±7.34	176.80±4.52	176.73±5.62	0.082
Body mass (kg)	82.44±7.90	66.18±6.92	71.62±11.07	0.001*
BMI (kg/m²)	24.85±1.86	21.18±2.15	22.85±2.68	0.005*
Baseline skeletal muscle mass (kg)	39.90±3.54	33.20±3.39	33.91±3.75	<0.001*
Training status	Trained cadets	Trained cadets	Trained cadets	—

Data were presented as mean±SD. *P* values were obtained using one-way ANOVA. BMI: Body mass index. HIIT: High-intensity interval training. All participants were physically active military cadets and members of university sports teams. *Significance was set at $p<0.05$.

Table 3: Within-group comparison of skeletal muscle mass following interventions (paired sample t-test).

Group	n	Pre-test Mean±SD	Post-test Mean±SD	Mean difference (Δ)	t	*P* value	Cohen's dz
Melatonin	10	39.90±3.54	39.70±3.77	-0.20±0.42	-1.50	0.168	0.47
HIIT	10	33.20±3.39	33.90±3.03	0.70±0.48	4.58	0.001*	1.45
HIIT+Melatonin	11	33.91±3.75	35.00±3.58	1.09±0.54	6.71	<0.001*	2.02

Mean difference was calculated as post-test minus pre-test values. Positive values indicate increased skeletal muscle mass. Cohen's dz represents the standardized mean change for paired observations. Effect sizes should be interpreted alongside statistical significance and sample size. *Significant at $p<0.05$. HIIT: High-intensity interval training.

following an overnight fast. Participants were instructed to avoid strenuous physical activity for at least 24 hours before assessment and to maintain normal hydration status. Participants were instructed to maintain their habitual dietary patterns throughout the intervention period and to refrain from consuming additional nutritional supplements that could influence body composition outcomes.

Data normality was assessed using the Shapiro–Wilk test. Within-group changes were analyzed using paired-samples t-tests. Between-group differences in post-intervention skeletal muscle mass were examined using analysis of covariance (ANCOVA), with baseline values entered as covariates (Table 2). Bonferroni-adjusted pairwise comparisons were performed when significant group effects were identified. Effect sizes were reported as Cohen's dz for paired comparisons and partial eta squared (η^2). Statistical significance was set at $p<0.05$. ANCOVA assumptions were examined by testing the group×baseline skeletal muscle mass interaction for homogeneity of regression slopes, evaluating the linear relationship between the covariate and outcome, and inspecting residual normality and homoscedasticity.

Results

Baseline participant characteristics were presented in Table 2. Age and height did not significantly differ between groups ($p>0.05$). However, body mass, BMI, and baseline skeletal muscle mass differed significantly across groups ($p<0.05$), supporting the use of baseline-adjusted analysis for post-intervention skeletal muscle mass. Descriptive statistics and within-group analyses for skeletal muscle mass were presented in Table 3, and the corresponding graphical representation of pre-test and post-test values was shown in Figure 1. No significant changes in skeletal muscle mass were observed in the Melatonin group following the intervention ($p>0.05$). In contrast, significant increases were observed in both the HIIT and HIIT+Melatonin groups ($p<0.05$).

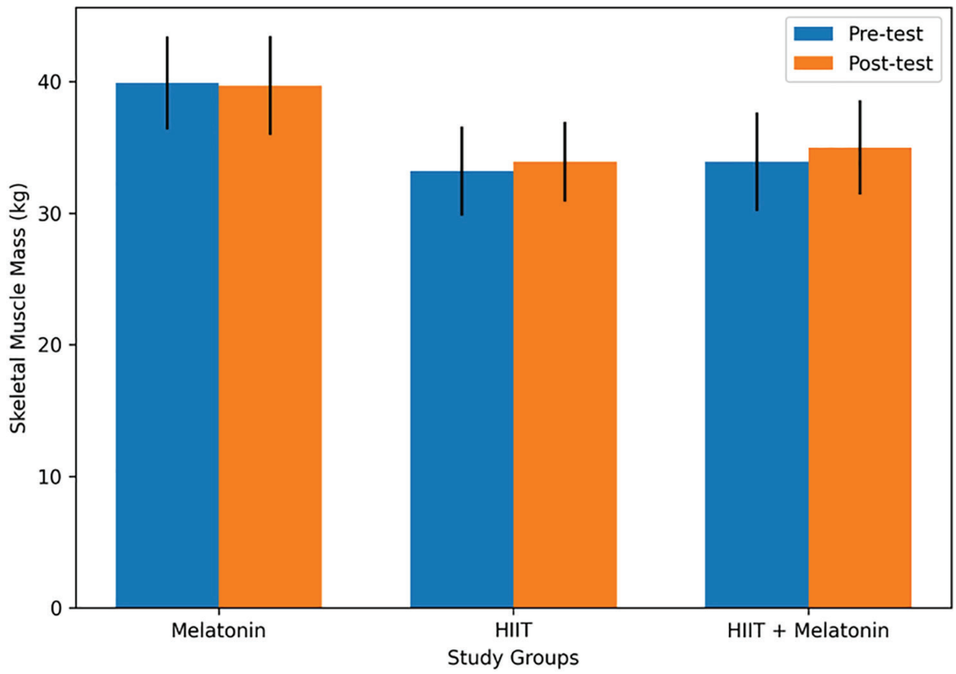

Figure 1: Pre-test and post-test skeletal muscle mass across study groups. Values were presented as mean±SD. HIIT: High-intensity interval training.

Table 4: Analysis of covariance (ANCOVA) for post-test skeletal muscle mass adjusted for baseline values.

Source	SS	df	MS	F	*P* value	Partial η^2
Pre-test skeletal muscle mass	332.83	1	332.83	1456.16	<0.001*	0.982
Group	4.10	2	2.05	8.97	0.001*	0.399
Error	6.17	27	0.23	—	—	—

Pre-test skeletal muscle mass was entered as the covariate. Partial η^2 represents effect size. *Significant at $p<0.05$.

Table 5: Bonferroni-adjusted pairwise comparisons for adjusted post-test skeletal muscle mass.

Comparison	Mean difference	95%CI	Adjusted *p* value
HIIT vs. Melatonin	0.67	-0.03-1.36	0.065
HIIT+Melatonin vs. Melatonin	1.08	0.42-1.74	0.001*
HIIT+Melatonin vs. HIIT	0.42	-0.12-0.95	0.173

Mean differences were adjusted for baseline skeletal muscle mass. Confidence intervals and *p* values were Bonferroni-adjusted. *Significant at $p<0.05$. HIIT: High-intensity interval training.

One-way ANCOVA demonstrated a significant main effect of group on post-test skeletal muscle mass after adjustment for baseline skeletal muscle mass [$F(2,27)=8.97$, $p=0.001$, partial $\eta^2=0.399$]. ANCOVA results were presented in Table 4. Bonferroni-adjusted pairwise comparisons were presented in Table 5. The HIIT+Melatonin group showed significantly higher adjusted post-test skeletal muscle mass than the Melatonin group. The adjusted difference between HIIT+Melatonin and HIIT did not reach statistical significance after Bonferroni correction.

ANCOVA diagnostic checks and analyses indicated that the homogeneity of regression slopes assumption was not fully satisfied [group×baseline skeletal muscle mass interaction: $F(2,25)=4.64$, $p=0.019$]. Residual homoscedasticity was acceptable based on Levene's test ($p=0.963$) and the Breusch-Pagan test ($p=0.712$). Residual normality was not fully supported by the Shapiro-Wilk test ($p=0.017$). Therefore, the ANCOVA findings were retained as baseline-adjusted estimates but interpreted conservatively in conjunction with the observed within-group changes and the study limitations.

Discussion

The present study investigated the isolated and combined effects of HIIT and melatonin supplementation on skeletal muscle mass in physically active military cadets. The main findings indicated that skeletal muscle mass increased significantly in both exercise groups, whereas melatonin supplementation alone did not significantly alter muscle mass. After adjustment for baseline skeletal muscle mass, the combined HIIT and melatonin group showed a significant adjusted advantage over the melatonin-only group; however, the adjusted difference between the combined group and the HIIT-only group was not statistically significant after Bonferroni correction.

The absence of significant improvements in the melatonin-only group may indicate that melatonin supplementation, without a concomitant exercise stimulus, is insufficient to induce substantial skeletal muscle hypertrophy. Although melatonin has been recognized for its antioxidant, anti-inflammatory, and neuroprotective properties, its direct anabolic effects on skeletal muscle remain limited (11-15). Farhud and Tahavorgar reported that melatonin primarily contributed to metabolic regulation and circadian rhythm modulation rather than direct enhancement of muscle protein synthesis (6). Similarly, Khalili *et al.* emphasized the role of melatonin in neural recovery and physiological regulation rather than structural muscular adaptations (9). These findings may help explain why melatonin supplementation alone did not significantly increase skeletal muscle mass in the present study (6, 9).

In contrast, participants in the HIIT group demonstrated significant increases in skeletal muscle mass following the intervention period. Previous evidences consistently showed that HIIT induced important physiological adaptations through repeated exposure to high metabolic stress and elevated exercise intensity. Gibala *et al.* and MacInnis and Gibala reported that interval-based exercise stimulated mitochondrial biogenesis, neuromuscular adaptations, and anabolic signaling pathways associated with muscular remodeling (2, 3). Likewise, Weston *et al.* demonstrated that low-volume HIIT protocols could substantially improve fitness-related outcomes even within short intervention periods (4). These physiological adaptations may collectively contribute to enhanced skeletal muscle remodeling and may partially explain the significant increases in skeletal muscle mass observed in the present study (2-4).

Beyond these adaptations, HIIT may influence skeletal muscle remodeling through several complementary physiological mechanisms. Repeated exposure to high-intensity exercise was shown to enhance muscle protein turnover, increase neuromuscular recruitment, and stimulate cellular pathways involved in muscular adaptation. HIIT may also promote mitochondrial adaptations and improve the efficiency of energy utilization within skeletal muscle tissue. Although these mechanisms were not directly assessed in the present study, they provide plausible biological explanations for the observed increases in skeletal muscle mass among participants who completed the HIIT intervention (16, 17).

The effectiveness of HIIT may be particularly relevant in military populations. Haddock *et al.* reported that high-intensity functional training programs improved physical readiness and fitness performance in military personnel (1). Because military cadets are frequently exposed to physically demanding conditions, HIIT may provide an efficient and time-effective training strategy for improving muscular and metabolic adaptations (1, 18). The combined HIIT and melatonin intervention was associated with the largest numerical increase in skeletal muscle mass. This pattern may reflect a possible supportive role of melatonin in recovery-related processes when combined with an adequate exercise stimulus. Previous evidences suggested that melatonin may contribute to recovery-related physiological functions, including sleep regulation and oxidative stress modulation. Reiter *et al.* and Pandi-Perumal *et al.* described melatonin as an important biological regulator involved in circadian rhythm synchronization, antioxidant defense, and recovery-related physiological responses (7, 8).

Although melatonin is not generally regarded as a direct anabolic agent, several biological properties may support exercise-related adaptations. Melatonin possesses antioxidant and anti-inflammatory effects that may attenuate exercise-induced oxidative stress and facilitate recovery processes. Furthermore, melatonin contributes to circadian rhythm regulation and sleep promotion, both of which are important for physiological recovery following strenuous exercise. Consequently, melatonin may create a more favorable recovery environment that supports, rather than directly induces, exercise-related muscular adaptations (7, 8).

The greater numerical increase observed in the HIIT+Melatonin group may reflect a potential interaction between exercise-induced adaptive responses and recovery-related mechanisms associated with melatonin supplementation. Improved recovery, enhanced sleep regulation, and reduced oxidative stress may theoretically facilitate adaptation to repeated high-intensity exercise stimuli. However, because the adjusted difference between the combined intervention and the HIIT-only group did not remain statistically significant after Bonferroni correction, any proposed synergistic effect should be interpreted cautiously and requires confirmation in larger placebo-controlled studies.

Because sleep quality was assessed as part of the broader research project using the Pittsburgh Sleep Quality Index (PSQI), the greater numerical response observed in the combined intervention group may be partially related to recovery-related mechanisms; however, this interpretation remains exploratory and was not directly tested in the present analysis. Previous systematic reviews have shown that exercise interventions, particularly HIIT, can positively influence sleep quality and recovery status. Min *et al.* demonstrated beneficial effects of HIIT on sleep-related outcomes (19), while Silva *et al.* reported that regular physical activity improves sleep quality and physiological recovery (20). Furthermore, Dattilo *et al.* suggested that sleep plays a critical role in muscular recovery through endocrine and molecular pathways involving anabolic hormone regulation (21). Adequate sleep duration and sleep efficiency have also been associated with improved recovery and athletic performance. Hirshkowitz *et al.* emphasized the physiological importance of sufficient sleep duration (22), while Halson highlighted the role of sleep optimization in athletic recovery and performance enhancement (10).

The ANCOVA findings indicated that intervention type influenced post-test skeletal muscle mass after controlling for baseline values. However, diagnostic checks showed that the homogeneity of regression slopes assumption and residual normality were not fully satisfied. Therefore, the adjusted ANCOVA findings should be interpreted as supportive but not definitive. The findings are partially consistent with previous studies demonstrating favorable body composition adaptations following interval-based exercise interventions (23-28). Rabiee *et al.* previously reported improvements in body composition following HIIT interventions combined with nutritional strategies (23).

Collectively, these findings suggest that HIIT appeared to be the main factor associated with improvements in skeletal muscle mass, whereas melatonin supplementation may have provided supportive recovery-related benefits when combined with exercise. Nevertheless, several limitations should be acknowledged. First, the study did not include a true non-intervention control group. Given the structured military environment, including routine physical training and standardized daily activities, it cannot be concluded that all observed changes were exclusively attributable to the study interventions. Second, placebo supplementation and participant blinding were not feasible because of logistical and administrative constraints in the military setting, which may have introduced expectation bias.

Third, the sample size was relatively small, and dietary intake was not objectively monitored using food records or dietary recalls. Fourth, skeletal muscle mass was assessed using bioelectrical impedance analysis, which may be influenced by hydration fluctuations despite standardized testing procedures. Fifth, baseline differences in body mass, BMI, and skeletal muscle mass were present across groups. These baseline differences further support the need for cautious interpretation of between-group comparisons. Although ANCOVA was used to adjust for baseline skeletal muscle mass, the ANCOVA assumption checks were not fully satisfied; therefore, causal and comparative interpretations should remain cautious. Future studies with larger sample sizes, longer intervention periods, true non-intervention and placebo-controlled groups, participant blinding, objective dietary monitoring, and additional biomarkers of muscle metabolism, recovery, and sleep physiology are warranted.

Conclusion

A four-week HIIT intervention was associated with significant increases in skeletal muscle mass among physically active military cadets, whereas melatonin supplementation alone did not significantly improve muscle mass. The combined HIIT and melatonin intervention showed the largest numerical increase and a significant adjusted difference compared with melatonin alone; however, it was not significantly superior to HIIT alone after Bonferroni correction. Although these findings suggest that melatonin may potentially have a supportive role when combined with exercise-based training, the results should be interpreted cautiously because of the absence of a true control group, lack of placebo and participant blinding, small sample size, baseline group differences, and incomplete fulfillment of ANCOVA assumptions.

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Authors' Contribution

MR designed the study and wrote the manuscript. SM and HO contributed to data collection and training supervision. MI performed statistical analysis and critically revised the manuscript. All authors approved the final version. Artificial intelligence tools were used solely for English language editing and manuscript refinement. All scientific interpretations, analyses, and conclusions were verified and approved by the authors.

Conflict of Interest

The authors declare no conflict of interest.

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