



Effects of Supplementation and Resistance Training on Inflammation, Muscle Damage, and DOMS in Untrained Individuals

Muhammad Hafid Nur Afdholi^{1*}, Kartika Nugraheni¹, Luthfia Dewi¹, Ali Rosidi¹, Sri Haryono²

¹Universitas Muhammadiyah Semarang, Indonesia

²Universitas Negeri Semarang, Indonesia

*Correspondence: E-mail: hafidafdholi@gmail.com

ABSTRACT

Resistance training elevates biomarkers of inflammation, muscle damage, and delayed onset muscle soreness (DOMS), particularly in untrained individuals. However, evidence regarding the combined effects of whey protein, β -hydroxy- β -methyl butyrate (HMB), and creatine monohydrate (CrM) supplementation during resistance training remains limited. This study aimed to evaluate the effects of combined supplementation and resistance training on interleukin-6 (IL-6), myoglobin, blood lactate concentration, and DOMS in untrained individuals. A single-blind randomized trial was conducted over an eight-week period involving 44 untrained males aged 20-22 years recruited using consecutive sampling and allocated into four groups, including P0 (resistance training only), P1 (whey protein + HMB), P2 (whey protein + CrM), and P3 (whey protein + HMB + CrM). IL-6 and myoglobin were assessed at Weeks 1 and 8, blood lactate concentration was assessed at Weeks 4 and 8, and DOMS was evaluated at 24 and 48 hours after the resistance training sessions performed during Weeks 1, 4, and 8. IL-6 did not differ significantly across groups ($p > 0.05$). Myoglobin concentrations showed small and inconsistent responses, although a significant reduction was observed in P3 (-4.50% ; $p = 0.0420$; median difference = -236.2 pg/mL, 98.83% CI $[-478.6, 59.2]$). Blood lactate concentration changed significantly over time ($p < 0.001$; $\eta^2 = 0.824$), while DOMS scores progressively decreased throughout the intervention ($p < 0.0001$; $\eta^2 = 0.624$). Overall, repeated resistance training appeared to be the primary factor driving physiological adaptations, whereas supplementation effects were generally limited. More frequent post-exercise sampling should be considered in future studies to better characterize the temporal pattern of the assessed marker, and stricter analytical procedures to further clarify the effects of combined whey protein, HMB, and creatine monohydrate supplementation during resistance training adaptations.

ARTICLE INFO

Article History:

Submitted/Received Mei 2026

First Revised July 2026

Accepted August 2026

Publication Date September 2026

Keyword:

DOMS, inflammation, muscle damage, resistance training, supplementation

INTRODUCTION

Physical fitness has increasingly gained public attention, and resistance training is widely practiced because of its ability to improve muscular strength, muscle mass, and metabolic health (Booth et al., 2012; Schoenfeld, 2010; Walsh, 2018). Resistance exercise induces physiological stress and inflammatory responses that contribute to muscle remodelling and adaptation (Tidball, 2017; Yang et al., 2018). These responses are commonly reflected by increases in inflammatory and muscle damage biomarkers, including interleukin-6 (IL-6) and myoglobin, which have been associated with muscle fiber microdamage, metabolic stress, and delayed onset muscle soreness (DOMS) (Brancaccio et al., 2007; Cipryan, 2017; Feng et al., 2024; Kanda et al., 2013; Pedersen & Febbraio, 2008). In addition, blood lactate concentration provides insight into the metabolic demands associated with resistance exercise and training adaptation (Benítez-Muñoz et al., 2024; Brooks, 2023). Therefore, evaluating IL-6, myoglobin, blood lactate concentration, and DOMS may provide a more comprehensive understanding of physiological responses to resistance training.

Nutritional supplementation is one strategy proposed to attenuate exercise-induced physiological stress and promote recovery, particularly among untrained individuals (Barnes, 2023; Naderi et al., 2025). Whey protein, β -hydroxy- β -methylbutyrate (HMB), and creatine monohydrate (CrM) are widely used in resistance training because they support muscle protein synthesis, reduce protein degradation, and enhance phosphocreatine availability for muscular contraction and recovery (Bell et al., 2025; Holeček, 2017; Wilson et al., 2013).

Previous investigations have reported beneficial effects of whey protein, HMB, and creatine supplementation on training adaptations. Increases in lean body mass, muscle fiber hypertrophy, muscular strength, and contractile protein accretion have been reported following combined whey protein and creatine supplementation during an 11-week resistance training program (Cribb et al., 2007). Improvements in skeletal muscle mass and isokinetic muscular function have also been observed following whey protein supplementation combined with a 4-week supervised resistance training program performed at 60-70% 1RM under dietary control (Kim et al., 2023). Furthermore, combined whey protein and HMB supplementation during a 12-week periodized resistance-training program consisting of linear and non-linear periodization phases resulted in greater increases in leg fat-free mass compared with whey protein alone (Stahn et al., 2020). Beyond performance-related adaptations, protein supplementation has also been reported to influence selected markers of muscle damage and exercise-induced soreness, including myoglobin responses and perceived muscle soreness following eccentric exercise (Nieman et al., 2020).

However, previous studies have generally focused on performance adaptations, body composition outcomes, or isolated recovery markers. Consequently, limited evidence is available regarding whether combined whey protein, HMB, and creatine monohydrate supplementation can simultaneously influence inflammatory, muscle damage, metabolic, and perceptual responses during resistance training adaptation in previously untrained individuals. Moreover, the combined effects of these supplements on IL-6, myoglobin, blood lactate concentration, and DOMS have not been comprehensively evaluated within a single resistance-training intervention.

Therefore, this study aimed to evaluate the effects of whey protein, HMB, and creatine monohydrate supplementation combined with resistance training on IL-6, myoglobin, blood lactate concentration, and DOMS in untrained individuals. It was hypothesized that combined supplementation would result in lower IL-6 concentrations, lower myoglobin concentrations,

reduced blood lactate accumulation, and lower DOMS scores compared with resistance training alone. By integrating inflammatory, muscle damage, metabolic, and perceptual outcomes within a single experimental framework, this study may provide a more comprehensive understanding of the physiological responses associated with nutritional supplementation during resistance training adaptation. The findings are expected to contribute to the growing body of evidence regarding nutritional strategies to support recovery and adaptation during resistance training and may provide practical implications for exercise practitioners and sports nutrition professionals.

METHODS

This study was a quantitative investigation employing a true experimental randomized controlled trial design with four parallel groups. Participants were randomized into four groups: P0 (resistance training without supplementation), P1 (whey + HMB + resistance training), P2 (whey + CrM + resistance training), and P3 (whey + HMB + CrM + resistance training). Ethical approval was obtained from the Ethics Committee of the Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang (No. 457/KE/06/2025), issued on June 19, 2025.

Participants

A total of 44 healthy young adult males participated in this study and were randomly assigned into four groups ($n = 11/\text{group}$). Baseline characteristics of participants were comparable across groups, including age (20.64-21.82 years), body weight (61.34-63.75 kg), height (168.31-170.09 cm), body mass index (BMI) (21.68-22.17 kg/m²), and muscle mass (34.73-36.06 kg), with no significant differences observed between groups ($p > 0.05$). Inclusion criteria were male, aged 18-25 years, untrained (not participating in resistance training for ≥ 6 months), not consuming whey/HMB/CrM for ≥ 3 months prior to the study, having normal nutritional status, being healthy without chronic disease, and willing to complete the intervention protocol.

Exclusion criteria included cardiovascular disease, musculoskeletal disorders, history of injury or surgery within the previous 6 months, and allergies to dairy products. All participants were recreationally active but had no structured resistance training background. Participants were able to communicate effectively and follow all training and assessment procedures throughout the intervention period.

Sampling Procedures

Recruitment was carried out through online flyers using consecutive sampling. Participants who voluntarily agreed to participate in the study were screened prior to enrolment based on the predefined inclusion and exclusion criteria. An a priori sample size calculation was performed using G*Power 3.1. Assuming a large effect size (Cohen's $d = 0.80$), a one-tailed hypothesis, a significance level of $\alpha = 0.05$, and 80% statistical power, a minimum total sample size of 40 participants was required. To account for an anticipated 10% attrition rate, the target sample size was increased to 44 participants. This sample size was also consistent with previous randomized controlled trials investigating HMB, creatine, whey protein supplementation, and resistance training in untrained individuals, which included approximately 9-11 participants per group (Jowko et al., 2001; Shirato et al., 2016). All recruited participants completed the study and were included in the final analysis.

All participants were informed regarding the study procedures and provided written informed consent before participation. After eligibility screening and informed consent

procedures had been completed, participants were randomly assigned to one of four study groups using simple randomization. Group allocation was performed manually by the research team following participant enrolment to provide each participant with an equal opportunity to be assigned to any intervention group. Transportation expenses, gym facility fees, trainer supervision costs, and supplementation provided throughout the intervention period were fully covered by the researchers. Participants also received a token of appreciation upon completion of the study.

Materials and Apparatus

All supplementation products were prepared in standardized daily isocaloric sachets and administered according to a consistent protocol throughout the intervention period. Participants in the supplementation groups were not informed of the specific supplementation composition assigned to their group. However, participant blinding was not feasible for the control group because no placebo supplementation was provided. Whey protein supplementation was administered at a dose of 25 g/day using L-Men Platinum (Nutrifood Indonesia, Jakarta, Indonesia), which provided approximately 0.7 g/day of HMB as part of the product formulation. CrM was administered separately at a dose of 3 g/day using L-Men Isopower Creatine (Nutrifood Indonesia, Jakarta, Indonesia). The whey protein and CrM doses were selected according to the manufacturer's recommended serving sizes and are consistent with commonly used dosages reported in sports nutrition literature (Jäger et al., 2017; Kreider et al., 2017). The determination of one-repetition maximum (1-RM) and the resistance training program utilized standardized resistance training equipment, including shoulder press, bench press, lat pulldown, squat, and deadlift apparatus. All training sessions were supervised by certified trainers to ensure proper technique and participant safety.

The levels of IL-6 were analysed using a Human IL-6 ELISA Kit (ABclonal, Woburn, Massachusetts, USA) and myoglobin levels were analysed using a Human Myoglobin ELISA Kit (ABclonal, Woburn, Massachusetts, USA) using a sandwich ELISA method with standards, controls, duplicate samples, and both intra-assay and inter-assay validation. Samples yielding concentrations outside the assay detection range were intended to be diluted and reanalysed according to the manufacturer's recommendations to ensure measurements remained within the linear range of the standard curve. Outlier values were evaluated against assay controls and duplicate measurements prior to analysis, and blood lactate concentration was assessed based on the previous study (Bruno et al., 2026) and were measured using Point of Care Testing (POCT) with the Accutrend Plus device (Roche Diagnostics, Mannheim, Germany). Delayed onset muscle soreness (DOMS) was assessed using a 0-10 cm Visual Analog Scale (VAS) (Koo & Yang, 2025).

Procedures

The research procedure is presented in Figure 1. Independent variables included supplementation of whey protein (25 g/day), HMB (0.7 g/day), CrM (3 g/day), and progressive resistance training (60-80% 1RM, 3x/week for 8 weeks). Dependent variables included IL-6, myoglobin, and blood lactate concentration, as well as DOMS scores.

Supplements were consumed immediately after training on training days, and on non-training days were consumed in the morning after breakfast. Participants were instructed to maintain their habitual dietary patterns and physical activity levels throughout the intervention period and to avoid consuming any additional nutritional supplements not provided by the research team. Adherence to the supplementation protocol was monitored weekly through participant self-reports during scheduled training sessions. No participant

reported the use of external supplements during the intervention, and all participants completed the supplementation protocol as prescribed.

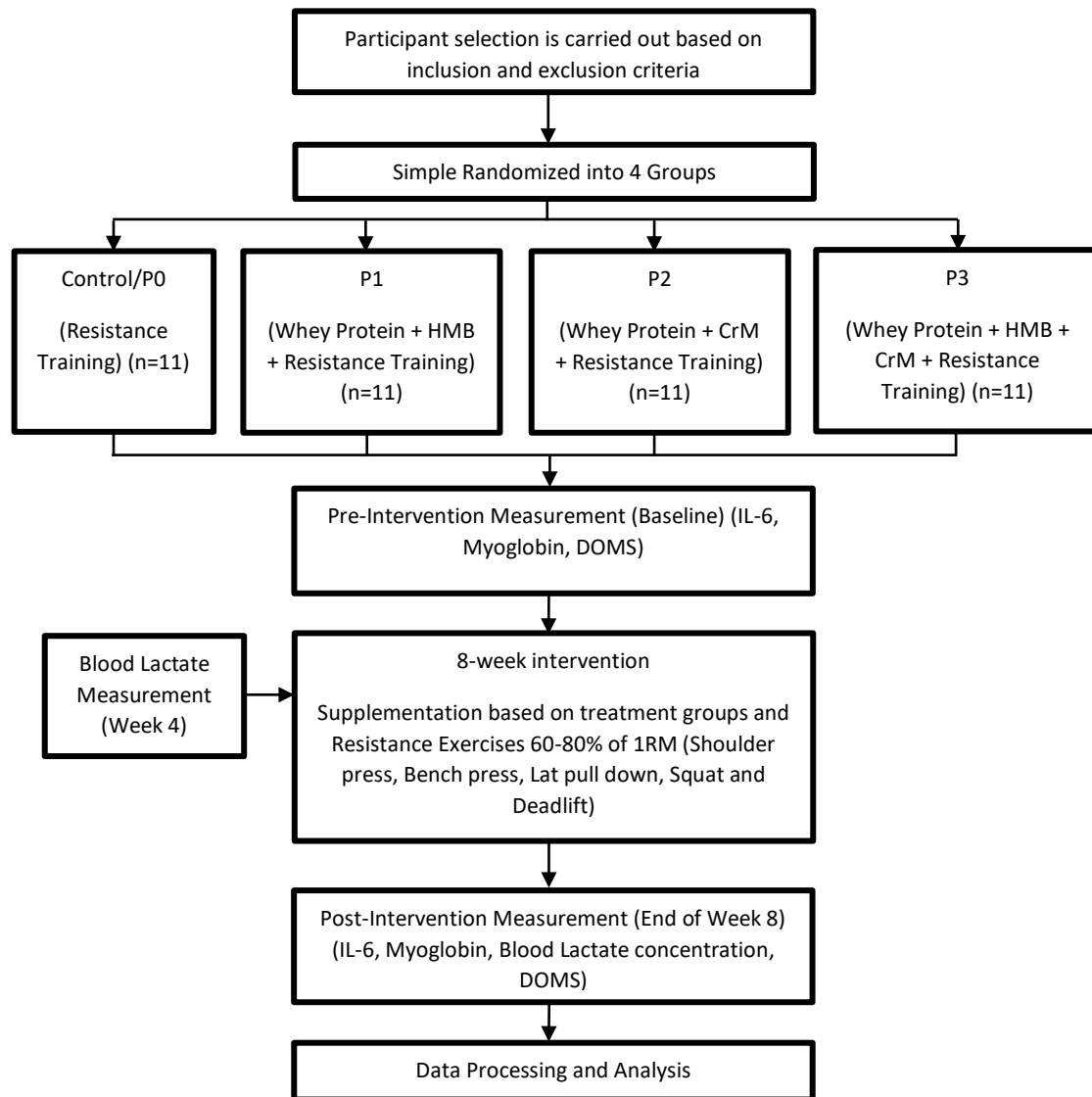


Figure 1. Research Design and Protocol

The determination of one-repetition maximum (1-RM) followed the 2021 ACSM guidelines (ACSM, 2021). Participants completed a 5-minute general warm-up, followed by a specific warm-up consisting of one set of 10 repetitions at 40-50% of the estimated 1-RM and one set of 5 repetitions at 60-70% of the estimated 1-RM. The 1-RM test was conducted through progressive single-attempt loading, starting at 80-85% of the estimated 1-RM for the first attempt and 90-95% for the second attempt. The load was then increased by 2.5-10 kg until the participant could perform only one repetition with proper technique. Each participant was given a maximum of five attempts with 3-5 minutes of rest between attempts. The procedure was carried out with spotting and supervision by a certified trainer.

The 1-RM measurement was conducted in the first week (baseline) and the eighth week (post-intervention). The baseline 1-RM assessment was used as the basis for designing the eight-week resistance training program, with weekly training loads determined according to intensity percentages (60-80% of 1-RM) (Lyristakis et al., 2022), derived from each participant's individual 1-RM value. This ensured that every participant received a training

load appropriate to their initial capacity. The 1-RM measurement in the eighth week was conducted to evaluate muscle strength adaptations and to confirm changes in maximal capacity following the intervention period.

The resistance training program was designed based on NSCA guidelines (NSCA, 2022), and conducted three times per week (Monday, Wednesday, and Friday) for eight weeks. Each session began with a 3-5-minute general warm-up followed by a specific warm-up consisting of two sets at 40-60% of 1-RM. Five main exercises were performed in sequence: shoulder press, bench press, lat pulldown, squat, and deadlift. Training intensity was progressively increased as follows: 60% 1-RM (weeks 1-2), 70% 1-RM (weeks 3-4), 75% 1-RM (weeks 5-6), and 80% 1-RM (weeks 7-8), with a consistent volume of 3 sets $\times$ 10 repetitions. Deadlifts were performed only during the Friday sessions, while the other exercises were performed in all training sessions. Rest periods were set at 90-120 seconds between sets and 3 minutes between exercises. Each session concluded with a 5-10-minute static stretching cooldown. All training sessions were supervised by certified trainers to ensure proper technique and safety.

Blood collection was performed in weeks 1 and 8, 30 minutes after exercise to capture the acute inflammatory response (Croft et al., 2009; Fischer, 2006; Nash et al., 2023). Blood lactate concentration were measured pre-exercise and ≤ 5 minutes post-exercise (Schraner et al., 2020) in weeks 4 and 8. Blood lactate concentration was assessed in a representative subsample of participants ($n = 20$; five participants per group) because of logistical and resource constraints. Five participants from each group were randomly selected using manual simple randomization prior to the intervention, and the same individuals were assessed at all subsequent lactate measurement time points. DOMS was assessed 24 and 48 hours after the resistance training sessions conducted during Weeks 1, 4, and 8.

Design or Data Analysis

Statistical analyses were performed using GraphPad Prism 11 (GraphPad Software, San Diego, California, USA). Data normality was assessed using the Shapiro-Wilk test. IL-6 and myoglobin were analysed using paired t-tests or Wilcoxon signed-rank tests for within-group comparisons, and Kruskal-Wallis tests followed by Dunn's post hoc tests for between-group comparisons. Blood lactate concentration and DOMS were analysed using mixed-design repeated-measures ANOVA, with Greenhouse-Geisser correction applied when the assumption of sphericity was violated. Post hoc analyses using Tukey's or Dunn's tests were conducted according to data distribution and analytical requirements. Statistical significance was set at $p \leq 0.05$, effect sizes were reported as partial eta squared (η^2), and 95% confidence intervals (95% CI) with corresponding lower and upper bounds were provided where appropriate to facilitate interpretation of the findings

RESULTS

As illustrated in Figure 2, IL-6 levels are presented as mean $\pm$ SD and did not differ significantly between pre- and post-intervention measurements in any treatment group after the 8-week intervention period. Panel E presents delta values representing the difference between post- and pre-intervention measurements using paired data only. Delta values also showed no significant differences between groups. However, these findings should be interpreted cautiously because IL-6 concentrations may have been influenced by pre-analytical limitations related to sample handling and biomarker stability, which could have obscured potential biological responses.

As illustrated in Figure 3, myoglobin levels are presented as mean $\pm$ SD and tended to decrease after the 8-week intervention in several treatment groups. A statistically significant

pre- to post-intervention reduction was observed in the whey + HMB + CrM + resistance training group (P3) (-4.50% ; $p = 0.042$; median difference = -236.2 pg/mL, 98.83% CI $[-478.6, 59.2]$). The other groups did not demonstrate significant changes. However, because all measured concentrations exceeded the analytical detection range of the ELISA assay, these findings should be interpreted with considerable caution and are presented primarily as descriptive patterns rather than definitive quantitative evidence of intervention effects. Delta values also did not differ significantly between groups.

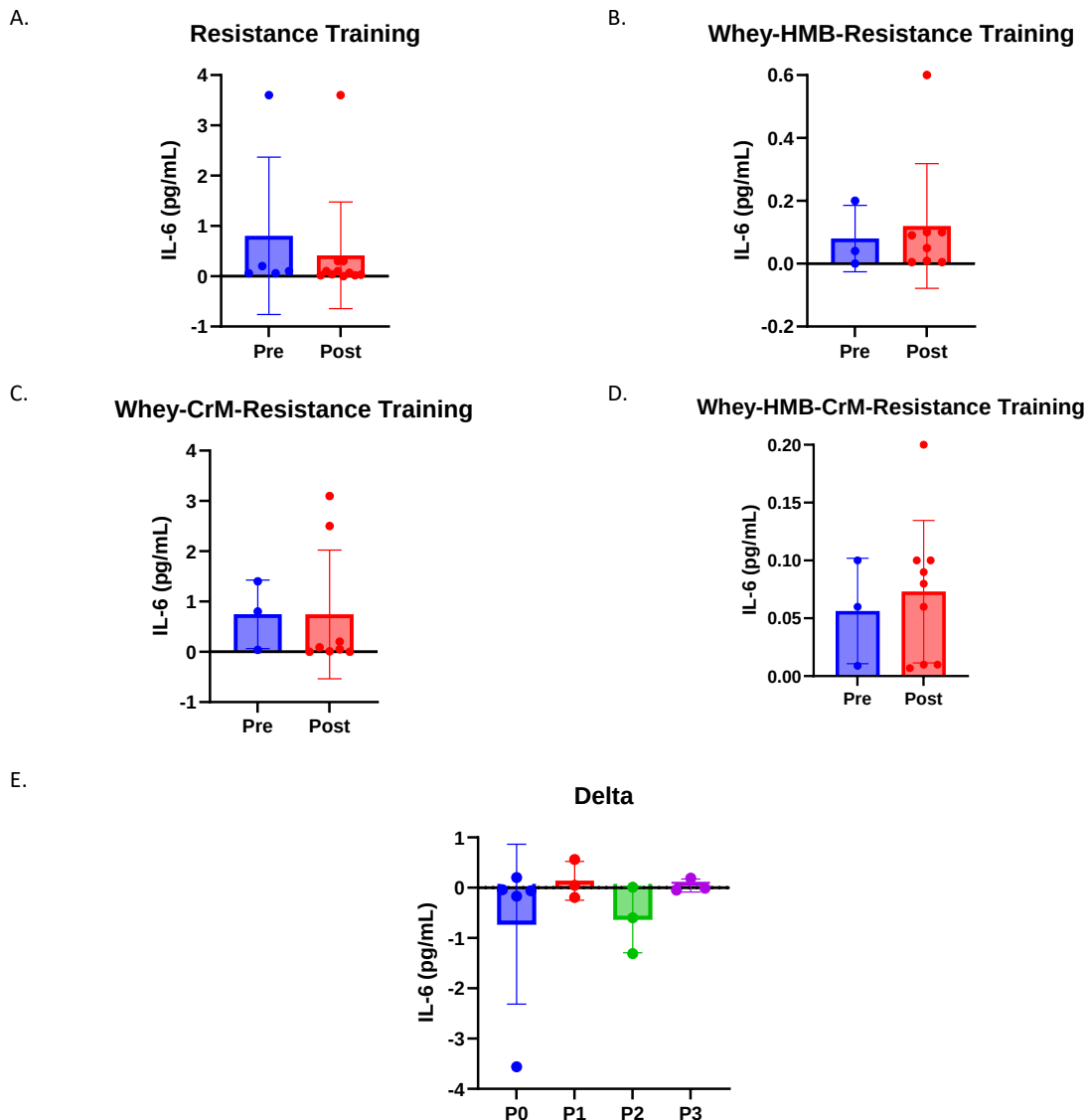


Figure 2. Interleukin-6 Levels

Individual participant responses corresponding to this outcome are presented in Supplementary Figure 1.

As illustrated in Figure 4, blood lactate concentrations were assessed in a representative subsample of participants ($n = 20$; five participants per group) and are presented as mean $\pm$ SD. Blood lactate concentrations changed significantly over time during the intervention period ($p < 0.001$; $\eta^2 = 0.824$). A significant main effect of group was also observed ($p = 0.032$; $\eta^2 = 0.414$). However, no significant time $\times$ group interaction was identified ($p > 0.05$), indicating that changes in blood lactate concentrations over time did not differ significantly

among groups. Post hoc analysis did not demonstrate significant between-group differences at corresponding measurement time points.

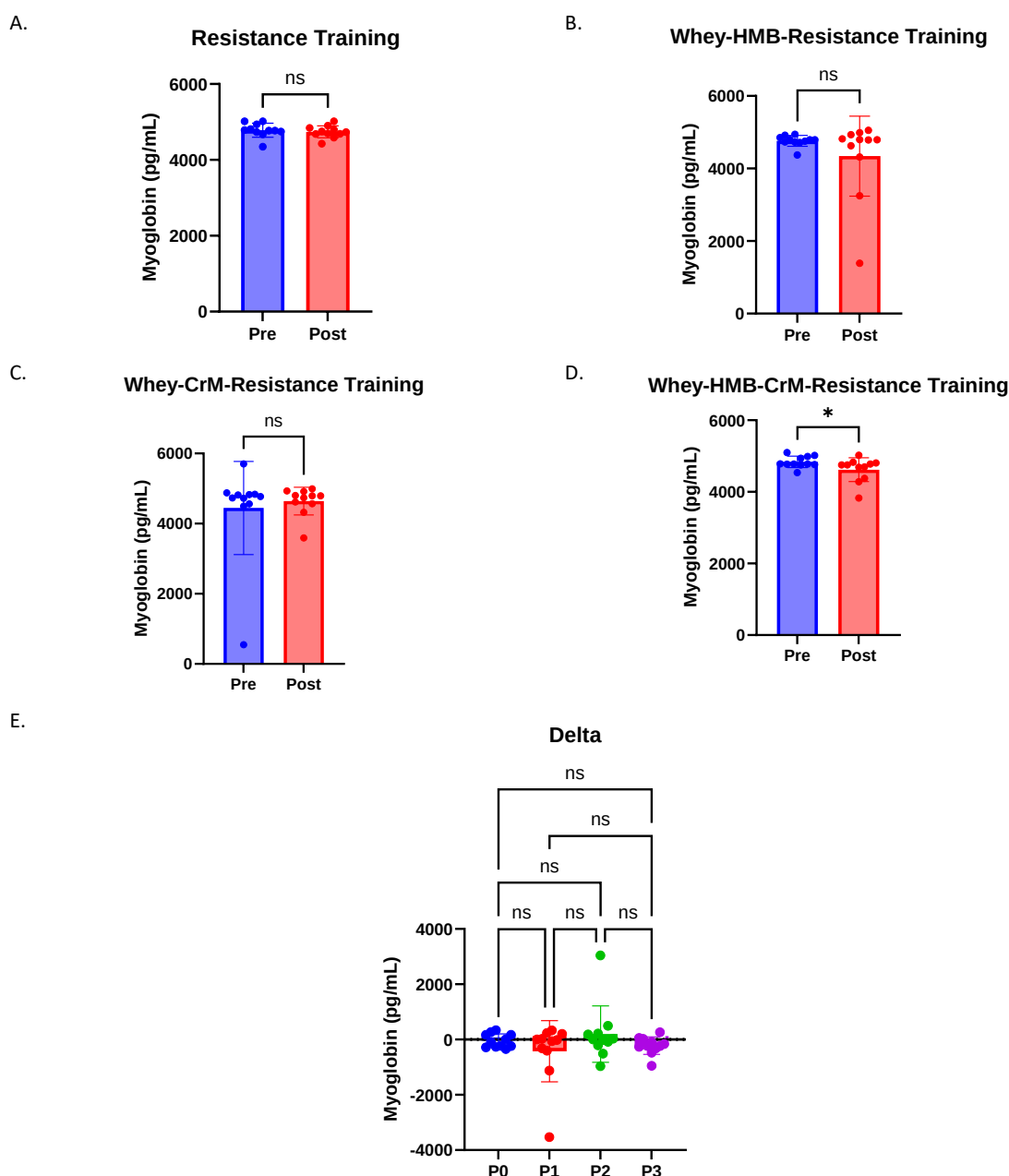


Figure 3. Myoglobin Levels

(*) indicates statistical differences between pre- and post-intervention values within the same group. (ns) indicates non-significant differences. Individual participant responses corresponding to this outcome are presented in Supplementary Figure 2.

Nevertheless, post-exercise lactate concentrations increased markedly in all groups at week 4 relative to pre-exercise values, with percentage increases ranging from approximately 180% to 268%, reflecting a robust acute metabolic response to resistance exercise. Significant within-group increases from week 4 pre- to post-exercise measurements were confirmed in all groups, with mean differences of -5.96 mmol/L (95% CI $[-8.48, -3.44]$) in P0, -8.02 mmol/L (95% CI $[-15.07, -0.97]$) in P1, -5.94 mmol/L (95% CI $[-8.57, -3.31]$) in P2, and -7.46 mmol/L (95% CI $[-11.09, -3.83]$) in P3. At week 8, blood lactate concentrations remained elevated

after exercise across all groups; however, numerically smaller percentage increases were observed in P2 and P3 (143.46% and 175.16%, respectively) compared with P0 (250.50%). Corresponding within-group comparisons from pre- to post-exercise at week 8 yielded mean differences of -10.70 mmol/L (95% CI $[-20.29, -1.11]$) in P0, -8.20 mmol/L (95% CI $[-14.01, -2.39]$) in P2, and -6.08 mmol/L (95% CI $[-11.78, -0.38]$) in P3. Given the absence of a significant time $\times$ group interaction, these observations should be interpreted as descriptive trends rather than evidence of differential intervention effects.

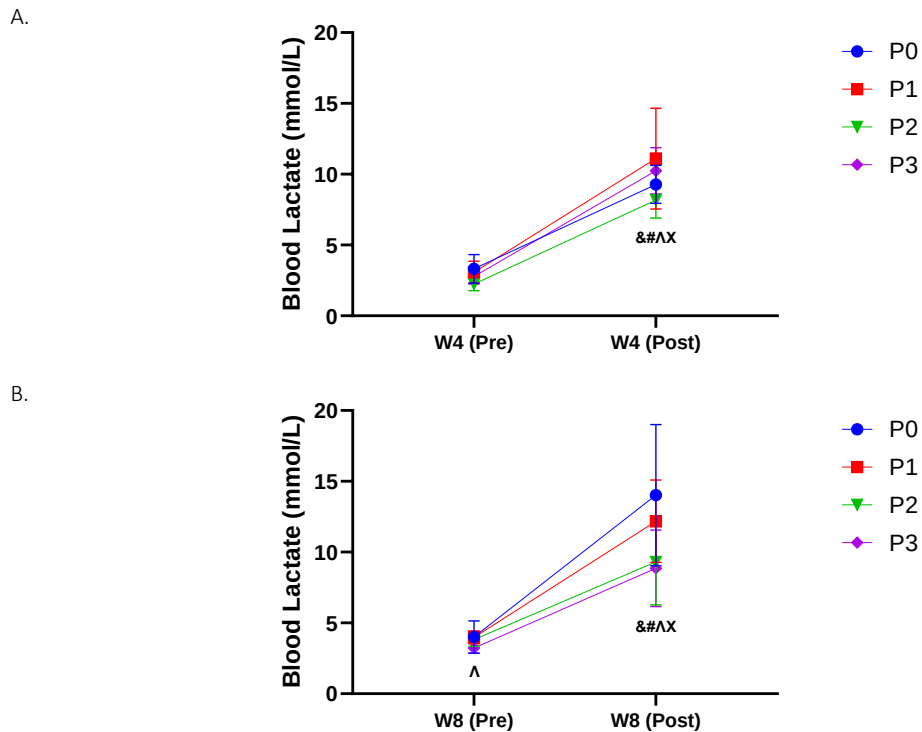


Figure 4. Blood Lactate Concentration

&, #, Δ, and X indicate statistical differences compared with Week 4/W4 (Pre) within groups P0, P1, P2, and P3, respectively. Individual participant responses corresponding to this outcome are presented in Supplementary Figure 3.

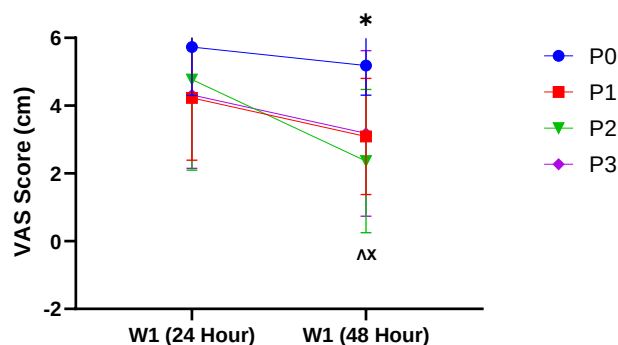
As illustrated in Figure 5, DOMS scores are presented as mean $\pm$ SD and decreased progressively throughout the intervention period across all groups. Significant main effects of time ($p < 0.0001$; $\eta^2 = 0.624$) and group ($p = 0.0043$; $\eta^2 = 0.277$) were observed. However, no significant time $\times$ group interaction was identified ($p > 0.05$), indicating that changes in DOMS scores over time did not differ significantly among groups. A significant subject effect was also detected ($p < 0.0001$; $\eta^2 = 0.418$).

Elevated DOMS scores at 24 and 48 h post-exercise were observed across groups at week 4. Within-group post hoc comparisons demonstrated reductions in DOMS scores from week 1 (24 h) to week 8 (48 h), with mean differences of 3.55 score units (95% CI $[2.04, 5.05]$) in P0, 3.41 score units (95% CI $[1.84, 4.98]$) in P1, 3.86 score units (95% CI $[1.26, 6.47]$) in P2, and 3.77 score units (95% CI $[1.88, 5.67]$) in P3.

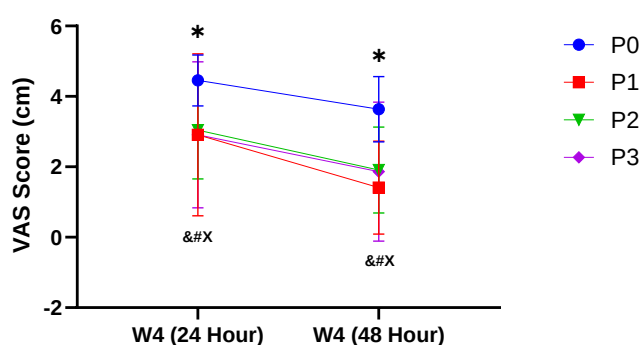
By week 8, lower DOMS scores were observed in the creatine-containing groups (P2 and P3) compared with the control group (P0). At week 8 (24 h), the mean differences between P0 and P2 and between P0 and P3 were 1.09 score units (95% CI $[0.15, 2.03]$) and 1.55 score units (95% CI $[0.42, 2.67]$), respectively. Similar differences were observed at week 8 (48 h), with mean differences of 1.27 score units (95% CI $[0.19, 2.35]$) for P0 versus P2 and 1.55 score

units (95% CI [0.42, 2.67]) for P0 versus P3. Given the absence of a significant time $\times$ group interaction, these observations should be interpreted as descriptive trends rather than evidence of differential intervention effects.

A.



B.



C.

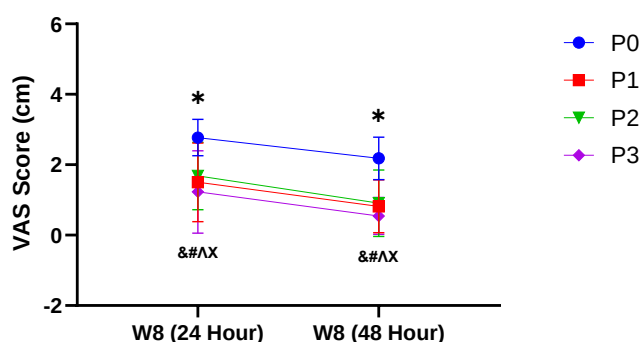


Figure 5. DOMS Scores

&, #, ^, and X indicate statistical differences compared with Week 1/W1 (24 h) within groups P0, P1, P2, and P3, respectively. (*) Indicates statistical differences between groups at the same observation time point. Individual participant responses corresponding to this outcome are presented in Supplementary Figure 4.

DISCUSSION

This study aimed to evaluate the effects of whey protein, HMB, and creatine monohydrate supplementation combined with resistance training on inflammatory responses, muscle damage, metabolic stress, and delayed onset muscle soreness (DOMS) in untrained individuals. Overall, the findings suggest that resistance training induced physiological adaptations throughout the intervention period; however, supplementation effects varied across biomarkers. IL-6 responses could not be interpreted conclusively because of substantial pre-analytical limitations, whereas myoglobin changes were relatively small and

inconsistent across groups. In contrast, blood lactate concentration and DOMS showed clear time-related adaptations, suggesting that exercise-induced physiological adaptations may have predominated during repeated resistance training exposure. Although supplementation effects were generally limited, creatine-containing interventions tended to show smaller increases in lactate and lower DOMS scores during the later stages of adaptation.

As presented in Figure 2, IL-6 responses showed no clear pattern of change across groups and time points. Physiologically, IL-6 is a cytokine that responds rapidly to exercise stimuli, particularly during the acute phase, and typically exhibits a transient post-exercise increase before returning to baseline (Docherty et al., 2022; Nash et al., 2023). However, IL-6 also has low stability, a short half-life, and high sensitivity to variations in temperature, processing delay, and storage conditions. Prolonged exposure to room temperature may lead to epitope degradation and reduced ELISA detection signals (Gong et al., 2019; Kenis et al., 2002; Simpson et al., 2020). Previous studies have shown that pre-analytical variability, including collection procedures, separation time, and storage duration, may obscure true inflammatory responses (Tvedt et al., 2015). Consequently, the absence of observable IL-6 responses in the present study should be interpreted cautiously, as methodological limitations may have obscured potential exercise-induced alterations in IL-6 concentrations. These findings highlight the importance of strict pre-analytical control when assessing low-concentration inflammatory biomarkers such as IL-6.

Although IL-6 responses could not be interpreted conclusively, evaluation of muscle damage markers such as myoglobin remains important for characterizing physiological responses to repeated resistance exercise. As shown in Figure 3, myoglobin responses demonstrated relatively small and inconsistent changes across intervention groups. Interpretation of these findings, however, is fundamentally limited by methodological constraints in the measurement process. All obtained values fell outside the valid detection range of the ELISA method used (39-2500 pg/mL) (ABclonal Technology, 2026), whereas sample concentrations ranged from approximately 4700-5000 pg/mL. This out-of-range (OOR) condition may cause signal saturation and loss of linearity relative to the standard curve, thereby limiting quantitative validity (Andreasson et al., 2015). Although polynomial interpolation was applied to estimate concentrations, nonlinear distortions caused by signal saturation cannot reliably restore quantitative accuracy. Furthermore, sample dilution was not performed before the initial ELISA analysis, and reanalysis using appropriately diluted samples was not feasible because of limited sample availability. The absence of dilution therefore increased the potential for technical bias. Accordingly, the findings are better interpreted as relative patterns of change rather than definitive quantitative estimates.

Despite these limitations, most groups demonstrated relatively stable or slightly reduced myoglobin levels, whereas P2 showed a numerical increase. No significant between-group differences were observed, suggesting that the interventions did not produce clear differential effects on muscle damage responses. Although P3 exhibited a numerical reduction in myoglobin concentration, the substantial analytical limitations associated with the ELISA measurements preclude definitive conclusions regarding a potential intervention effect.

Interpretation of myoglobin responses is further complicated by the mismatch between blood sampling timing and the known kinetics of myoglobin release. Blood samples were collected less than one hour post-exercise, whereas myoglobin typically peaks several hours after exercise and may remain elevated for up to 24 hours (Augusto et al., 2025). Consequently, the sampling protocol may have underestimated the magnitude of muscle damage responses. In addition, repeated exposure to resistance exercise may have

contributed to the repeated bout effect, a protective adaptation associated with reduced muscle damage during subsequent exercise sessions (Calvo-rubio et al., 2024). Similar attenuated post-exercise myoglobin responses following repeated training exposure have been reported previously (Ross et al., 1983; Roxin et al., 1984).

In addition to muscle damage, resistance exercise also imposes considerable metabolic demands. Therefore, blood lactate responses were examined to further characterize the physiological adaptations induced by repeated resistance training. As illustrated in Figure 4, blood lactate concentration increased substantially following exercise across all groups, particularly during the early phase of the intervention. No significant interaction between time and intervention group was observed, indicating that lactate response patterns were relatively similar across groups and that supplementation with whey protein, HMB, and creatine monohydrate did not substantially modify overall lactate responses during resistance exercise. This finding suggests that repeated resistance training, rather than supplementation, was the primary factor driving metabolic adaptations throughout the intervention.

In contrast, the main effect of time showed a large effect size ($\eta^2 = 0.824$), indicating strong metabolic adaptations during repeated resistance training exposure. Together with the absence of a significant time and intervention interaction, this finding suggests that these adaptations were primarily driven by repeated resistance training rather than supplementation. Post-exercise blood lactate concentration increased markedly at week 4, consistent with the high anaerobic metabolic demands of resistance exercise. However, by week 8, creatine-containing groups tended to exhibit smaller lactate increases compared with the control group, which may indicate improved metabolic adaptation during the later stages of training. This pattern is broadly consistent with previous evidence demonstrating that repeated resistance training may improve lactate utilization and clearance as physiological adaptations occur (Benítez-Muñoz et al., 2024; Brooks, 2023).

Although a significant main effect of group was observed ($\eta^2 = 0.414$), post hoc analysis did not reveal significant pairwise differences between groups. This discrepancy may reflect relatively small differences between group means despite the overall group effect, suggesting that supplementation did not produce sufficiently distinct metabolic responses to separate the intervention groups statistically. These findings are consistent with previous studies showing that lactate responses are influenced more strongly by exercise intensity and metabolic adaptation than by nutritional interventions. Furthermore, because blood lactate analysis was conducted in a representative subsample, caution is warranted when generalizing these findings to the entire study population.

In addition to metabolic adaptations, repeated resistance exercise exposure also influences perceptual recovery responses, particularly delayed onset muscle soreness (DOMS). Although lactate is not considered a direct cause of DOMS, both variables may reflect broader physiological adaptations induced by repeated resistance training. As presented in Figure 5, DOMS scores progressively decreased throughout the intervention period across all groups. No significant interaction between time and intervention group was observed, with a very small effect size ($\eta^2 = 0.020$), indicating that the overall pattern of DOMS reduction was relatively similar among groups. This finding suggests that improvements in DOMS primarily reflected exercise-induced adaptation rather than differential supplementation effects. Nevertheless, the main effect of time was highly significant with a large effect size ($\eta^2 = 0.624$), indicating substantial reductions in muscle soreness as the intervention progressed.

These findings are physiologically consistent with the repeated bout effect, in which repeated exposure to mechanical stress progressively reduces muscle damage and

inflammatory responses during subsequent exercise sessions (Hody et al., 2019). Experimental studies have suggested that repeated exposure to resistance exercise may attenuate muscle damage and soreness responses during subsequent exercise sessions through multiple physiological adaptations associated with the repeated bout effect (Peake et al., 2017). Previous studies have consistently demonstrated that eccentric exercise initially induces substantial muscle damage and soreness responses, but these responses become attenuated following repeated exposure (Nieman et al., 2020; Peake et al., 2017).

Although the interaction effect was not significant, a significant main effect of group ($\eta^2 = 0.277$) indicated that average DOMS levels differed between intervention groups. In particular, creatine-containing groups (P2 and P3) demonstrated lower DOMS scores during the later stages of adaptation compared with the control group. However, because no time $\times$ group interaction was observed, these differences should be interpreted cautiously as general group tendencies rather than evidence of distinct adaptation trajectories.

DOMS is a multifactorial phenomenon involving structural muscle damage, inflammatory responses, and pain perception mechanisms (Hody et al., 2019; Peake et al., 2017). Consequently, supplementation effects on biochemical recovery markers do not necessarily translate directly into reductions in perceived muscle soreness. This interpretation is consistent with previous findings showing that supplementation may influence muscle damage biomarkers without consistently reducing DOMS severity (Nieman et al., 2020).

Recent evidence also suggests that supplementation effects on DOMS remain inconsistent and are influenced by multiple moderating factors. Meta-analytic findings indicate that amino acid supplementation may reduce DOMS under specific conditions, although the magnitude of effect depends heavily on dosage, duration, exercise characteristics, and participant status (Salem et al., 2024). Similarly, recent studies have reported that supplementation effects on recovery-related outcomes do not consistently translate into clinically meaningful reductions in perceived soreness (Ma et al., 2025; Meng, 2025).

The subject effect on DOMS responses was also relatively large ($\eta^2 = 0.418$), indicating substantial inter-individual variability. This finding is consistent with evidence that DOMS responses vary considerably between individuals even under identical exercise conditions (Cheung et al., 2003). Variability in DOMS perception may reflect differences in physiological and perceptual responses among individuals (Connolly et al., 2003).

Overall, the findings of the present study suggest that physiological adaptations induced by repeated resistance training represented the dominant factor influencing inflammatory, metabolic, and perceptual responses throughout the intervention period. Supplementation with whey protein, HMB, and creatine monohydrate may have provided modest supportive benefits during the later stages of adaptation, particularly in relation to lower lactate accumulation and DOMS scores in creatine-containing groups; however, these observations should be interpreted cautiously given the absence of significant interaction effects.

This study has several strengths, including the use of a randomized trial design with repeated measurements across multiple physiological domains. The simultaneous assessment of inflammatory, metabolic, muscle damage, and perceptual outcomes provided a comprehensive overview of physiological responses to resistance training and supplementation in untrained individuals. Nevertheless, several limitations should be acknowledged. IL-6 interpretation was strongly influenced by pre-analytical limitations, whereas myoglobin analysis was constrained by analytical validity issues related to out-of-range ELISA measurements and suboptimal sampling timing. In addition, the relatively small sample size and substantial inter-individual variability may have reduced statistical sensitivity. External factors, including habitual dietary intake and physical activity outside the

intervention, were also not fully controlled and may have contributed to variability in the observed responses.

Future research should implement stricter pre-analytical and analytical procedures, particularly when assessing low-concentration inflammatory biomarkers and muscle damage markers. This includes the use of appropriate sample dilution procedures and optimized post-exercise blood sampling schedules. Studies involving larger sample sizes, repeated post-exercise sampling intervals, and tighter control of external variables are warranted to more accurately characterize the physiological effects of whey protein, HMB, and creatine monohydrate supplementation during resistance training adaptations.results

CONCLUSION

Repeated resistance training was the primary factor driving physiological adaptations in untrained individuals, particularly in blood lactate concentration and delayed onset muscle soreness (DOMS). Supplementation with whey protein, HMB, and creatine monohydrate did not demonstrate clear modulatory effects on IL-6 and myoglobin responses, although interpretation of these biochemical outcomes should be considered in light of methodological limitations related to biomarker analysis, sampling timing, and the subsample-based assessment of blood lactate. However, creatine-containing groups tended to exhibit smaller increases in lactate and lower DOMS scores during the later stages of adaptation, suggesting a modest supportive role during repeated resistance training. Overall, exercise-induced adaptations appeared to exert greater influence on physiological responses than supplementation alone in untrained individuals.

From a practical perspective, coaches, sports practitioners, and sports nutritionists should prioritize structured and progressive resistance training programs when aiming to improve physiological adaptations in untrained individuals. Whey protein, HMB, and creatine monohydrate supplementation may be considered as complementary strategies to support adaptation during prolonged resistance training, particularly for reducing perceived muscle soreness and attenuating lactate accumulation, although their additional benefits appear modest relative to the adaptations induced by resistance training itself.

ACKNOWLEDGMENT

The authors would like to express their gratitude to all participants for their commitment to completing the eight-week intervention program. Appreciation is also extended to the staff of the Prof. Soegijono Physical Fitness Laboratory, Faculty of Sports Science, Universitas Negeri Semarang, as well as the Biomolecular and Clinical Pathology Laboratory, Universitas Muhammadiyah Semarang, for their technical support in the implementation of the study and sample analysis.

AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

REFERENCES

- ABclonal Technology. (2026). Human Myoglobin (MYO) ELISA Kit (RK01912). <https://abclonalbio.com/elisa-kits/HumanMyoglobinMYOELISAKit/RK01912>
- ACSM. (2021). ACSM's guidelines for exercise testing and prescription (G. Liguori, Y. Feito, C. Fountaine, & B. A. Roy (eds.); Eleventh). Philadelphia : Wolters Kluwer.

- Andreasson, U., Perret-Liaudet, A., Van Waalwijk Van Doorn, L. J., Blennow, K., Chiasserini, D., Engelborghs, S., ... & Teunissen, C. E. (2015). A practical guide to immunoassay method validation. *Frontiers in neurology*, 6, 179.
- Augusto, R. D. S., Dill, A., Souza, E., Nogueira, T. L., Gomes, D. V., Paiva, J., ... & Santos, C. G. (2025). Kinetics of Serum Myoglobin and Creatine Kinase Related to Exercise-Induced Muscle Damage and ACTN3 Polymorphism in Military Paratroopers Under Intense Exercise. *Journal of functional morphology and kinesiology*, 10(4), 381.
- Barnes, M. J. (2023). Nutrition-based strategies to reduce exercise-induced muscle damage and soreness. *Nutrients*, 15(11), 2523.
- Bell, H. L., Jackson, K. G., Crompton, L. A., Givens, D. I., & Lovegrove, J. A. (2025). The impact of whey protein on plasma branched-chain amino acids and glycaemic control in humans. A narrative review. *Nutrition Research Reviews*, 1-13.
- Benítez-Muñoz, J. A., Cupeiro, R., Rubio-Arias, J. Á., Amigo, T., & González-Lamuño, D. (2024). Exercise influence on monocarboxylate transporter 1 (MCT1) and 4 (MCT4) in the skeletal muscle: A systematic review. *Acta Physiologica*, 240(3), e14083.
- Booth, F. W., Roberts, C. K., & Laye, M. J. (2012). Lack of exercise is a major cause of chronic diseases. *Comprehensive physiology*, 2(2), 1143-1211.
- Brancaccio, P., Maffulli, N., & Limongelli, F. M. (2007). Creatine kinase monitoring in sport medicine. *British medical bulletin*, 81(1), 209-230.
- Brooks, G. A. (2023). What the lactate shuttle means for sports nutrition. *Nutrients*, 15(9), 2178.
- Bruno, J., Thapa, R. K., Santos, Â., Montoro-Bombú, R., & Sarmento, H. (2026). Validity and reliability of portable blood lactate analyzers: A systematic review with COSMIN and GRADE assessment. *Sports Medicine-Open*, 12(1), 37.
- Calvo-Rubio, M., Garcia-Dominguez, E., Tamayo-Torres, E., Soto-Rodríguez, S., Olaso-Gonzalez, G., Ferrucci, L., ... & Gómez-Cabrera, M. C. (2024). The repeated bout effect evokes the training-induced skeletal muscle cellular memory. *Free Radical Biology and Medicine*, 225, 247-254.
- Cheung, K., Hume, P. A., & Maxwell, L. (2003). Delayed onset muscle soreness: treatment strategies and performance factors. *Sports medicine*, 33(2), 145-164.
- Cipryan, L. (2017). IL-6, antioxidant capacity and muscle damage markers following high-intensity interval training protocols. *Journal of human kinetics*, 56, 139.
- Connolly, D. A., Sayers, S. E., & Mchugh, M. P. (2003). Treatment and prevention of delayed onset muscle soreness. *Journal of strength and conditioning research*, 17(1), 197-208.
- Cribb, P. J., Williams, A. D., Stathis, C. G., Carey, M. F., & Hayes, A. (2007). Effects of whey isolate, creatine, and resistance training on muscle hypertrophy. *Medicine & Science in Sports & Exercise*, 39(2), 298-307.
- Croft, L., Bartlett, J. D., MacLaren, D. P., Reilly, T., Evans, L., Matthey, D. L., ... & Morton, J. P. (2009). High-intensity interval training attenuates the exercise-induced increase in plasma IL-6 in response to acute exercise. *Applied Physiology, Nutrition, and Metabolism*, 34(6), 1098-1107.
- Docherty, S., Harley, R., McAuley, J. J., Crowe, L. A., Pedret, C., Kirwan, P. D., ... & Millar, N. L. (2022). The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. *BMC Sports Science, Medicine and Rehabilitation*, 14(1), 5.
- Feng, L., Li, B., Yong, S. S., Wu, X., & Tian, Z. (2024). Exercise and nutrition benefit skeletal muscle: From influence factor and intervention strategy to molecular mechanism. *Sports Medicine and Health Science*, 6(4), 302-314.

- Fischer, C. P. (2006). Interleukin-6 in acute exercise and training: what is the biological relevance?. *Exercise immunology review*, 12, 6-33.
- Gong, Y., Liang, S., Zeng, L., Ni, Y., Zhou, S., & Yuan, X. (2019). Effects of blood sample handling procedures on measurable interleukin 6 in plasma and serum. *Journal of clinical laboratory analysis*, 33(7), e22924.
- Hody, S., Croisier, J. L., Bury, T., Rogister, B., & Leprince, P. (2019). Eccentric muscle contractions: risks and benefits. *Frontiers in physiology*, 10, 536.
- Holeček, M. (2017). Beta-hydroxy-beta-methylbutyrate supplementation and skeletal muscle in healthy and muscle-wasting conditions. *Journal of cachexia, sarcopenia and muscle*, 8(4), 529-541.
- Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., ... & Antonio, J. (2017). International society of sports nutrition position stand: protein and exercise. *Journal of the International Society of Sports Nutrition*, 14(1), 20.
- Jówo, E., Ostaszewski, P., Jank, M., Sacharuk, J., Zieniewicz, A., Wilczak, J., & Nissen, S. (2001). Creatine and β -hydroxy- β -methylbutyrate (HMB) additively increase lean body mass and muscle strength during a weight-training program. *Nutrition*, 17(7-8), 558-566.
- Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Mori, Y., & Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise Immunology Review*, 19, 72–85.
- Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Mori, Y., & Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise Immunology Review*, 19, 72–85.
- Kenis, G., Teunissen, C., De Jongh, R., Bosmans, E., Steinbusch, H., & Maes, M. (2002). Stability of interleukin 6, soluble interleukin 6 receptor, interleukin 10 and CC16 in human serum. *Cytokine*, 19(5), 228-235.
- Kim, C. B., Park, J. H., Park, H. S., Kim, H. J., & Park, J. J. (2023). Effects of whey protein supplement on 4-week resistance exercise-induced improvements in muscle mass and isokinetic muscular function under dietary control. *Nutrients*, 15(4), 1003.
- Koo, M., & Yang, S. W. (2025). Visual analogue scale. *Encyclopedia*, 5(4), 190.
- Kreider, R. B., Kalman, D. S., Antonio, J., Ziegenfuss, T. N., Wildman, R., Collins, R., ... & Lopez, H. L. (2017). International Society of Sports Nutrition position stand: safety and efficacy of creatine supplementation in exercise, sport, and medicine. *Journal of the international society of sports nutrition*, 14(1), 18.
- Lyristakis, P. M., Wundersitz, D. W., Zadow, E. K., Mnatzaganian, G., & Gordon, B. A. (2022). The influence of considering individual resistance training variables as a whole on muscle strength: A systematic review and meta-analysis protocol. *Plos one*, 17(1), e0262674.
- Ma, Y., Yan, R., Li, Y., Li, D., Sun, X., Chen, T., & Liu, X. (2025). The impact of nutritional intervention and resistance training on muscle strength and mass in healthy older adults—a comparative analysis. *Frontiers in nutrition*, 12, 1640858.
- Meng, K. (2025). Effect of timing of branched-chain amino acid supplementation on muscle recovery after resistance training in healthy males. *American Journal of Men's Health*, 19(2), 15579883251332731.
- Naderi, A., Rothschild, J. A., Santos, H. O., Hamidvand, A., Koozehchian, M. S., Ghazzagh, A., ... & Podlogar, T. (2025). Nutritional Strategies to Improve Post-exercise Recovery and

- Subsequent Exercise Performance: A Narrative Review: A. Naderi. *Sports Medicine*, 55(7), 1559-1577.
- Nash, D., Hughes, M. G., Butcher, L., Aicheler, R., Smith, P., Cullen, T., & Webb, R. (2023). IL-6 signaling in acute exercise and chronic training: Potential consequences for health and athletic performance. *Scandinavian Journal of Medicine & Science in Sports*, 33(1), 4-19.
- Nieman, D. C., Zwetsloot, K. A., Simonson, A. J., Hoyle, A. T., Wang, X., Nelson, H. K., ... & Guérin-Deremau, L. (2020). Effects of whey and pea protein supplementation on post-eccentric exercise muscle damage: a randomized trial. *Nutrients*, 12(8), 2382.
- NSCA-National Strength & Conditioning Association (Ed.). (2021). *Essentials of strength training and conditioning*. Human kinetics.
- Peake, J. M., Neubauer, O., Della Gatta, P. A., & Nosaka, K. (2017). Muscle damage and inflammation during recovery from exercise. *Journal of applied physiology*, 122(3), 559-570.
- Pedersen, B. K., & Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiological reviews*, 88(4), 1379-1406.
- Ross, J. H., Attwood, E. C., Atkin, G. E., & Villar, R. N. (1983). A study on the effects of severe repetitive exercise on serum myoglobin, creatine kinase, transaminases and lactate dehydrogenase. *QJM: An International Journal of Medicine*, 52(2), 268-279.
- Roxin, L. E., Venge, P., & Friman, G. (1984). Variations in serum myoglobin after a 2-min isokinetic exercise test and the effects of training. *European journal of applied physiology and occupational physiology*, 53(1), 43-47.
- Salem, A., Ben Maaoui, K., Jahrami, H., AlMarzooqi, M. A., Boukhris, O., Messai, B., ... & Chtourou, H. (2024). Attenuating muscle damage biomarkers and muscle soreness after an exercise-induced muscle damage with branched-chain amino acid (BCAA) supplementation: A systematic review and meta-analysis with meta-regression. *Sports medicine-open*, 10(1), 42.
- Schoenfeld, B. (2020). Science and development of muscle hypertrophy. *Human Kinetics*.
- Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W., & Wackerhage, H. (2020). Metabolite concentration changes in humans after a bout of exercise: a systematic review of exercise metabolomics studies. *Sports medicine-open*, 6(1), 11.
- Shirato, M., Tsuchiya, Y., Sato, T., Hamano, S., Gushiken, T., Kimura, N., & Ochi, E. (2016). Effects of combined β -hydroxy- β -methylbutyrate (HMB) and whey protein ingestion on symptoms of eccentric exercise-induced muscle damage. *Journal of the International Society of Sports Nutrition*, 13(1), 7.
- Simpson, S., Kaislasuo, J., Guller, S., & Pal, L. (2020). Thermal stability of cytokines: A review. *Cytokine*, 125, 154829.
- Stahn, A. C., Maggioni, M. A., Gunga, H. C., & Terblanche, E. (2020). Combined protein and calcium β -hydroxy- β -methylbutyrate induced gains in leg fat free mass: a double-blinded, placebo-controlled study. *Journal of the International Society of Sports Nutrition*, 17(1), 16.
- Tidball, J. G. (2017). Regulation of muscle growth and regeneration by the immune system. *Nature Reviews Immunology*, 17(3), 165-178.
- Tvedt, T. H. A., Rye, K. P., Reikvam, H., Brenner, A. K., & Bruserud, Ø. (2015). The importance of sample collection when using single cytokine levels and systemic cytokine profiles as biomarkers—a comparative study of serum versus plasma samples. *Journal of Immunological Methods*, 418, 19-28.

- Walsh, N. P. (2018). Recommendations to maintain immune health in athletes. *European journal of sport science*, 18(6), 820-831.
- Wilson, J. M., Lowery, R. P., Joy, J. M., Walters, J. A., Baier, S. M., Fuller Jr, J. C., ... & Rathmacher, J. (2013). β -Hydroxy- β -methylbutyrate free acid reduces markers of exercise-induced muscle damage and improves recovery in resistance-trained men. *British Journal of Nutrition*, 110(3), 538-544.
- Yang, C., Jiao, Y., Wei, B., Yang, Z., Wu, J. F., Jensen, J., ... & Kuo, C. H. (2018). Aged cells in human skeletal muscle after resistance exercise. *Aging (Albany NY)*, 10(6), 1356.