Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 12 May 2026
Revised 20 June 2026
Accepted 12 July 2026

Natural Resources for Human Health 2026, Vol. 6 (6s): 1300–1321

KEYWORDS:
Running, moderate intensity exercise, inflammatory biomarkers, exercise, intervention.

Training Status Modifies Muscle-Damage and Inflammatory Biomarker Responses During a 6-Week Moderate-Intensity Treadmill-Running Programme in Healthy Young Men: A Non-Randomised Controlled Study.

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ABSTRACT:

Background: A single exercise session leads to increased muscle damage (CK and LDH) and inflammatory biomarkers (IL-6 and CRP), peaking in hours and returning to baseline over a 24-hour period, regular training induces a chronic anti-inflammatory adaptation. The magnitude of this biomarker response to repeated moderate-intensity treadmill exercise according to activity status remains not fully characterized.

Objective: This study was conducted to compare the levels of muscle damage markers; CK and LDH at baseline (t0), 3 weeks (t1) and 6 weeks (t2) following moderate intensity running exercise among healthy young men and to determine the levels of inflammatory biomarkers; IL-6 and CRP at baseline (t0), 3 weeks (t1) and 6 weeks (t2) following moderate intensity running exercise among healthy young men.

Methods: This study was a non-randomized, controlled design with 36 participants divided into four groups based on baseline physical activity levels: active-intervention, active-control, sedentary-intervention and sedentary-control (n = 9 per group). Active status ≥150 min/week of moderate intensity physical activity, and sedentary status < 120 min /week, within each group of participants were matched and assigned to intervention or control. The intervention consisted of a supervised 6-week exercise program (3 sessions

per week) and adherence to the program was monitored through attendance (100%). The blood samples were taken at baseline, week 3, and week 6 between 07:00–09:00 pm, 24 h after the last exercise session to minimize acute post exercise effects. CK (primary score), LDH, CRP and IL-6 levels were measured. Mixed repeated-measures ANOVA (time × group), Greenhouse–Geisser correction (where sphericity was violated), and polynomial trend contrasts were used to analyze the data with a significance at $p < 0.05$.

Results: For all four biomarkers, there was a significant Time main effect (all $p < 0.001$), a significant Time × Group interaction (all $p < 0.001$), and a significant between-subjects Group difference (all $p < 0.001$). These effects were driven by the Sedentary-intervention group showed a marked transient rise at t1 followed by partial normalisation at t2: CK 107.72 ± 22.02 → 242.48 ± 47.84 → 140.89 ± 63.25 U/L; LDH 207.16 ± 38.48 → 345.52 ± 68.19 → 203.28 ± 73.31 U/L; CRP 0.85 ± 0.47 → 3.33 ± 0.65 → 1.23 ± 0.49 mg/L; and IL-6 3.27 ± 0.77 → 8.04 ± 1.15 → 2.49 ± 1.00 pg/mL. The Active-intervention, Active-Control, and Sedentary-Control groups were not significantly different over time. The interactions were carried by highly significant quadratic Time × Group components (CK $F(3,32) = 22.75$, $\eta^2p = 0.68$; LDH $F(3,32) = 87.11$, $\eta^2p = 0.89$; CRP $F(3,32) = 261.81$, $\eta^2p = 0.96$; IL-6 $F(3,32) = 263.70$, $\eta^2p = 0.96$; all $p < 0.001$).

Conclusion: In this small, non-randomized study, the sedentary-intervention group had showed significant change in CK, LDH, CRP and IL-6 levels at t1, followed by attenuation at t2, while The active-intervention, active-Control, and sedentary-control groups were not significantly different over time. These descriptive patterns are similar to the transient muscle-stress and inflammatory response to a novel running stimulus in sedentary individuals, and partial recovery during the programme.

1. INTRODUCTION:

Physical activity is a physiological regulator of the inflammatory response with a physiological relationship between skeletal muscle contraction and beneficial modulation of the inflammatory response on a systemic level (El Assar et al., 2022). Regular moderate-intensity aerobic exercise has a positive impact on immune system function as well as reducing the risk for multiple chronic diseases, such as cardiovascular diseases (CVD) and diabetes (Cabral et al., 2020). The American College of Sports Medicine (ACSM) recommends at least ≥150 minutes of moderate-intensity aerobic exercise, three days per week at 64%-76% HR-max contributes significantly to preventing chronic disease (Arslan & Aydın, 2025; Bushman, 2020). There are two different and sequential biological responses to physical exercise that are sometimes confused. Single bout of exercise produces acute response, which is reflected by a transient response of the organism, while repeated exercise produces distinct chronic responses; in both cases the response may be beneficial, maintaining, or detrimental, depending on the stimuli (Gronwald et al., 2020; Nash et al., 2023; Ringleb et al., 2025). This difference is most pronounced in the case of interleukin-6 (IL-6): While released transiently from contracting muscle, it functions as an anti-inflammatory myokine and drives metabolic adaptation in healthy subjects, in chronic disease it is elevated chronically in the systemic circulation (Nash et al., 2023). Therefore, it is critical to differentiate between acute excursions and chronic adaptations when interpreting biomarker data of any training study (Ringleb et al., 2025).

Physical activity can also be a physiological stressor that can alter tissue homeostasis and induce an inflammatory response in the short and long term (Romagnoli et al., 2016). The induced inflammatory response is one of the key factors that stimulate recovery and physiological adaptation (Podgórski et al., 2021). Exercise-induced muscle damage could be assessed by biochemical and immunological markers reflecting metabolic stress and inflammatory response to exercise (Podgórski et al., 2021). The symptoms include weakness in muscle strength, loss of motion, local swelling, muscle soreness, and elevated blood levels of muscle enzymes like creatine kinase (CK) and lactate dehydrogenase (LDH) (Fatouros & Jamurtas, 2016). In addition, muscle damage triggers an inflammatory reaction, which increases C-reactive protein (CRP) and cytokines such as interleukin-6 (IL-6) (Sciberras et al., 2015). In exercise, IL-6 is believed to act as a hormone secreted by contracting skeletal and has adaptive

metabolic and anti-inflammatory properties, regulating substrate availability and energy metabolism. It stimulates glucose uptake and enhances production of glucose in the liver during exercise (Sabzevari Rad & Panahzadeh, 2024). In response to IL-6, the liver synthesizes CRP, which is an acute-phase protein widely used as a marker of systemic inflammation (Waśkiewicz et al., 2025). In the exercise context, elevated CRP levels are usually seen after high-intensity exercise compared to low- intensity exercise or moderate-intensity exercise (Palacios et al., 2015). Enzymes released from the skeletal muscle into the bloodstream during or after exercise are called muscle damage markers, which are an indirect, non-specific markers of exercise-induced muscle damage. These markers tend to increase during strenuous exercise due to stress and permeability of muscle fibres. Creatine kinase is one of the most commonly used indicators of muscle damage because it is a sensitive marker of increased muscle fibre membrane permeability. Creatine kinase has a greater response than some of the other muscle damage markers, and is relatively simple and inexpensive to measure (Stožer et al., 2020). Furthermore, the peak levels of CK following exercise is not clearly defined and may be between 24 and 72 hours; however, CK is still an important marker of skeletal muscle damage (Yparraguirre Salcedo et al., 2024). Lactate dehydrogenase is another enzyme that is frequently used as a marker of muscle damage, and is involved in the interconversion of pyruvate and lactate through the nicotinamide adenine dinucleotide (NAD^+/NADH) pathway. LDH levels can be elevated as a result of cellular damage, increased membrane permeability, or tissue necrosis (Aranda, 2010; Palacios et al., 2015). These markers are mainly used for assessing muscle damage and physiological stress during exercise, and not to prevent damage (Yparraguirre Salcedo et al., 2024).

Muscle damage triggers an inflammatory response, where inflammatory cells gradually infiltrate the damaged muscle fibres over a period of days to weeks that later trigger the repair mechanism and recovery process after exercise-induced damage, especially following strenuous exercise (Bessa et al., 2016). Therefore, the inflammatory response to muscle damage could initiate repair and adaptation tissue processes, and could be informative as a tool for tracking recovery from exercise (Palacios et al., 2015). Furthermore, while training status can markedly affect physiological response to exercise, there have been few studies that investigated CK, LDH, CRP and IL-6 during repeated bouts of moderate-intensity running, while considering two variances: the effect of the training intervention and the effect of the participants' pre-existing activity status. Most research designs combine them, either by comparing active with sedentary subjects at one-time point, or an intervention with a control group without stratifying on baseline activity. Thus, it is crucial to identify reliable biomarkers to assess training program efficacy and the exercise effects on individual responses (Bischof et al., 2024; Cipryan, 2017; Małkowska et al., 2026; Teixeira et al., 2021). This seems to be one of the first controlled biomarker studies in Palestine, but more than just geographic, this contribution is methodological because the factorial design enables the effect of the intervention and the effect of being active or sedentary to be estimated separately, as well as the interaction between the two factors over time. Thus, this study evaluated the effects of running on a treadmill at moderate intensity on 6-week muscle damage levels and inflammatory biomarkers (CK, LDH, CRP, IL-6) in healthy young men with varying activity levels before the intervention (sedentary vs. active). Furthermore, we hypothesized a time × intervention × activity status interaction: that (1) biomarkers would increase temporarily and then decrease over the course of the programme (time effect); (2) the increase and decrease of these biomarkers would be greater on sedentary participant compared to active participants (activity status: intervention effect); and (3) the time course would be different (activity status: time × intervention interaction).

MATERIAL AND METHODS

2.1 Study design and settings:

This study was a non-randomised controlled, quasi-experimental repeated-measures study. The participants were recruited from February to March of 2026 using posters around campus and an email to the student mailing list of the department. Participants were not randomly assigned to each group (active and sedentary); instead, researchers matched pairs based on baseline creatine kinase concentration. In each matched pair, one individual was assigned to the intervention group and the other to the control group, with similar values at the start of the study for both groups. Laboratory staff involved in the measurements were blinded to the status of the participants. The study was not registered prospectively. Participants then completed a fatigue induction protocol using treadmill-running conducted at Golden Gym, Hebron University, Palestine, where the temperature was maintained between 22-25°C. Participants arrived at the gym 4 hours before their usual bedtime between (7-8 pm). This study was not prospectively registered in a public trials registry. It was a research study that was not required to be registered at the host institution and did not require prospective registration by the approving research ethics committee when the study was carried out.

2.2 Participant selection:

Participants were first classified as active or sedentary on the basis of a questionnaire, and were subsequently allocated to the intervention or control condition. All participants were informed in writing about the study's objectives, methodology, risks, and benefits. They also provided written consent assuring the confidentiality of their information and promising not to release any participant's details. A total of 36 adult male participants aged 19-27 years from the local population were enrolled in this study, divided into two main groups (intervention group = 18) and (control group =18). The intervention group and control group will be subdivided into group A (active n=9) and group B (sedentary n=9) based on specific inclusion and exclusion criteria as shown in Figure 1. An overview of the study design is presented in Figure. 2.

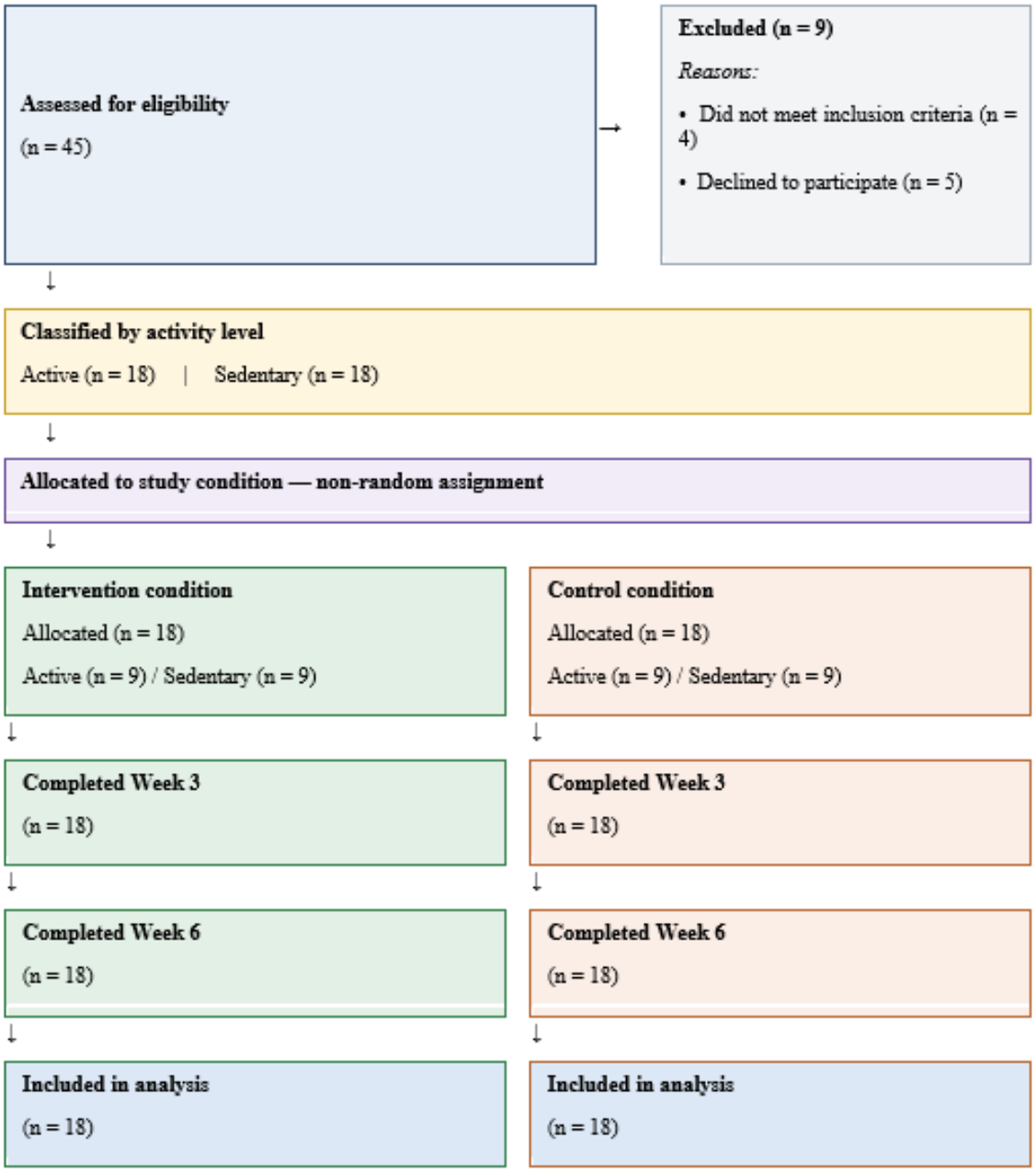
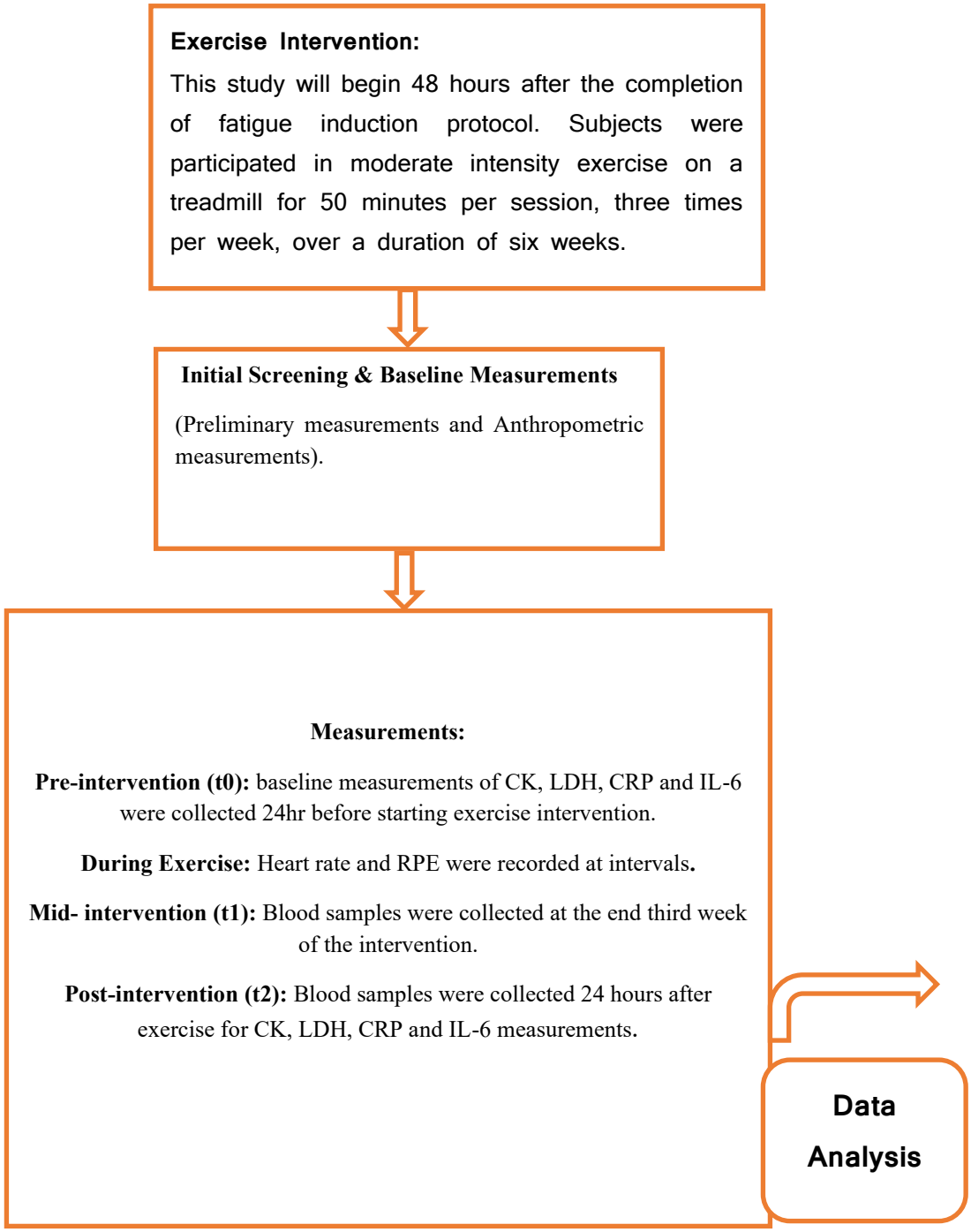


Figure 1: A flowchart for selecting participants and group allocation.

Natural Resources for Human Health
eISSN: 2583-1194



2.3 Criteria for inclusion:

Healthy men aged 19–27 years were eligible.

2.3.1 Criteria for selecting active and sedentary individuals:

Volunteers were stratified using two criteria: physical-activity level and sedentary behaviour. Both were based on answers to the International Physical Activity Questionnaire (IPAQ) adapted for healthy adults. This "adapted" version includes 15 items divided into 5 domains, reflecting the type of exercise (running, cycling, resistance training), intensity (light, moderate, or vigorous), and duration (three days per week) of active participants, as well as active time spent on transport, household activities, recreation and leisure, and sedentary time spent sitting. Sedentary individuals: were individuals who did not participate

in any form of exercise training cut-off point (total exercise time < 120 min /week) (Zhang et al., 2024). Active individuals: were considered to be physically active; they had been engaging in physical activity cut-off point of ≥150 min, three days per week for three months continuously (Kyral et al., 2019). Only subjects with a body mass index (BMI) between 18.5 and 24.9 kg/m² were included in this study(Burman et al., 2022). Participants were categorized by a questionnaire and categorized as sedentary (<120 min /week) or active (>150 min/week or more). Type of exercise (running, cycling, resistance training), intensity (light, moderate, vigorous), and duration (three days per week) of exercise performed for active participants. Each participant was required to consume their final meal at least 120 min prior to commencing exercise, with the last meal scheduled at 5:00 PM (dietary intake was not standardized). Medically qualified personnel were included as co-researchers to provide safety oversight during all study activities. An electrocardiogram (ECG) test was performed as part of the screening process, with interpretation carried out by a medically qualified co-researcher. An Automated External Defibrillator (AED) was available on site throughout the research activity to ensure immediate emergency response capability. Family medical history from each participant was obtained using a structured questionnaire to identify risk factors for sudden cardiac death.

2.3.2 Control group: Participants usual activity was monitored throughout the study using questionnaire at all three time points (baseline, week 3, and week 6) using IPAQ and active controls were asked to continue with their regular structured exercise during the intervention period and no significant changes in the reported activity was found within or between groups over time. There was no cross-contamination of conditions were observed (Kim et al., 2015).

2.4 Criteria for exclusion:

The exclusion criteria were as follows:

A current injury or history of lower limb injury in the past 2 years, including anterior cruciate ligament tear, muscle strain, or meniscus tear. Back muscle dysfunctions, such as myofascial pain syndrome and back muscle strains. Participants were asked at each blood collection point (baseline, week 3, week 6) to fill in a short health status checklist before the blood collection to ensure that exclusion criteria were not present in the stated time frame: acute infection/fever (2 weeks before), immunization (4 weeks prior), strenuous or unaccustomed exercise (24 h to 72 h before), NSAID or anti-inflammatory medication (72 h prior), antioxidant or dietary supplements (2 weeks prior), caffeine (morning of collection), alcohol (24 h to 72 h prior), and inadequate sleep (last night), these factors may materially alter CK, LDH, CRP or IL-6 were also excluded (Xu et al., 2018).

2.5 Sample size:

A sensitivity analysis was performed to assess the minimum observable effect size (MES) for the main outcome. For the time × group interaction, with a fixed sample size of 36 participants (36 = four groups × nine participants per group) using G*Power 3.1.9.7 software, the calculation result indicated the need for 28 participants to confirm the consistency and validity of the research findings. To avoid potential dropout and ensure the robustness of the study results, the sample size was increased by 30% and adjusted to 36 participants, yielding 36.4. The number of individuals per group was set at 36 (nine in each group) to ensure four equal-sized groups, even though the factorial design required 37 participants. The number of individuals per group was set at 36 (nine per group) to ensure that each group was of equal size. Three repeated measurements (baseline, week 3, week 6), a significance level of $\alpha = 0.05$, a power of $(1 - \beta) = 0.80$, an assumed correlation coefficient between repeated measurements of 0.5, and a and a nonsphericity correction of $\varepsilon = 0.64$ (calculated Greenhouse-Geisser value for CK) (Kyral et al., 2019). The MES was determined to be a moderate to large effect (Cohen's f-value ≈ 0.31, partial $\eta^2 \approx 0.09$). The effect size was not assumed from external data; rather, the MES was calculated using a fixed and realistic sample size, a conventional significance level, and a power of η^2. The observed effect was obtained from the study's specific CK interaction. This lower limit was significantly exceeded due to the pronounced interaction between time and group (partial $\eta^2 = 0.55$; $f \approx 1.10$), providing good statistical power for the underlying analysis. Plasma creatine kinase was the pre-specified primary outcome and the effect of interest was the time × intervention × activity-status interaction.

2.6 Sample collection and processing:

Three blood samples were taken from the vein at baseline 24 h after (fatigue induction protocol on a separate day) 24 h after week 3, and 24 h after week 6 between 07:00–09:00 pm by a registered nurse, and participants had refrained from alcohol, caffeine and NSAIDs 24h before blood sampling. Control samples were taken at the same time of day and under similar conditions. Heparinized plasma was used for the measurement of CK/LDH and serum was used for the measurement of

CRP/IL-6. Samples were centrifuged at 1,000×g for 10 min. After centrifugation, approximately 1.25 ml of serum and 1.25 of plasma were obtained from each participant, aliquoted and stored at −80 °C for 2 weeks before processing, after a single freeze–thaw cycle prior to analysis. Visible haemolysed samples were excluded and re-collected. Calibrators provided by the manufacturer were used to calibrate assays; internal quality controls were added to each assay run, with intra and inter-assay coefficients of variance were ≤ 15%.

2.7 Experimental protocol:

The selected participants underwent an initial assessment that included preliminary measurements consisting of the following:

2.7.1 Anthropometric data measurements were collected for all participants before the fatigue induction including: height, weight, body mass index, body fat percentage, blood pressure, heart rate and Electrocardiogram.

2.7.2 Maximal oxygen consumption test: VO_2 max was estimated through the use of the Harvard Step Test in a separate test after anthropometric measurements 48 hr prior to fatigue induction protocol. Each subjects were required to step on a 45 cm platform at 30 steps/min to a maximum of 5 minutes or until they voluntary exhaustion. Fatigue was stipulated as failure to sustain the rate of stepping during 15 seconds. Resting heart rate was measured before the test during a brief warm-up and cool-down exercise. Immediate post-exercise heart rate was measured and VO_2 max predicted by a nomogram which depended on both the heart rate and body weight by measured the middle scale by looking at the intersection with the drawn line. The results were then adjusted through age-based correction factor (17 -35 years, correction factor =1) (Khurde et al., 2021; Pescatello, 2014). Estimated VO_2 max was used as a baseline descriptive variable to describe cardiorespiratory fitness and support interpretation of training status differences between groups.

2.7.3 Moderate-intensity running exercise protocol:

Participants were instructed to avoid exercise and to refrain from taking any anti-inflammatory medications for 48 hours before the running exercise protocol. Participants completed a moderate-intensity treadmill running program of 44-48 minutes each session depends on participants, three sessions each week for 6 weeks, supervised by the researcher (Singh et al., 2022). The exercise protocol was performed by each participant separately but the sessions were arranged at the same time. The intervention was not presented as a group class, but each participant completed the protocol individually to ensure consistency in data collection and exertion levels for each individual (Shore et al., 2021). Each session started with a 5 minute warm up at 2-4 miles per hour (mph) or around 50-60% of the age-predicted maximum heart rate. This period was used to warm up the participants and build up their heart rate gradually towards the main training session. By the end of the warm-up, participants should be slightly winded but still able to talk. At this stage, the RPE was kept between 9-11 on the Borg 6-20 RPE scale (Kim et al., 2015; Yoder et al., 2025). After a warm-up, the speed on the treadmill was increased by 2 minutes (2 km\h) until participants reached a heart rate between 65% and 75% of their age-predicted maximum. The heart rate was recorded throughout the trial with a pulse rate meter and was used to record the cardiovascular response during the incremental phase (Faricier et al., 2024). This phase typically required two to four stages, equivalent to 4–8 minutes, depending on the participant, before proceeding to 30 minutes of moderate-intensity treadmill running. A treadmill incline was kept at 0% during the intervention to ensure exercise protocol standardization. The use of a flat surface minimized exercise intensity variations and made physiological stress in the participants equal (Green et al., 2002). At the end of each session, a 5-minute cool-down was conducted at 50-60% of the participants' maximum heart rate using light walking or slow running. This stage was designed to aid in recovery, relaxation and to minimize muscle discomfort after exercise. Participants continued to run at an RPE of 9-10 on the Borg 6-20 RPE scale during the cool down, which helped them return to resting physiological levels (Yoder et al., 2025). During this stage, participants were also asked to concentrate on deep breathing and relaxation (Yoder et al., 2025).

During the exercise sessions, participants' heart rates were maintained within the target range of 65–75% of maximum heart rate and were continuously monitored using a heart rate monitor. Should a participant's heart rate fall outside of the target zone, the researcher allowed the treadmill speed to be increased incrementally until the HR was brought back into the target zone (Wang & Hunt, 2023). To monitor the level of exertion and to keep the participants safe, rating of perceived exertion was assessed during each exercise session using the Borg 6-20 RPE scale. RPE was measured prior to exercise, after every 5 minutes during exercise, before the end of the exercise, and after the exercise. The regular testing could be used to adjust the intensity of the exercises and that fatigue monitoring was more reliable during the six-week intervention. Attendance was monitored and documented, and where possible, on a structured attendance sheet made by the researcher, otherwise on a digital log sheet.

This "double tracking" approach enabled effective monitoring of session attendance and completeness of data (Kilpatrick et al., 2009). All exercise sessions occurred during the time period between 7:00 and 9:00 PM to reduce physiological response changes that occur across different time periods of the day, such as heart rate, core temperature, and perceived exertion. The evening schedule also ensured a consistency of participants' daily routines and minimized pre-exercise nutritional status variability. Participants were asked to eat a standardized meal 2-3 hours prior to each session, as this was done to control for the energy available to the athletes before each session and to minimize the effect of diet on exercise performance (Kim et al., 2015). As shown in figure 3.

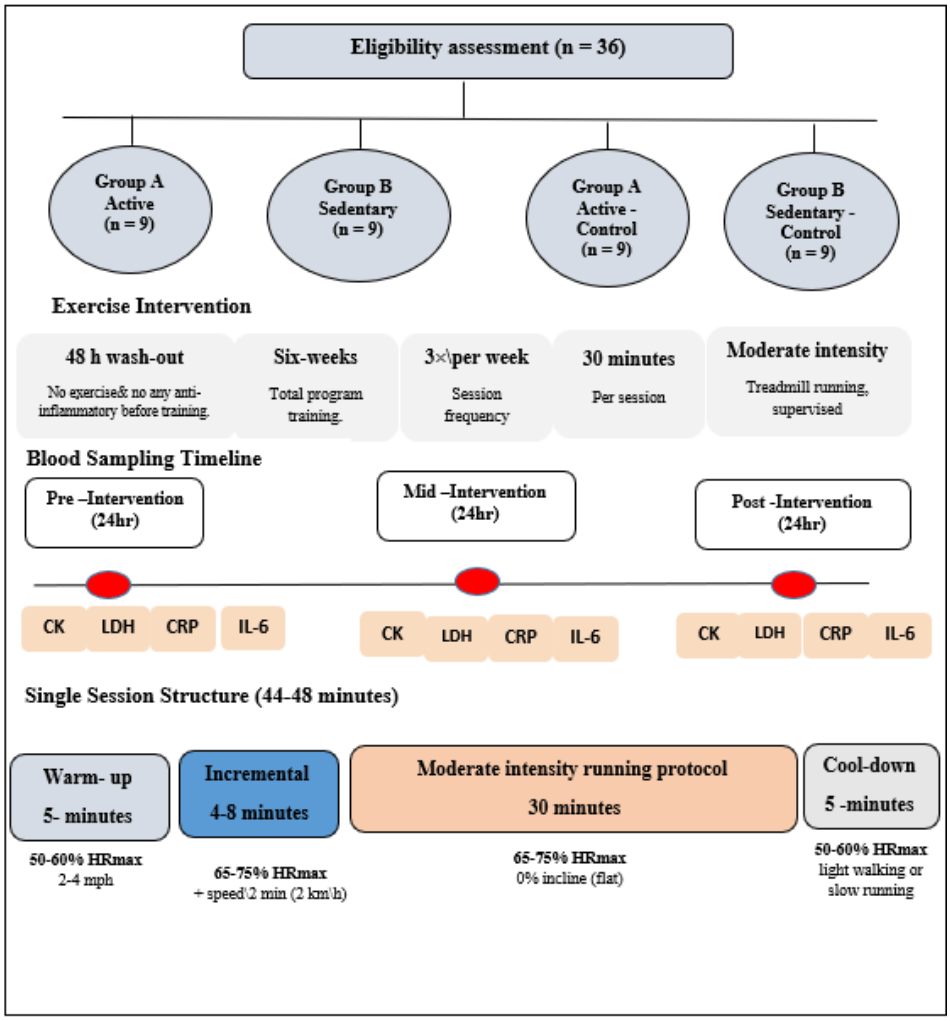


Figure 3: flowchart of exercise-intervention protocol.

2.8 Biomarkers selection:

The following biomarkers (CK, LDH, CRP and IL-6) were selected based on previous studies that have been established them as the most widely used non-specific, indirect biomarkers used for assigning exercise response. CK and LDH, which are indirect non-specific markers related to leakage of intramuscular enzymes into the blood. IL-6 and CRP were used as inflammatory markers (Yparraguirre Salcedo et al., 2024). These markers are indicative for muscle stress and inflammation. Creatine kinase was chosen as the primary outcome variable because it is the most indirect sensitive exercise-induced muscle stress, while LDH, IL-6 and CRP were used as secondary outcome variables.

2.9 Biomarkers Measurements:

2.9.1 CK: the CK level was analyzed and measured using UV- spectrophotometer (RT-9700, Rayto Life and Analytical Sciences, Shenzhen, china) according to manufacturer instruction. The CK was measured using CK-NAC liquid kit (ref. 990524), which

contains a substrate in solution form and buffer. The test was performed according to kit procedure: 1000 µl of the reagent solution consist of (800 µl of working reagent + 200µl of substrate) was incubated at 37°C for 5 min then 40 µl of plasma sample were added to working reagent. The assay was performed using the kit for the determination of CK CK-NAC Liquid (IFCC method; catalogue no. 99 05 24, Química Clínica Aplicada S.A. [QCA] Amposta, Spain), which was read at 340 nm and 37 °C; the detection limit of the assay was 2 U/L, and samples greater than 1500 U/L were diluted 1:10 in 0.9% saline and then reassayed and the results multiplied by 10. The coefficient of variation of the within-run variation and between-run variation were 1.8% and 3.5%, respectively. Calibration of the assay was performed using the manufacturer's Calibrator for Autoanalysers (catalogue no. 99 62 80) when the reagent lot had changed or when results of the control sera were outside the specified range, and Seriscann normal and Seriscann abnormal control sera were used in every analytical run. Enzyme activity was quantified based on the change in absorbance, with results reported in U/L.

2.9.2 LDH: lactate dehydrogenase was analyzed and measured using UV- spectrophotometer (RT-9700, Rayto life and analytical sciences, Shenzhen, china) according to manufacturer instruction. The LDH was measured using LDH SCE mode. liquiUV kit (ref.12214), which contain a substrate in solution form and buffer. The test was performed according to kit procedure: 1000 µl of the reagent solution consist of (800 µl of working reagent + 200µl of substrate) was incubated at 37°C for 5 min then 20 µl of plasma sample were added to working reagent. Assay reading was done at 340 nm at 37 °C and required about 3 min 30 s for reaction. The manufacturer's data indicated that the range of the assay was 24 U/L to 1000 U/L, and that samples above this range should be diluted 1/5–1/10 in saline, then re-assayed and the result multiplied by the dilution factor. Intra-run coefficients of variation were 0.8% and 1.2% at the concentrations of 439 U/L and 919 U/L, respectively. The assay was calibrated according to the manufacturer's guidelines and a two-level internal quality control material (Standatrol S-E) was analysed in each test run. Enzyme activity was quantified based on the change in absorbance, with results reported in U/L.

2.9.3 CRP: C-reactive protein was analyzed and measured using the FinecareTM CRP quantitative test (Model no: FS-11, Wondfo, Guangzhou Wondfo Biotech, Guangzhou, China), which is a fluorescence immunoassay, sandwich immunodetection kit, which containing buffer and test cartridge (the florescence-labeled detector antibody on the conjugate pad). 5µl of serum sample was mixed with the buffer and then 75µl was taken for the mixture (sample + buffer) into test cartridge, the immune complex between CRP and antibody reflect the amount of antigen and FinecareTM FIA meters showed CRP concentration in serum sample displayed as mg/L. Measurement range of 0.5 - 200 mg/L and limit of detection of 0.5 mg/L, and the correlation coefficient (r = 0.987), within- and between-lot coefficients of variation were ≤ 15%. Each test cartridge lot was automatically calibrated with the lot-specific ID chip supplied by the manufacturer and the Finecare CRP control was analysed as an internal quality control.

2.9.4 IL-6: interleukin-6 was analyzed and measured using the FinecareTM IL-6 quantitative test (Model no: FS-113, Wondfo, Guangzhou Wondfo Biotech, Guangzhou, China), a fluorescence immunoassay, sandwich immunodetection kit, which contains buffer and test cartridge (the fluorescence-labeled detector antibody on the conjugate pad). 75µl of serum sample was mixed with the buffer and then 75µl was taken for the mixture (sample + buffer) into test cartridge, the immune complex between IL-6 and the antibody reflect the amount of antigen and FinecareTM FIA meters showed IL-6 concentration in the serum sample displayed as pg\ml. Measurement range of 0.3–4000 pg/mL and a limit of detection of 0.3 pg/mL, and the correlation coefficient (r = 0.980), within- and between-lot coefficients of variation were ≤ 15%. Each test cartridge lot was automatically calibrated with the lot-specific ID chip supplied by the manufacturer and the Finecare IL-6 control was analysed as an internal quality control.

2.10 Statistical analysis:

All the data were tabulated in Microsoft Excel 2016, both clinical findings and laboratory results of the participants. All individuals were informed about the collection of data and their informed consent was given before the data were collected. The data were initially organized in excel to tabulate data for participants who met the inclusion criteria. The data were then exported to the IBM SPSS Statistics version 25 to perform statistical analysis. Descriptive data are presented as mean ± SD (standard deviation). The Shapiro–Wilk test was used to assess normality, and the level of statistical significance was defined as $p < 0.05$. The Shapiro–Wilk W-statistics and p-values are reported in Supplementary Table S1. A mixed repeated-measures ANOVA was used to examine the effects of time, participant group, and the time × group interaction on CK, LDH, CRP, and IL-6 levels. Time was used as a within-subjects factor and measured at baseline (t0), mid-the intervention (t1) and post-intervention (t2). The between-subjects factor was participant group consisting of the active group, active-control group,

sedentary group, and sedentary-control group. Mauchly's test was used to assess the assumption of sphericity. Sphericity was violated and Greenhouse–Geisser corrected values were reported. Polynomial contrast analysis was also conducted to determine the trend for biomarker changes over time, such as linear and quadratic trends. The presence of significant time × group interaction was considered as evidence of differences in biomarker response among participant groups throughout the exercise period. All statistical tests were performed at a p-value of $p < 0.05$, with the results being analysed in the context of both statistical significance and the magnitude of effects to give a complete representation of physiological and inflammatory responses to exercise.

2.10 Ethical approval: This study was submitted to the Research Ethical Committee of the Universiti Teknologi MARA and approved under no. REC/01/2026 (PG/FB/67).

3. Results:

3.1 Participants characteristics:

A total of 36 participants were enrolled for this study and successfully completed the six-week treadmill-running programme and all scheduled assessments. The results are presented as Mean ± SD for age, height, weight, BMI, blood pressure systolic and diastolic, heart rate, body fat and VO_2 max, as shown in Table 1. All the participants were within the target age range (19-27 years) and their BMI values were within the normal BMI range (18.5 to 24.9 kg/m²). Additionally, blood pressure, resting heart rate and ECG findings were within normal limits for all participants to ensure that no any participant had chronic or cardiovascular disease. It was observed that both active and active control participants achieved better results in body fat percentage and VO_2 max (55.7 ± 2.2 $mL \cdot kg^{-1} \cdot min^{-1}$) compared to sedentary participants and sedentary control participants.

Table1: Baseline characteristics by activity status and intervention condition.

Variable	Sedentary-intervention (n=9) Mean ± SD	Sedentary-control (n=9) Mean ± SD	Active-intervention (n=9) Mean ± SD	Active-control (n=9) Mean ± SD
Age of participant (years)	20.00 ± 0.87	20.67 ± 1.41	20.56 ± 1.13	20.89 ± 1.05
Height (cm)	170.22 ± 5.45	172.67 ± 4.15	175.00 ± 4.58	175.00 ± 4.58
Weight (kg)	61.44 ± 4.30	62.89 ± 3.26	69.33 ± 5.12	71.00 ± 5.24
BMI (kg/m²)	21.19 ± 0.62	21.10 ± 1.13	22.58 ± 0.48	23.16 ± 0.55
Blood pressure systolic (mmHg)	118.00 ± 4.24	118.67 ± 2.06	117.22 ± 2.86	117.89 ± 3.06
Blood pressure diastolic (mmHg)	70.44 ± 2.40	69.11 ± 2.52	69.67 ± 2.18	71.44 ± 2.74
Heart rate (bpm)	86.67 ± 4.03	67.00 ± 7.45	66.44 ± 2.30	70.33 ± 2.65
Body Fat (%)	13.82 ± 0.70	13.86 ± 1.51	11.93 ± 0.71	13.58 ± 0.87
VO_2 max (ml/kg/min)	45.56 ± 1.98	49.46 ± 3.53	55.67 ± 2.18	52.33 ± 1.64

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Table 2. Descriptive Statistics of Muscle Damage and Inflammatory Biomarkers Across Study Groups at Different Exercise Time Points.

Time Point	Participant Group	CK (U/L) Mean ± SD	LDH (U/L) Mean ± SD	CRP (mg/L) Mean ± SD	IL-6 (pg/ml) Mean ± SD
Baseline (Pre - intervention, 24 h)	Active-intervention participants	105.89 ± 8.18	315.00 ± 9.47	1.03 ± 0.16	1.43 ± 0.23
	Active-Control participants	105.67 ± 7.76	314.78 ± 9.09	1.02 ± 0.14	1.40 ± 0.19
	Sedentary-intervention participants	107.72 ± 22.02	207.16 ± 38.48	0.85 ± 0.47	3.27 ± 0.77
	Sedentary Control participants	120.02 ± 30.32	215.33 ± 24.91	0.62 ± 0.34	3.14 ± 0.80
Mid-intervention (3 Weeks, 24 h)	Active-intervention participants	107.22 ± 10.01	317.00 ± 11.93	1.11 ± 0.19	1.52 ± 0.26
	Active Control participants	106.44 ± 8.31	316.00 ± 9.57	1.03 ± 0.16	1.44 ± 0.22
	Sedentary-intervention participants	242.48 ± 47.84	345.52 ± 68.19	3.33 ± 0.65	8.04 ± 1.15
	Sedentary Control participants	124.44 ± 42.66	198.81 ± 20.91	0.44 ± 0.22	3.51 ± 0.67
Post- intervention (6 Weeks, 24 h)	Active -intervention participants	107.00 ± 7.63	317.00 ± 9.45	1.23 ± 0.49	1.03 ± 0.16
	Active- Control participants	106.00 ± 7.84	315.56 ± 9.13	1.02 ± 0.14	1.40 ± 0.19
	Sedentary -intervention participants	140.89 ± 63.25	203.28 ± 73.31	1.23 ± 0.49	2.49 ± 1.00
	Sedentary Control participants	122.84 ± 32.27	215.31 ± 15.81	0.58 ± 0.32	3.38 ± 0.72

Table 2 displays descriptive values of the biomarkers at each time point. The active-intervention and active-control groups had generally similar mean values for the levels of CK and LDH in the blood at baseline: 105.89 ± 8.18 U/L and 105.67 ± 7.76 U/L, respectively, for CK; and 315.00 ± 9.47 U/L and 314.78 ± 9.09 U/L, respectively, for LDH. The baseline LDH was descriptively lower in both sedentary groups than in the active groups, while the mean IL-6 was higher in the sedentary groups than the active groups. CK, LDH, CRP and IL-6 levels were significantly elevated at t1 in the sedentary-intervention group as compared to baseline (CK 242.48 ± 47.84 U/L; LDH 345.52 ± 68.19 U/L; CRP 3.33 ± 0.65 mg/L; IL-6 8.04 ± 1.15 pg/mL) and were relatively modest in the active-intervention and active-control groups between time points. At the post-intervention (t2), values in the sedentary-intervention group were lower than at t1 (CK 140.89 ± 63.25 U/L; LDH 203.28 ± 73.31 U/L; CRP 1.23 ± 0.49 mg/L; IL-6 2.49 ± 1.00 pg/mL), while the sedentary-control group showed only minor fluctuations. Descriptively, the sedentary-intervention group showed the greatest apparent increase at t1, followed by a decrease at t2, while the active groups showed little change.

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Table 3. Mixed Repeated-Measures ANOVA for CK, LDH, CRP and IL-6 Levels Across Time and Participant Groups and Polynomial contrast: Quadratic Trend Significant.

Marker	Effect / contrast	Test used	F (df1, df2)	P- value	η^2p
CK *(U/L)*	Time	Greenhouse–Geisser	14.53 (1.27, 40.61)	<0.001	0.31
	Time × Group	Greenhouse–Geisser	13.00 (3.81, 40.61)	<0.001	0.55
	Group (between-subjects)	Between-subjects	18.27 (3, 32)	<0.001	0.63
	Time – quadratic	Polynomial contrast	25.08 (1, 32)	<0.001	0.44
	Time × Group – quadratic	Polynomial contrast	22.75 (3, 32)	<0.001	0.68
LDH *(U/L)*	Time	Greenhouse–Geisser	26.89 (1.70, 54.38)	<0.001	0.46
	Time × Group	Greenhouse–Geisser	36.41 (5.10, 54.38)	<0.001	0.77
	Group (between-subjects)	Between-subjects	29.59 (3, 32)	<0.001	0.74
	Time – linear	Polynomial contrast	0.003 (1, 32)	0.958	0.00
	Time – quadratic	Polynomial contrast	64.39 (1, 32)	<0.001	0.67
	Time × Group – linear	Polynomial contrast	0.057 (3, 32)	0.982	0.01
	Time × Group – quadratic	Polynomial contrast	87.11 (3, 32)	<0.001	0.89
CRP *(mg/L)*	Time	Greenhouse–Geisser	73.43 (1.54, 49.26)	<0.001	0.70
	Time × Group	Greenhouse–Geisser	87.65 (4.62, 49.26)	<0.001	0.89
	Group (between-subjects)	Between-subjects	25.73 (3, 32)	<0.001	0.71
	Time – linear	Polynomial contrast	5.25 (1, 32)	0.029	0.14
	Time – quadratic	Polynomial contrast	212.97 (1, 32)	<0.001	0.87
	Time × Group – linear	Polynomial contrast	2.55 (3, 32)	0.073	0.19
	Time × Group – quadratic	Polynomial contrast	261.81 (3, 32)	<0.001	0.96
IL-6 *(pg/mL)*	Time	Sphericity assumed	163.53 (2, 64)	<0.001	0.84
	Time × Group	Sphericity assumed	123.04 (6, 64)	<0.001	0.92
	Group (between-subjects)	Between-subjects	76.49 (3, 32)	<0.001	0.88
	Time – linear	Polynomial contrast	5.98 (1, 32)	0.020	0.16
	Time – quadratic	Polynomial contrast	352.11 (1, 32)	<0.001	0.92
	Time × Group – linear	Polynomial contrast	5.51 (3, 32)	0.004	0.34
	Time × Group – quadratic	Polynomial contrast	263.70 (3, 32)	<0.001	0.96

Note that η^2p (partial eta-squared) was calculated using the reported F and design degrees of freedom [$\eta 2\ p = (F \times df1) / (F \times df1 + df2)$], and that within-subject contrast error df = 32.

Creatine Kinase:

As the result showed in Table 3, the significant interactions indicate that the pattern of change in CK over time was significantly different between groups. The exercise intervention had a different effect on active versus sedentary subjects. CK increased significantly in sedentary individuals at (t1). Over the time the active groups had relatively comparable CK levels. The changes of CK were not linear. But CK increased during (t1) and then decreased post-intervention (t2), as shown in figure 4. This results in a quadratic (curved) response curve, consistent with exercise-induced muscle damage and recovery. Therefore, the differences in the CK levels in the different groups of participants over time were analyzed using a mixed repeated measures ANOVA. Mauchly's test indicated that the assumption of sphericity was violated ($W = 0.424$, $\chi^2 = 26.59$, $p < .001$) and so Greenhouse–Geisser corrected data were utilized. The main effect of time on the CK level was significant ($F (1.27, 40.61) = 14.53$, $p < .001$), and the differences in the concentration of the CK level between the 3 exercise time points were significant. An overall difference in the level of CK in the various groups of participants was also detected, $F (3, 32) = 18.27$, $p < .001$. Importantly, a significant time × group interaction effect was observed, $F (3.81, 40.61) = 13.00$, $p < .001$, indicating that the alterations to CK during time differed significantly among the groups. There was a significant quadratic trend over time and time × group interaction ($p < .001$) as revealed by polynomial contrast analysis. This supports the notion of a temporary elevation in CK levels during (t1) and subsequent partial recovery at (t2) measurement, particularly in sedentary participants.

Lactate dehydrogenase:

The change in LDH levels over time in the four groups were investigated with a mixed repeated measures ANOVA (Table 3). Mauchly's test ($W = 0.823$, $\chi^2 = 6.03$, $p = .049$) indicated that the sphericity assumption was violated. Therefore, Greenhouse–Geisser corrected values were used in the interpretation. The analysis revealed a significant main effect of time for LDH ($F1.70, 54.38 = 26.89$, $p < .001$), indicating that there were significant differences in LDH levels between the three exercise time points, as shown in figure 5. The main effect of participant group $F (3, 32) = 29.59$, $p < .001$, was also significant, showing general differences in LDH level between active and sedentary participants. Additionally, a significant time × group interaction was observed, $F (5.10, 54.38) = 36.41$, $p < .001$, suggesting that the dynamics of LDH levels over time were relatively different among groups. Polynomial contrast analysis revealed a significant quadratic trend for both time and the time × group interaction ($p < .001$), with a pronounced increase in LDH levels during the (t1), and a partial recovery at the (t2) assessment. This type of reaction was most often seen in sedentary participants with the highest LDH levels at mid-Intervention. By contrast, the control group had the most stable LDH profile at each measurement time point.

C- Reactive Protein:

A mixed repeated-measures ANOVA was performed to explore the difference in the CRP level across three time points (t0), (t1) and (t2). Mauchly's test suggested a violation of the sphericity assumption ($W = 0.701$, $\chi^2 = 11.03$, $p = .004$), so Greenhouse–Geisser adjusted data were utilized for interpretation. We found a significant main effect of time on CRP levels, $F (1.54, 49.26) = 73.43$, $p < .001$. This suggests that there were substantial fluctuations in CRP levels throughout the study period. There was also a significant main effect of participant group, $F (3, 32) = 25.73$, $p < .001$, showing differences in CRP levels among active and sedentary participants. Also a significant time × group interaction effect was identified, $F (4.62, 49.26) = 87.65$, $p < .001$, showing that the variations in CRP over time differed significantly across groups, as shown in figure 6. The polynomial contrast analysis revealed linear ($p= 0.029$) and quadratic ($p < .001$) time effects, with a significant increase in CRP levels at (t1) and a partial recovery at (t2). This non-linear response pattern was most evident in the sedentary participants with the highest concentration of CRP at (t1), and the highest inflammatory response in this group during (t1). In contrast, the active group had the most consistent CRP profile at all assessment time points with minimal changes following exercise at three time points; (t0), (t1) and (t2.

Interleukin-6

A mixed repeated measures ANOVA was conducted to determine IL-6 levels changed over time. Mauchly's test indicated that the assumption of sphericity was not violated ($W = 0.930$, $\chi^2 = 2.25$, $p = .325$), consequently, sphericity-assumed data were used for interpretation. There was a significant main effect of time on IL-6 $F (2, 64) = 163.53$, $p < .001$, which indicated a considerable change in IL-6 levels throughout the course of the study. The main effect of participant group, $F (3, 32) = 76.49$, $p < .001$, showed general differences in IL-6 levels between the active and sedentary groups. There was also a significant time x group interaction effect for IL-6, $F (6, 64) = 123.04$, $p < .001$, whereby the change in IL-6 was different across time in the groups, as shown in figure 7. The time effects were significant and included a linear time effect as well as a significant quadratic time effect, with the most significant effect being the quadratic time effect, which indicates a sharp increase in IL-6 concentrations during (t1) and a partial decrease following exercise at (t2) ($p < .001$). This nonlinear response was particularly apparent in sedentary participants, who had the highest IL-6 levels during (t1), and therefore higher exercise-induced inflammatory and immune activation. The profile of IL-6 in the active group was more stable across all assessment time points; (t0), (t1) and (t2).

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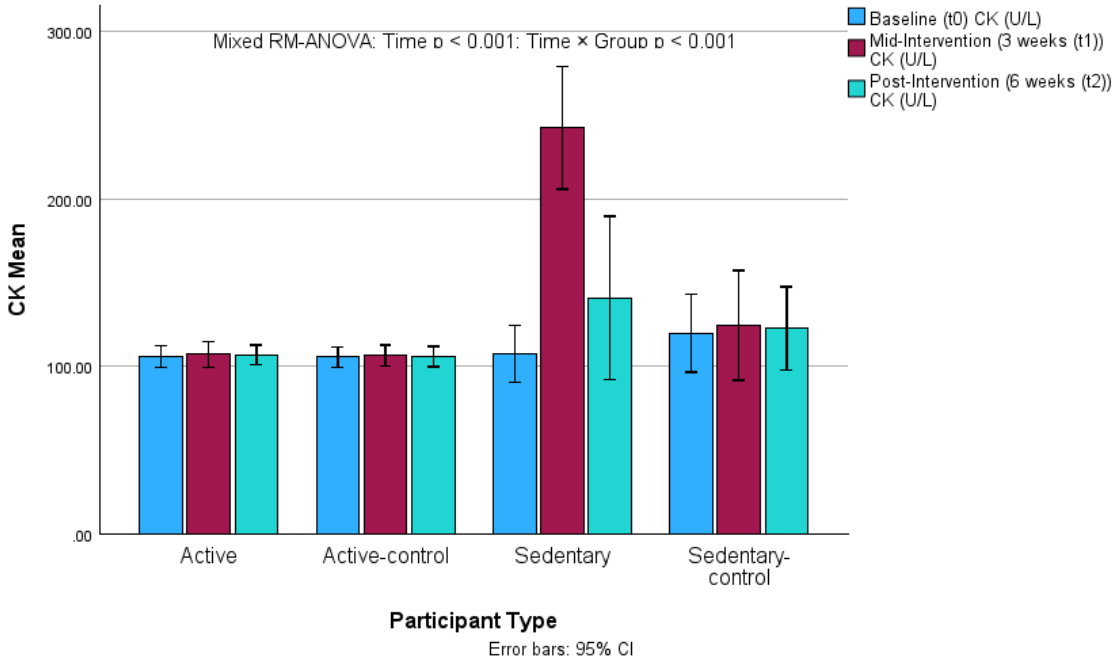

Figure 4: The levels of CK were significantly different at various time points and between different participant groups.

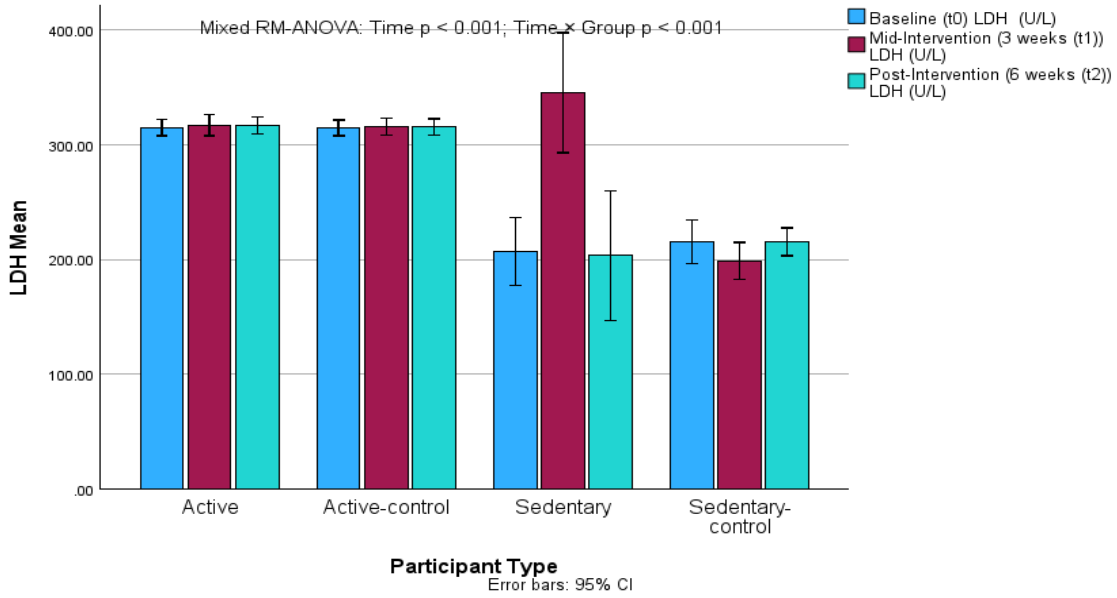

Figure 5: Mean values of serum lactate dehydrogenase (LDH, U/L) in the four groups of participants at (t0), (t1) and (t2).

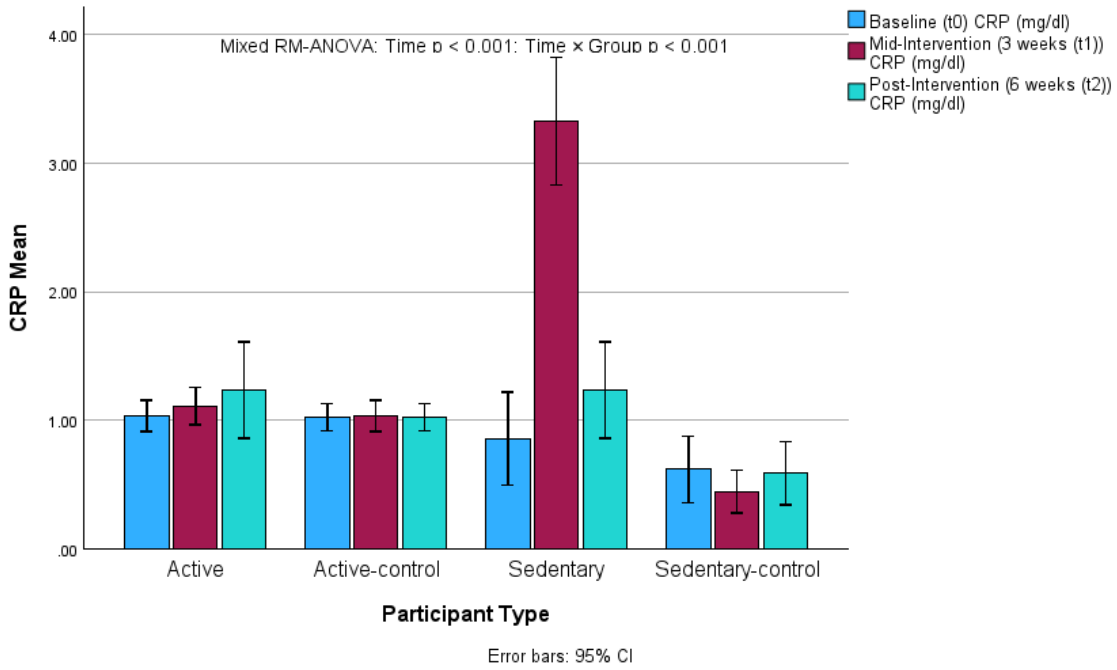

Figure 6: Mean values of serum C-reactive Protein (mg/dl) in the four groups of participants at (t0), (t1) and (t2).

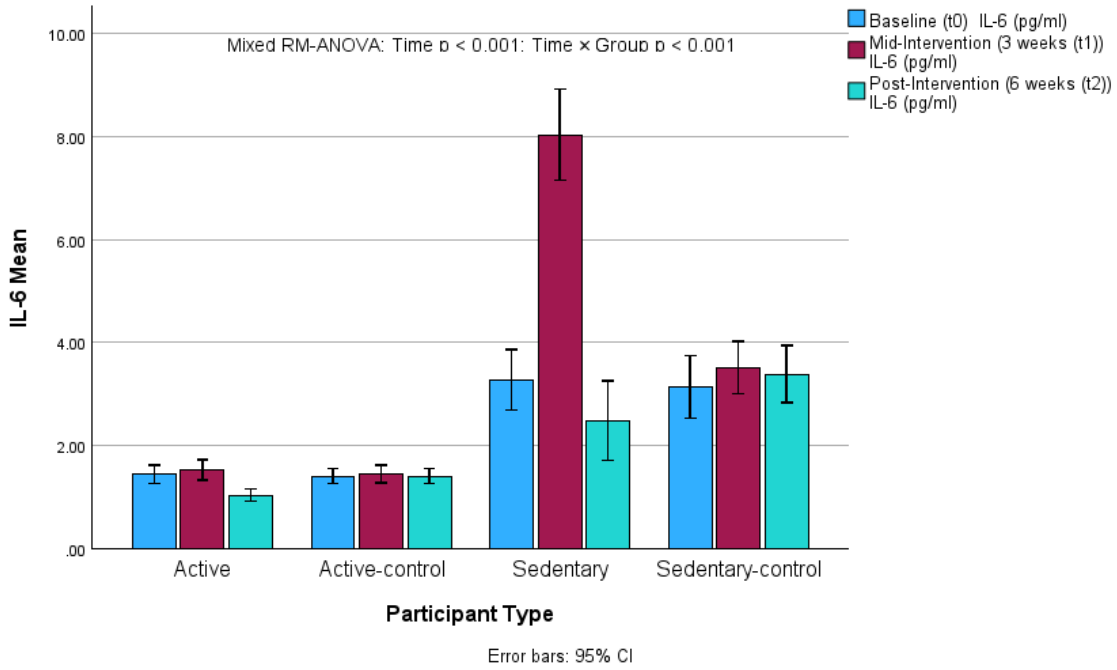

Figure 7: Mean values of serum Interleukin-6 (pg/ml) in the four groups of participants at (t0), (t1) and (t2). Errors bars indicate 95% confidence intervals (n = 9/group).

4. DISCUSSION:

This study was conducted to compare the levels of muscle damage markers; CK and LDH at (t0), (t1) and (t2) following moderate intensity running exercise among healthy young men and to determine the levels of inflammatory biomarkers; IL-6 and CRP at (t0), (t1) and (t2) following moderate intensity running exercise among healthy young men. A noticeable baseline

difference was found between sedentary and sedentary control groups in heart rate. Both groups were categorized as non-active, but the sedentary group had a higher heart rate compared to the sedentary control group (86.67 ± 4.03 bpm vs. 67.00 ± 7.45 bpm). Heart rate was obtained manually by the use of a pulse oximeter and was only collected as a baseline descriptive variable and not a primary outcome. This variation can be due to the individual variability or to some uncontrolled pre-testing variables such as anxiety, sleep quality, hydration status, recent activity and caffeine consumption (Sajjadieh et al., 2020).

The main finding of this study that muscle damage markers (CK, LDH) and inflammation markers (CRP, IL-6) followed a non-linear over time and highly dependent on training level. All biomarkers showed a statistically significant main time effect, a statistically significant main group effect, and a statistically significant time × group interaction ($p < 0.001$); however, in all cases, the time × group interaction was driven by a quadratic interaction ($p < 0.001$). In practice, the biomarkers increased significantly during the t1 and decreased by the t2. Descriptively, the sedentary group demonstrated the greatest biomarker elevations at (t1), with CK increased to 242.48 ± 47.84 U/L, LDH to 345.52 ± 68.19 U/L, CRP to 3.33 ± 0.65 mg/L, and IL-6 to 8.04 ± 1.15 pg/mL. These values were lower at (t2), indicating a transient physiological stress response and partial adaptation or recovery. There were relatively small changes over time for the active-intervention and active-control groups. However, the small magnitude of the changes in the active-intervention group, statistically significant, should be noted in the blood markers LDH, CRP, and IL-6, as described by (Putro et al., 2026; Stožer et al., 2020), characterized by a non-linear, transient pattern, may reflect an indirect disruption of the sarcolemma results from extreme exercise loading and leads to a local injury reaction that causes a hepatic acute phase response and release of cytosolic CK and LDH into the circulation, which leads to increased membrane permeability. This confirms the results of this study where the increases in all four parameters were highest in the sedentary intervention group at t1, with the levels of CK 242.48 ± 47.84, LDH 345.52 ± 68.19, CRP 3.33 ± 0.65, and IL-6 8.04 ± 1.15 pg/ml, which is considered a marker of increased sensitivity of the sedentary muscles to the novel mechanical stimulus.

Bessa et al. (2016) have shown that high-intensity exercise leads to a temporary increase in muscle damage and inflammatory markers such as CK, LDH, CRP, and IL-6. In professional athletes, the onset and disappearance of CK and LDH from the blood were more rapid, indicating efficient recovery and adaptation to repeated training. The inflammatory response was concentrated primarily in skeletal muscle, suggesting that the elevated levels of inflammatory biomarkers after exercise may simply be a normal recovery process, rather than chronic systemic inflammation. These findings support the use of CK, LDH, CRP, and IL-6 as indirect indicators of muscle fatigue and recovery during exercise (Bessa et al., 2016). Markus et al. (2022) showed that the 60-minute downhill running protocol resulted in significant time-dependent changes in CK and LDH enzyme levels, with CK and CRP peaking during the recovery period, while interleukin-6 showed a transient inflammatory response (Markus et al., 2022). The fact that the response was not maintained through 6 weeks of training, but rather reduced by 6 weeks of training, is best explained by the repeated bout effect and training adaptation.

A study conducted by Doma, K et al. confirmed that single bout of damaging exercise provides protection against another bout, resulting in a reduction of the increase in CK and myoglobin levels as well as the inflammatory response and decrease in force output in subsequent bouts (Doma et al., 2023). Repeat bout of exercise lead to progressive reduction in muscle damage as a result of structural adaptations of the sarcolemma and the cytoskeleton, neural adaptations, and a dampened inflammatory response, which means that the same or more running load results in a smaller biomarker signal with repeated exposures (Calvo-Rubio et al., 2024). This protection emerged early in the study and was maintained throughout the intervention in the already-active participants, who had a well-established training history, and is an example of a reduced acute response to running exercise. The IL-6 results are notable, as this measure showed the greatest intergroup difference of any other marker. The prototypical exercise-responsive myokine is IL-6: it is released by contracting muscle in response to calcium flux, glycogen depletion and metabolic stress, and the downstream actions of exercise-derived IL-6 are metabolic and largely anti-inflammatory (as opposed to the chronic disease-derived sustained pro-inflammatory IL-6) (Ringleb et al., 2025). A dramatic transient IL-6 peak in sedentary men is thus consistent with more muscle stress, but ultimately it is an adaptive and signalling response. The parallel transient elevation in CRP, which is an acute-phase reactant and has a slower time course, is a mechanistically expected part of the cytokine response and is a well-established consequence of endurance exercise-induced inflammation (Waśkiewicz et al., 2025). An interesting feature of the data is the baseline difference in LDH and IL-6 between groups: active participants had higher resting LDH but lower resting IL-6 than sedentary participants. Resting LDH is higher in active persons, likely due to metabolic and enzymatic adaptations resulting from chronic aerobic activity, while lower resting IL-6 levels are in line with the positive baseline inflammatory effects of regular exercise (Allard et al., 2023). The significant time × group interactions did

not result from significant changes in the active groups, but rather from the sedentary–experimental group reaching a high level in the t1 and returning to a low level at t2 near to baseline value, whereas other groups' values were remained stable throughout the study. These observations align with the literature showed that prolonged or strenuous running raised CK, LDH, CRP and IL-6, that the magnitude of the response depends on the severity of the stimulus, and athlete's individuals exhibit attenuated responses compared to sedentary (Bray et al., 2025; Chen et al., 2025; D'Alleva et al., 2025). The practical implication for exercise prescription is that sedentary individuals should expect a substantial but transient muscle-damage and inflammatory response during the first weeks of a running programme, and that gradual progression allows this response to attenuate as protective adaptation develops.

5. Conclusion:

In this small, non-randomized study, biomarker trajectories differed by activity status and intervention. The sedentary-intervention group had showed significant change in CK, LDH, CRP and IL-6 levels at t1, followed by attenuation at t2, while changes in the active groups were showed small changes across time points. These descriptive patterns are similar to the transient muscle-stress and inflammatory response to a novel running stimulus in sedentary individuals, and partial recovery during the programme.

6. Limitation of this study:

There are some limitations that should be noted. The sample consisted of healthy young men with nine participants in each group, limiting the statistical power for between-group comparisons and therefore the generalizability of the results to women, older adults and clinical population. non-random allocation and selection bias; baseline group differences; overlapping activity definitions; lack of blinding; possible infection, diet, sleep and recent-activity confounding; absence of objective activity monitoring; unreported adherence and achieved workload; reliance on age-predicted HRmax; only one 24-hour post exercise interval CK in particular shows large inter-individual variability and it is not linearly related to functional impairment, which is reflected in the large standard deviations in the sedentary–experimental group; adding it to LDH, IL-6 and CRP does not remove this issue. The 24 h post-exercise sampling of the markers was performed at three time points and may not reflect the time of maximal concentration of each marker as IL-6 peaks early and returns to near basal levels within hours, whereas CRP peaks later. Lastly, the study only evaluated blood markers and did not measure direct muscle function indices, or muscle soreness, so the connection between the biochemical response and muscle damage was also inferred.

Acknowledgments: The authors thank our members for volunteering for this study.

Conflict of interest and funding: The authors have not declared any conflict of interest.
Use of AI and AI-enabled technologies: The authors used AI tool (ChatGPT) for language and grammar editing of the manuscript text when preparing this work.

Table S1: Supplementary Table S1

Tests of Normality

	participant type	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
pre exercise (24 hrs) CK (U/L)	Active	0.127	9	.200*	0.97	9	0.897
	Active control	0.126	9	.200*	0.968	9	0.88
	Sedentary	0.145	9	.200*	0.95	9	0.687
	Sedentary control	0.269	9	0.06	0.826	9	0.04
pre exercise (24 hrs) LDH (U/L)	Active	0.077	9	.200*	0.993	9	0.999
	Active control	0.092	9	.200*	0.984	9	0.983
	Sedentary	0.154	9	.200*	0.949	9	0.682

	Sedentary control	0.197	9	.200*	0.936	9	0.544
pre exercise (24 hrs) CRP (mg/l)	Active	0.139	9	.200*	0.971	9	0.906
	Active control	0.156	9	.200*	0.938	9	0.557
	Sedentary	0.184	9	.200*	0.911	9	0.326
	Sedentary control	0.202	9	.200*	0.895	9	0.223
pre exercise (24 hrs) IL-6 (pg/ml)	Active	0.16	9	.200*	0.968	9	0.879
	Active control	0.142	9	.200*	0.978	9	0.951
	Sedentary	0.186	9	.200*	0.916	9	0.363
	Sedentary control	0.158	9	.200*	0.946	9	0.644
Mid-Exercise at the end of 3 weeks (24 h) CK (U/L)	Active	0.209	9	.200*	0.911	9	0.324
	Active control	0.171	9	.200*	0.939	9	0.575
	Sedentary	0.164	9	.200*	0.918	9	0.374
	Sedentary control	0.281	9	0.04	0.792	9	0.016
Mid-Exercise at the end of 3 weeks (24 hrs) LDH (U/L)	Active	0.166	9	.200*	0.959	9	0.785
	Active control	0.117	9	.200*	0.966	9	0.862
	Sedentary	0.145	9	.200*	0.964	9	0.843
	Sedentary control	0.161	9	.200*	0.944	9	0.629
Mid-Exercise at the end of 3 weeks (24 hrs) CRP (mg/l)	Active	0.143	9	.200*	0.98	9	0.964
	Active control	0.139	9	.200*	0.971	9	0.906
	Sedentary	0.132	9	.200*	0.981	9	0.971
	Sedentary control	0.172	9	.200*	0.971	9	0.904
Mid-Exercise at the end of 3 weeks (24 hrs) IL-6 (pg/ml)	Active	0.174	9	.200*	0.928	9	0.461
	Active control	0.19	9	.200*	0.923	9	0.414
	Sedentary	0.12	9	.200*	0.949	9	0.678
	Sedentary control	0.176	9	.200*	0.928	9	0.465
Post-Exercise after 6 weeks (24h) CK (U/L)	Active	0.117	9	.200*	0.972	9	0.915
	Active control	0.156	9	.200*	0.958	9	0.774
	Sedentary	0.362	9	0.001	0.61	9	<.001

	Sedentary control	0.244	9	0.131	0.756	9	0.006
Post-Exercise after 6 weeks (24h) LDH(U/L)	Active	0.098	9	.200*	0.991	9	0.997
	Active control	0.096	9	.200*	0.986	9	0.987
	Sedentary	0.128	9	.200*	0.95	9	0.695
	Sedentary control	0.195	9	.200*	0.948	9	0.669
Post-Exercise after 6 weeks (24h) CRP (mg/l)	Active	0.145	9	.200*	0.96	9	0.801
	Active control	0.156	9	.200*	0.938	9	0.557
	Sedentary	0.145	9	.200*	0.96	9	0.801
	Sedentary control	0.171	9	.200*	0.933	9	0.515
Post-Exercise after 6 weeks (24h) IL-6 (pg/ml)	Active	0.139	9	.200*	0.971	9	0.906
	Active control	0.142	9	.200*	0.978	9	0.951
	Sedentary	0.172	9	.200*	0.956	9	0.761
	Sedentary control	0.213	9	.200*	0.912	9	0.327

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

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