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Comparative Effects of Isometric and Isotonic Progressive Continuous Exercise on miRNA-155 Expression Following Sports-Related Muscle Injury

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ABSTRACT

Background: Background: Muscle injury often causes pain, inflammation, and reduced function. MicroRNA-155 (miRNA-155) is involved in inflammatory regulation and tissue regeneration after skeletal muscle injury. Exercise rehabilitation may influence these molecular responses, but evidence regarding different exercise modalities remains limited. Objective: To compare the effects of isometric progressive continuous exercise (MPCE) and isotonic progressive continuous exercise (NPCE) on miRNA-155 expression in athletes with grade 1–2 muscle injury.
Method: A true experimental randomized pretest–posttest control group design was used. Twenty-one athletes with grade 1–2 muscle injury were randomly assigned to MPCE (n = 7), NPCE (n = 7), or control (CON; n = 7). Intervention groups performed progressive continuous exercise once daily for 7 consecutive days, while the control group received no exercise intervention. Venous blood samples were collected before and after intervention, and miRNA-155 expression was analyzed using real-time quantitative polymerase chain reaction (qPCR). Data were analyzed using within-group tests, Kruskal–Wallis, and post-hoc analysis..
Result: In the MPCE group, miRNA-155 increased from 0.583 ± 0.5 to 3.700 ± 3.6, but the change was not significant (p = 0.066). In the NPCE group, miRNA-155 significantly decreased from 14.273 ± 1.3 to 5.645 ± 3.6 (p = 0.001). The control group showed no significant change from 1.119 ± 1.6 to 1.598 ± 1.4 (p = 0.310). Between-group analysis showed significant differences in baseline values (H = 13.406, p = 0.001) and change scores (H = 12.276, p = 0.002). Post-hoc analysis revealed significant differences between both intervention groups and control, while no difference was found between MPCE and NPCE.
Conclusion: Progressive continuous exercise significantly modulated miRNA-155 expression compared with the control group. However, no significant difference was found between MPCE and NPCE, and results should be interpreted with caution due to baseline imbalance. Further studies with larger samples and adjusted analyses are warranted.

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INTRODUCTION

Muscle injuries are among the most common injuries in sports, accounting for approximately one in three injury cases. Their incidence varies between 10–55% of all sports-related injuries (Järvinen et al., 2013). González et al. (2021) reported an average injury rate of 2.7 per 1000 hours of sports participation, with football presenting the highest rate at 7.21 per 1000 hours. Injury incidence was highest during training sessions (49.28%), compared with pre-competition and competition periods (40.72%). The most frequent types of injuries include lumbar muscle strains (12.4%), ankle sprains (11.98%), and bone fractures (9.31%) (González et al., 2021).

Muscle injury induces pain that leads to decreased function during local inflammation, which is characterized by an increase in inflammatory proteins, including enzymes such as cyclooxygenase-2 (COX-2) and cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (Leung & Cahill, 2010). Shortly after injury, a regenerative response follows to repair the tissue. The primary goal of early management is to reduce pain and inflammation. Failure in this initial management can result in delayed healing and progression to chronic conditions.

The management of muscle injuries in sports is complex and continuously evolving. The conventional approach widely used is the rest, ice, compression, and elevation (RICE) protocol (Vuurberg et al., 2018). More recently, updated frameworks such as protect, elevate, avoid anti-inflammatory drugs, compress, and educate (PEACE) and load, optimism, vascularization, and exercise (LOVE) have been introduced. These approaches differ primarily in their emphasis on rest versus protection and the use of ice and anti-inflammatory medications. However, both emphasize the importance of exercise in the rehabilitation process. Notably, the LOVE principle promotes earlier initiation of exercise depending on injury severity, which may enhance vascularization through regenerative angiogenesis (Liu et al., 2023).

Exercise therapy is a core intervention in physiotherapy, alongside physical, electromagnetic, and mechanical modalities. It exerts therapeutic effects by influencing cellular physiological processes, including reducing inflammation, alleviating pain, promoting tissue regeneration, and enhancing bioplasticity (Huang et al., 2013). Beyond physiological adaptations, exercise has also been shown to influence gene expression, thereby opening opportunities for integrating physiotherapy into genomic medicine (Cornwall et al., 2018).

The mechanisms underlying the effects of exercise can be explained through mechanobiology and bioenergetics. Mechanobiology involves the application of biophysics and biomechanics to understand biological and physiological functions at multiple levels, including protein interactions, gene transcription, cell migration, and intercellular communication (Lim et al., 2010). Physiotherapy utilizes mechanotherapy to stimulate these mechanobiological processes (Ng et al., 2017). Muscle contraction during exercise plays a key role in reducing inflammation (Clifford et al., 2019), decreasing pain (Naugle et al., 2016), and improving tissue function. Additionally, skeletal muscle acts as an endocrine organ, releasing cytokines and peptides known as myokines and exerkines, which exert autocrine, paracrine, and endocrine effects (Magliulo et al., 2021).

Muscle regeneration following injury involves the activation of satellite cells, which proliferate and differentiate to form new myofibers (Shadrach & Wagers, 2011). This regenerative process is highly dependent on the cellular environment, particularly the balance between pro-inflammatory and anti-inflammatory macrophages derived from myeloid cells. Exercise has been shown to modulate inflammation, as indicated by increased macrophage markers such as CD68 (Abreu & Kowaltowski, 2020). These processes are further regulated by microRNAs (miRNAs), small non-coding RNAs that play a critical role in gene expression. One such molecule, miRNA-155, is known to regulate immune cell activation and tissue regeneration (Nie et al., 2016; Chen et al., 2022).

MicroRNA-155 is a non-coding RNA, a class of small single-stranded RNA molecules that inhibit target gene function at the post-transcriptional stage of gene expression. MicroRNA-155 is analyzed using the real-time quantitative Polymerase Chain Reaction (qPCR) technique. The real-time qPCR test is a method used to detect genetic material from a tissue by employing DNA sequences called primers to selectively amplify the target genome. The instrument used is the Applied Biosystems qPCR system.

Previous studies have demonstrated that both endurance and resistance exercise can alter miRNA expression as part of the adaptive response to physical activity (Baggish et al., 2011). However, findings remain inconsistent, with some miRNAs being upregulated and others downregulated. Most existing research has focused on aerobic exercise using frequency, intensity, time, and type (FITT) parameters, which has been shown to increase the secretion of exerkines beneficial for tissue regeneration (da Silva et al., 2020).

Despite these advances, there is a lack of evidence regarding the specific effects of different types of muscle contraction—particularly isometric and isotonic contractions—on molecular mechanisms such as miRNA expression in the context of muscle injury. Although these contraction types are commonly applied in clinical physiotherapy practice for inflammatory conditions, their underlying cellular and molecular mechanisms remain insufficiently understood.

Therefore, this study is important to clarify the role of different muscle contraction types in modulating biological responses during muscle injury recovery. A deeper understanding of these mechanisms may strengthen the scientific basis of exercise therapy and support the development of physiotherapy interventions aligned with molecular and genomic approaches. The aim of this study is to analyze the differences in miRNA-155 expression in sports-related muscle injuries following isometric muscle contraction exercise compared with isotonic muscle contraction exercise using a progressive continuous dosage, as well as with a control group..

METHOD

This study employed a true experimental randomized pretest–posttest control group design. Participants were randomly allocated into three groups: two intervention groups and one control group. The researcher was not directly involved in either the intervention delivery or outcome assessment. All interventions were administered by professional physiotherapists at a physiotherapy clinic in Yogyakarta, Indonesia. Pre and post intervention data measurement miRNA-155 expression taken from blood plasma administered by expert biomedical technicians. Blood sample analysis with Quantitative PCR by laboratory expert in UGM Biomolecular Laboratory

The size of samples in this study were calculated using the Pocock formula based on a previous study by Cliford (2019) . A total of 21 athletes with muscle injuries voluntarily participated and completed a 7-day intervention program. Prior to randomization, all participants underwent baseline assessments, including interviews, physical examination, and injury grading. Sampling was conducted using purposive sampling based on predefined inclusion and exclusion criteria, followed by simple randomization using a lottery method for group allocation.

The inclusion criteria were: members of the University Sports Club of Universitas ‘Aisyiyah Yogyakarta, aged 18–25 years, diagnosed with grade 1 or grade 2 muscle injury, and not currently taking non-steroidal anti-inflammatory drugs (NSAIDs). Sports injury in this study was defined as muscle tissue damage caused by sports activity. Injury severity was classified as grade 1 or grade 2 based on physical examination and muscle ultrasonography (MUS). Exclusion criteria included grade 3 muscle rupture or tear and the presence of fracture signs. Participants were withdrawn if they did not complete the program or sought other forms of treatment during the study period.

The intervention consisted of Progressive Continuous Exercise (PCE), a structured exercise program involving either isometric or isotonic muscle contractions with progressively increased

dosage according to the FITT principle (frequency, intensity, time, and type), performed continuously without rest during each session. The frequency was once daily for 7 consecutive days. Exercise intensity increased gradually, starting from 30 repetitions on day 1 and increasing by 10 repetitions each subsequent day until day 7. For the isometric PCE group (MPCE), participants performed muscle contractions at 50% of one-repetition maximum (1RM) capacity for 30–40 seconds without any change in muscle length. For the isotonic PCE group (NPCE), participants performed alternating concentric and eccentric contractions using body weight resistance through a full joint range of motion, with each movement completed in approximately 5 seconds at a moderate tempo.

Venous blood samples were collected at baseline and again on day 7. Plasma was separated for laboratory analysis. MicroRNA-155, a small non-coding single-stranded RNA involved in post-transcriptional gene regulation, was analyzed using real-time quantitative polymerase chain reaction (qPCR) with an Applied Biosystems qPCR system.

RESULTS AND DISCUSSION

Results

A total of 21 participants were enrolled and equally distributed into three groups: MPCE (n = 7), NPCE (n = 7), and control (CON; n = 7). Baseline subject characteristics are presented in Table 1. The mean age of participants was comparable across groups. Mean body weight was highest in the MPCE group (64.8 ± 9.1 kg), followed by the control group (60.0 ± 3.5 kg), and lowest in the NPCE group (53.7 ± 13.6 kg). Mean body height was similar among groups, ranging from 168.4 ± 7.8 cm in the MPCE group to 169.5 ± 7.1 cm and 169.5 ± 7.5 cm in the NPCE and control groups, respectively. Baseline blood pressure values were also comparable between groups. The MPCE group had a mean blood pressure of 122.2/86.0 ± 10.7/5.0 mmHg, the NPCE group 121.8/84.87 ± 4.8/10.1 mmHg, and the control group 120.8/85.6 ± 6.2/11.4 mmHg. The mean body mass index (BMI) was 22.74 ± 3.6 kg/m² in the MPCE group, 18.79 ± 2.2 kg/m² in the NPCE group, and 21.41 ± 3.5 kg/m² in the control group. Overall, baseline demographic and anthropometric characteristics were generally similar among the three groups.

Table 1. Subject characteristics

Characteristic	MPCE (n=7) (Mean±SD)	NPCE (n=7) (Mean±SD)	CON (n=7) (Mean±SD)
Age	20,2±1,1	19,2±0,7	20,1±1,5
Body weight (kg)	64,8±9,1	53,7±13,6	60±3,5
Body height (cm)	168,4±7,8	169,5±7,1	169,5±7,5
Blood pressure, sistole/diastole (mmHg)	122,2/86±10,7/5	121,8/84,87±4,8/10,1	120,8/85,6±6,2/11,4
Body mass index	22,74±3,6	18,79±2,2	21,41±3,5

Within-group analysis of miRNA-155 expression is presented in Table 2. In the MPCE group, mean miRNA-155 increased from 0.583 ± 0.5 at baseline to 3.700 ± 3.6 following intervention; however, this change did not reach statistical significance (p = 0.066). In the NPCE group, miRNA-155 expression decreased significantly from 14.273 ± 1.3 to 5.645 ± 3.6 (p = 0.001), suggesting a notable downregulatory effect of the intervention. In the control group, miRNA-155 levels showed a slight, non-significant increase from 1.119 ± 1.6 to 1.598 ± 1.4 (p = 0.310).

Table 2. Within group analysis of miRNA-155

Group	Pre	Post	*p*
MPCE (n=7) (Mean±SD)	0,583±0,5	3,700±3,6	0,066*
NPCE (n=7)	14,273±1,3	5,645±3,6	0,001*

(Mean±SD)			
CON (n=7) (Mean±SD)	1,119±1,6	1,598±1,4	0,310**

***Paired T-test**

****Wilcoxon**

Between-group analysis using the Kruskal–Wallis test (Table 3) revealed significant differences in baseline miRNA-155 levels across the three groups (H = 13.406, p = 0.001). This baseline imbalance indicates that the groups were not fully comparable prior to intervention, which should be considered when interpreting subsequent between-group comparisons. Significant differences were also observed in the change scores of miRNA-155 following intervention (H = 12.276, p = 0.002).

Table 3. Between group analysis of miRNA-155

Variable	H score	*p*
Pre-value	13,406	0,001
Difference-value	12,276	0,002

Post-hoc analysis (Table 4) revealed no significant difference between the MPCE and NPCE groups either at baseline (p = 1.000) or in change values after intervention (p = 0.964), suggesting comparable response patterns between the two intervention groups. In contrast, both intervention groups differed significantly from the control group. Specifically, significant differences were observed between MPCE and CON at baseline (p = 0.004) and in change values (p = 0.002), as well as between NPCE and CON at baseline (p = 0.006) and in change values (p = 0.047).

Table 4. Post-Hoc analysis

Group		*p*
miRNA-155 pre-value	MPCE vc NPCE	1,000
	MPCE vs CON	0,004
	NPCE vs CON	0,006
miRNA-155 Difference value	MPCE vc NPCE	0,964
	MPCE vs CON	0,002
	NPCE vs CON	0,047

DISCUSSION

The present study indicated that both exercise interventions influenced miRNA-155 expression compared with the control condition. Within-group analysis showed a significant reduction in miRNA-155 only in the NPCE group, while changes in the MPCE and control groups were not statistically significant. Between-group comparisons revealed significant differences in baseline values and post-intervention change scores among the three groups. Post-hoc analysis further demonstrated that both intervention groups differed significantly from the control group, whereas no significant difference was found between MPCE and NPCE. These results suggest that progressive continuous exercise, particularly NPCE, may have a greater effect on modulating miRNA-155 expression following muscle injury.

Nie et al. (2016) reported that genetic deletion of miRNA-155 in mice substantially delayed muscle regeneration. However, miRNA-155 did not appear to directly regulate satellite cell proliferation or differentiation. Instead, miRNA-155 was expressed in myeloid cells, where it played a crucial role in appropriate myeloid cell activation and in regulating the balance between pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages during skeletal muscle regeneration. Mechanistically, miR-155 suppresses SOCS1, a negative regulator of the JAK-STAT signaling pathway, during the early inflammatory response after muscle injury.

MIRHG155 is the host gene encoding miR-155, originally identified as the B-cell Integration Cluster (BIC) gene. This gene consists of three exons spanning a 13 kb region on human chromosome 21 (Hsa21) and contains the primary non-coding transcript pri-miR-155 within exon 3, which is further processed into mature miR-155. The expression of miR-155 varies across cell types and tissue environments and is coordinated by multiple signaling pathways in response to cellular stimuli.

MicroRNAs are endogenous non-coding RNAs of approximately 21–25 nucleotides in length (Wahid et al., 2010). They function at the post-transcriptional level by suppressing gene expression through binding to messenger RNA (mRNA). In line with their expanding biological roles, miRNAs regulate numerous cellular processes, including proliferation, differentiation, and apoptosis (Gurtan & Sharp, 2013). In exercise adaptation, miRNAs are also involved in angiogenesis and inflammation (Abreu & Kowaltowski, 2020).

Exercise can induce epigenetic modifications that alter gene expression and are associated with changes in miRNA levels across different cell types (Z. Chen et al., 2020). These dynamic fluctuations in miRNA expression may enhance tissue adaptation to exercise through gene regulatory mechanisms. Such responses may also serve as indicators of the health benefits of exercise. Although most miRNAs are intracellular, many are also present in body fluids as circulating miRNAs (ci-miRNAs) (Touron et al., 2023).

Several miRNAs are considered important mediators of tissue adaptation to exercise training, including cardiac and skeletal muscle hypertrophy, as well as regeneration in adults and older populations (Ramasamy et al., 2015). Previous studies have shown that regular exercise influences miRNA regulation. Moreover, miRNAs appear to play essential roles in both acute and chronic responses to strength training and endurance training, in athletes, animal models, patients, and the general population. Their regulation in human skeletal muscle depends on exercise mode, intensity, and duration (Ultimo et al., 2018).

The current findings indicate that contracting muscle can modulate miRNA-155 expression. Muscle contraction may stimulate molecular responses through mechanotransduction, increased energy demand, and activation of hormones such as apelin, GDF-15, and oxytocin (Magliulo et al., 2021).

MiRNA-155 appears to have a dual biological role. Elevated expression has been associated with pro-inflammatory activity in several chronic diseases, including cancer (Mahesh & Biswas, 2019). Conversely, the absence of miRNA-155 has been shown to impair tissue regeneration following injury (Nie et al., 2016). In this study, isometric contraction appeared to reduce miRNA-155 expression, potentially lowering inflammation while maintaining sufficient levels to support tissue regeneration. These findings are consistent with Silva et al. (2019), who reported that muscle cells respond to exercise through both upregulation and downregulation of miRNA expression (Silva et al., 2019).

It should be noted that baseline miRNA-155 expression differed significantly among groups prior to intervention ($p = 0.001$), indicating a substantial baseline imbalance despite randomization. This imbalance may limit the validity of direct between-group comparisons and the interpretation of intervention effects. Additionally, the small sample size in each group ($n = 7$) may have reduced statistical power and increased susceptibility to pre-existing group differences. Future studies are encouraged to employ baseline-adjusted analyses, such as analysis of covariance (ANCOVA) or mixed-effects models, and to include larger sample sizes to account for pre-existing differences and provide more robust estimates of intervention effects.

CONCLUSION

This study demonstrated that progressive continuous exercise significantly influenced miRNA-155 expression in athletes with grade 1–2 muscle injury. Both isometric progressive

continuous exercise (MPCE) and isotonic progressive continuous exercise (NPCE) showed significant differences in miRNA-155 expression compared with the control group. However, post-hoc analysis revealed no statistically significant difference between the MPCE and NPCE groups ($p = 0.964$), indicating that the data do not support a conclusion of superiority of either intervention over the other. The observed changes in miRNA-155 expression suggest that progressive continuous exercise, regardless of modality, may play a role in modulating molecular responses associated with inflammation and tissue regeneration. These findings support the potential role of exercise therapy in regulating molecular and genomic mechanisms during muscle healing, though they should be interpreted with caution given the significant baseline imbalance in miRNA-155 levels among groups prior to intervention. Further studies with larger sample sizes, longer intervention periods, and baseline-adjusted analytical approaches are recommended to clarify the long-term effects of progressive exercise on muscle regeneration and inflammatory biomarkers.

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AUTHOR CONTRIBUTION STATEMENT

MAI, SMJ, JA, AA, and PU contributed to the study conception and design. MAI and SMJ conducted data collection and intervention procedures. MAI, SMJ, and PU performed data analysis and interpretation. SMJ drafted the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

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