RESEARCH PAPER

# Acute Responses of CK, LDH, CRP, and IL-6 Following Exercise-Induced Fatigue in Healthy Young Male Adults.

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**Abstract**:
**Background**: Treadmill-induced fatigue elicits muscle damage and inflammatory responses. These interactions can vary between active and sedentary individuals, as a result of physiological adaptation and recovery capacity.
**Purpose**: This study compared serum CK and LDH, and measured CRP and IL-6 pre- and post-fatigue in healthy male participants.
*Methods*: A total of 36 participants (aged between 19-27) were non-randomly assigned to two groups: intervention (9 sedentary and 9 active) and control (9 sedentary and 9 active). Venous blood was drawn before and 24 h after the protocol for analysis levels of CK, LDH, CRP and IL-6. The Shapiro–Wilk test was used to assess normality. Paired-samples t-tests were used with Cohen's *d* to analyse within-group changes, and a two-way between-subject's ANOVA was used with partial $\eta^2$ to analyse between-group differences change (Δ) with a significance at $p < 0.05$.
**Results**: All biomarkers significantly increased ($p < 0.001$) with large effect sizes was observed in the sedentary participants. There were no significant changes seen in control groups. The two-way ANOVA showed a significant effect of intervention for all biomarkers (partial $\eta^2 = 0.587–0.831$). For CK ($p = 0.033$), LDH ($p = 0.040$) and IL-6 ($p = 0.027$) in the sedentary participants; no significant interaction was seen for CRP ($p = 0.214$).
**Conclusion**: There was a significant muscle damage and inflammatory response following treadmill-induced fatigue in active and sedentary individuals, but higher in sedentary individuals. These findings confirm that the acute biochemical and inflammatory response to fatigue differs according to activity status.
Keywords: Treadmill fatigue, Biomarkers, Inflammation.
**How to cite this article:** Mohammed I. Islemeh.- , Maria Justine., Wan Mazlina Saad. Mahmoud . Ramlawi. . Acute Responses of CK, LDH, CRP, and IL-6 Following Exercise-Induced Fatigue in Healthy Young Male Adults.. Int J Drug Deliv Technol. 2026;16(79s):997-1015. DOI: 10.25258/ijddt.16.79s.172.

## 1. Introduction

Muscle Fatigue (MF) is a state in which maximal power or maximal capacity to generate force is reduced during or after repeated contractions [1]. Fatigue is a common experience in daily life and often occurs during or after intense exercise, prolonged physical activity, or sustained effort [2]. Fatigue is a key concept in sports science that has significant implications for sports

performance. Exercise-induced fatigue may be defined as a significant decrease in the capacity to sustain exercise performance [3]. In other words, fatigue is the failure to achieve a desired level of exercise intensity, which results in a decreased ability to engage in exercise performance or a failure to complete task [4]

Physical activity can also be a physiological stressor that can alter tissue homeostasis and induce inflammatory response both in short and long term [5]. One of the major factors that drives recovery and physiological adaptation is the induced inflammatory response [6]. markers reflecting metabolic stress and inflammatory response to exercise [6]. The symptoms of this condition 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) [7]. In addition, muscle damage may trigger an inflammatory reaction, which increases C-reactive protein (CRP) and cytokines such as interleukin-6 (IL-6) [8]. In exercise, IL-6 is believed to act as a hormone, regulating substrate availability and energy metabolism. It stimulates glucose uptake and enhances production of glucose in liver during exercise [9]. In response to IL-6, the liver synthesized CRP which is an acute-phase protein widely used as a marker of systemic inflammation [10]. In exercise context, CRP may increase after intensive exercise and higher increases are generally seen after high intensity exercise than with low or moderate intensity exercise [11]. Moreover, physiological indicators such as blood pressure, heart rate and maximal oxygen consumption ($\dot{V}O_2$ max) can be used also to evaluates the effectiveness of physical exercise [12, 13, 14]. Enzymes released from the skeletal muscle into the bloodstream during or after exercise are called muscle damage markers, which are an indirect indicator of exercise-induced muscle damage. These markers tend to raise during rigorous exercise due to stress and permeability of muscle fibres. Creatine kinase is one of the most widely used markers of muscle damage because it is a sensitive indicator of an increase in sarcolemma permeability, has a greater response than some of the other muscle damage markers, and is relatively simple and inexpensive to measure [15]. Furthermore, the peak period of CK concentrations following exercise is not clearly defined and may be between 24 and 72 hours (h); however, CK is still an important marker of skeletal muscle damage [16]. Lactate dehydrogenase is another enzyme that is frequently used as a marker of muscle damage, and it is an enzyme that 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 [17, 18]. These markers are mainly for assessing muscle damage and physiological stress during exercise, and not to prevent damage [19].

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 from exercise-induced damage, especially after strenuous exercise [20]. Therefore, the inflammatory reaction to muscle damage could initiate repair and adaption tissue process, and could be informative as a tool for tracking recovery from exercise [17].

Furthermore, while training status can markedly affect physiological response to exercise, few studies have investigated this response in both active and sedentary young adults. Thus, it is crucial to identify reliable biomarkers to assess training program efficacy and the exercise effects on individual responses [21, 22, 23]. Additionally, it is important to develop personalized programs to specific groups. This study is the first study conducted on Palestinian population" which is another drawback of the current literature is the lack of information from Middle Eastern people, especially Palestinian adults. Thus, there is a lack of data describing the acute changes in fatigue-induced muscle damage and inflammatory biomarkers including CK, LDH, CRP and IL-6 following treadmill-induced fatigue in active and sedentary young Palestinian males, so there is limited evidence for the physiological response to exercise-induced fatigue among Palestinians individuals. Investigating young adults from Palestine may enhance the general understanding of exercise-related biomarker responses and assist in determining whether findings from other populations are applicable to Palestinians. Furthermore, it will assessment of whether these responses are modulated by population-specific factors, including diet, lifestyle and physical activity status, as well as genetic variations [24]. We hypothesized that exercise-induced fatigue would significantly increase CK, LDH, CRP, and IL-6 levels, with greater responses among sedentary individuals.

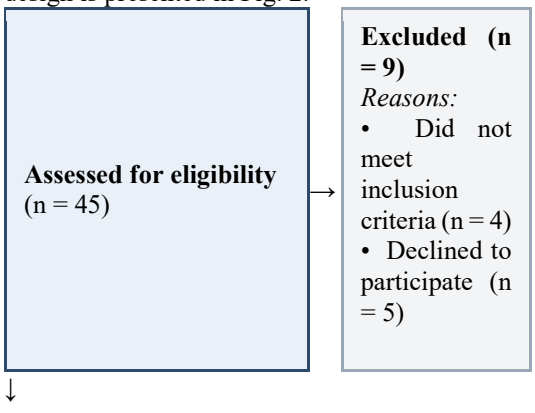
## 2. Material and methods:

2.1 Study design and settings:

This study was a 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:

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 participants' adult's male aged 19-27 years from local population were enrolled in this study, divided into two main groups (intervention group number (*n*)= 18) and (control group *n* =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 presented in Fig. 1. An overview of the study design is presented in Fig. 2.



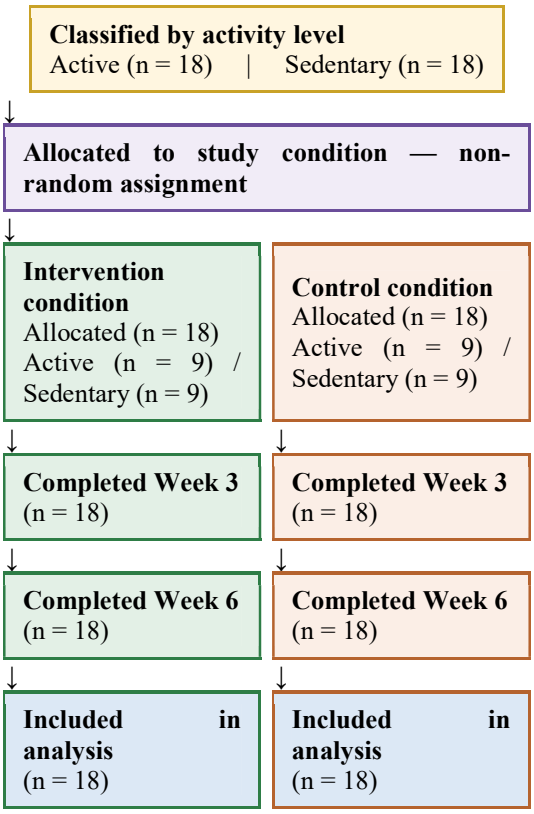
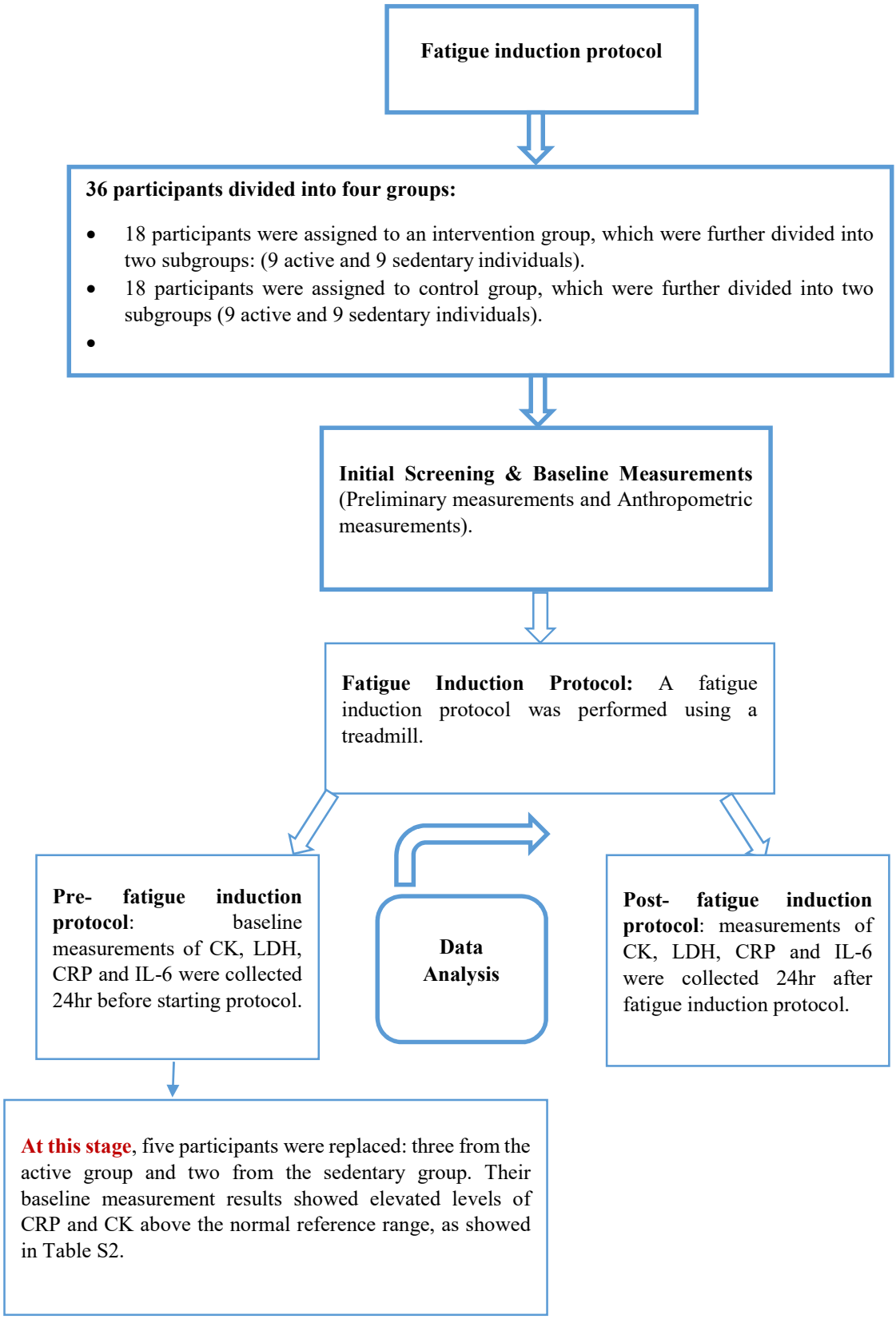




Figure 1: A flowchart for selecting participants and group allocation.




2.3 Criteria for inclusion:

Fig. 2: Study flowchart.

Adult males with age ranging from 19 to 27 years old.

2.3.1 Criteria for selecting active and sedentary individuals:

Sedentary individuals: were individuals who did not participate in any form of exercise training (total exercise time < 120 min /week) (Zhang et al., 2024). Active individuals: were considered to be physically active they have been engaging in physical activity for at least ≥150 min per week, three days per week for three months continuously (Kyral et al., 2019). Only subjects with body mass index (BMI) between of 18.5 to 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. Medically qualified personnel 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: Only subjects with body mass index (BMI) between of 18.5 to 24.9 kg/m² were included in this study. Participants usual activity was monitored throughout the study using questionnaire and active controls were asked to continue with their regular structured exercise during the study (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, recent infection or fever, chronic inflammatory/autoimmune disease, smoking or vaping, alcohol use, recent vaccination, medication and analgesic/NSAID use, antioxidant or dietary supplementation, recent strenuous exercise, and sleep deprivation, these factors may materially alter CK, LDH, CRP or IL-6 were also excluded (Xu et al., 2018).

2.5 Sample size:

G*Power 3.1.9.7 software was used to calculate the sample size of 36 participants. (36 = four groups × nine participants per group), 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. (Kyral et al., 2019).

2.6 Sample collection and processing:

A blood samples were collected from each individual 24 h before and 24 h after fatigue induction protocol 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 Fatigue induction 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 fatigue induction protocol including: height, weight, body mass index, body fat percentage, blood pressure, heart rate and Electrocardiogram.

2.7.2 Maximal oxygen consumption test: $\dot{V}O_2$ max was estimated through the use of the Harvard

Step Test in a separate test after anthropometric measurements. 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 $\dot{V}O_2$ max predicted by a nomogram which depended on both the heart rate and body weight [25, 26]. $\dot{V}O_2$ max was used as a baseline descriptive variable to describe cardiorespiratory fitness and support interpretation of training status differences between groups.

2.7.3 Fatigue induction protocol:

After a week of initial measurements, the participants applied the fatigue protocol, which included the following: Pulse rate measurement was used to monitor the heart rate and the rating of perceived exertion (RPE) was taken every 2 minutes on the Borg 6-20 RPE scale by each participant. The participants chose their own speed for 5 minutes on the treadmill as a warm-up and familiarization [26]. After that, the participants started running at a speed of 6 km/h with the treadmill incline set to 0% consistently throughout the experiment [27]. The participants were also instructed to avoid using the treadmill handles while running to prevent any reduction in the exerted effort [28] The speed was increased by 1 km/h every two minutes with strong verbal encouragement throughout the test period to motivate them to exert maximum effort. The speed was increased every two minutes until the participant rated their effort level at 13 (somewhat hard) on the Borg scale. At this point, the treadmill speed was not increased further, and participants continued running at a constant speed until they reached level 17 (very difficult). At this point, participants completed an additional two minutes of running to ensure they reached the threshold of voluntary exhaustion. Following this, participants concluded the protocol with a five-minute cool-down period of their self-selected speed [29]. All participants were considered to have reached voluntary exhaustion when they were unable to continue the task even with verbal encouragement from the researcher [30]. Exhaustion was considered to have been achieved, and the intervention was terminated when a score of 17 PRE on the Borg scale was reached and were unable to continue running [31].

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 [11]. This is due to their ability to rise rapidly during exercise induced fatigue and their ability to reflect the effect of physical activity on individuals.

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; results under the lower limit of detection (0.5) were [recorded as below detectable limit.

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 CRP control was analysed as an internal quality control; results under the lower limit of detection (0.3) were [recorded as below detectable limit.

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 Statistical Package for the Social Sciences (SPSS) version 25 to perform statistical analysis. Statistical significance was determined at the $p < 0.05$ level. Data are shown as mean ± *SD* values. The change scores (Δ) were normally distributed within all four groups (as shown in Table S1, Shapiro-Wilk test and inspection of the Q-Q plots), justifying the use of parametric test analyses. Paired-samples t-tests were used to examine changes in muscle-damage (CK, LDH) and inflammatory (IL-6, CRP) biomarkers from pre- to post-fatigue induction, and Cohen's *d* served as the effect size. The difference between groups in the fatigue-induced change (Δ = post − pre) was explored with a two-way between-subject's ANOVA, with activity level (active vs. sedentary) and intervention (intervention vs. control) fixed factors and their interaction included; Levene's test was used to confirm homogeneity of variances and partial eta squared ($\eta^2$) was reported as the effect size. Bonferroni adjusted simple main effects were used to follow up significant interactions. All tests were two-sided and, due to the small sample size, the activity and interaction effects are considered exploratory.

2.11: 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 fatigue induction protocol. The results presented as Mean ± *SD* for age, height, weight, BMI, blood pressure systolic and diastolic, heart rate, body fate and $\dot{V}O_2$ max, as shown in table1. 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□$m^{-2}$). Additionally, blood pressure, resting heart rate and ECG findings were

within normal limits for all participants to ensure that no any participant have chronic or cardiovascular disease. It was observed that both active and active control participants achieved better results outcome in body fat percentage and $\dot{V}O_2$ max compared to sedentary participants and sedentary control participants. Among the 18 participants underwent the treadmill-induced fatigue protocol, 12 were able to perform the additional 2 minutes after reaching RPE 17, while 6 participants could not continue the protocol. For the active group, 7 participants completed the extra 2 minutes and 2 participants stopped early, while for the sedentary group 5 participants completed the extra 2 minutes and 4 participants stopped early. Because the protocol was designed to each participant's level of volitional exhaustion, all participants reached a similar level of maximum effort end-point, and therefore the completion level was not used as an analytical variable.

Table 1: Descriptive characteristics and outcomes by participant type (n = 9 per group).

| **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 | 174.00 ± 4.46 |
| Weight (kg) | 61.44 ± 4.30 | 62.89 ± 3.26 | 69.33 ± 5.12 | 71.00 ± 5.24 |
| BMI (kg□m$^{-2}$) | 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 |
| Pre-fatigue CK (U/L) | 113.70 ± 11.72 | 111.06 ± 18.68 | 90.25 ± 9.19 | 81.53 ± 15.49 |
| Pre-fatigue LDH (U/L) | 262.06 ± 25.1 | 257 ± 25.1 | 186.06 ±18.55 | 192.20 ± 13.8 |
| Pre-fatigue CRP (mg/L) | 1.96 ± 0.28 | 2.09 ± 0.47 | 1.07 ± 0.27 | 1.28 ± 0.36 |
| Pre-fatigue IL-6 (pg/mL) | 2.81 ± 0.47 | 2.81 ± 0.46 | 1.51 ± 0.28 | 1.17 ± 0.15 |
| Post-fatigue CK (U/L) | 160.83 ± 6.79 | 113.22 ± 11.95 | 120.15 ± 12.16 | 88.78 ± 10.07 |
| Post-fatigue LDH (U/L) | 380.42 ± 21.94 | 255.29 ± 28.62 | 266.20 ± 21.45 | 195.77 ± 20.10 |
| Post-fatigue CRP (mg/L) | 3.67 ± 0.40 | 1.91 ± 0.58 | 2.61 ± 0.38 | 1.49 ± 0.47 |
| Post-fatigue IL-6 (pg/mL) | 5.97 ± 0.42 | 3.08 ± 0.70 | 3.79 ± 0.45 | 1.45 ± 0.35 |

Values are shown as the mean ± SD; n = number of participant per group. BMI: body mass index; $\dot{V}O_2$ max: maximal oxygen, BP, blood pressure, consumption; CK: creatine kinase; LDH: lactate dehydrogenase; CRP: C-reactive protein; IL-6: interleukin-6; bpm: beats per minute; SD: standard deviation.

All values expressed as mean ± standard deviation ($N$ = 36; $n$ = 9 per group) in Table 1. Groups were

classified according to activity level (active vs. sedentary) and intervention status (intervention vs. control). Pre- and post-fatigue values represent the concentration of each biomarker before and after inducing fatigue. Continuous variables were checked for normality by the Shapiro–Wilk test and plots of distributions, and are presented as mean ± *SD*. Table 1 contains only descriptive characteristics, while within-group changes after fatigue and between-group comparisons of the fatigue-induced change (Δ) are presented in Tables 2 and 3, respectively.

Table 2: Pre–Post Changes in Biomarkers by Group (paired t test)

| **Group** | **Biomarker** | **Pre Mean ± *SD*** | **Post Mean ± *SD*** | **Mean Difference** | ***t* (df= 8)** | ***p*-value** | **Cohen's *d*** |
|---|---|---|---|---|---|---|---|
| Active-Intervention | CK | 90.25 ± 9.19 | 120.15 ± 12.16 | +29.90333 | -7.022 | <.001[a] | +2.34 |
| | LDH | 186.06 ± 18.55 | 266.20 ± 21.45 | +80.14111 | -10.042 | <.001[a] | +3.35 |
| | CRP | 1.07 ± 0.27 | 2.61 ± 0.38 | +1.54778 | -8.048 | <.001[a] | +2.68 |
| | IL-6 | 1.51 ± 0. | 3.79 ± 0. | +2.28111 | -10.864 | <.001[a] | +3.62 |
| Active-Control | CK | 81.53 ± 15.49 | 88.77 ± 10.07 | +7.24444 | -1.595 | .149 | +0.53 |
| | LDH | 192.20 ± 13.38 | 195.70 ± 20.10 | +3.56556 | -.440 | .671 | +0.15 |
| | CRP | 1.28 ± 0.36 | 1.49 ± 0.47 | +0.21111 | -1.142 | .286 | +0.38 |
| | IL-6 | 1.17 ± 0.15 | 1.45 ± 0.35 | +28333 | -2.000 | .080 | +0.67 |
| Sedentary-Intervention | CK | 113.70 ± 11.72 | 160.83 ± 6.79 | +47.13222 | -9.373 | <.001[a] | +3.12 |
| | LDH | 262.06 ± 25.10 | 380.42 ± 21.94 | +118.3611 | -9.047 | <.001[a] | +3.02 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | CRP | 1.96 ± 0.28 | 3.67 ± 0.40 | +1.70778 | -9.414 | <.001[a] | +3.14 |
| | IL-6 | 2.81 ± 0.47 | 5.97 ± 0.42 | +3.16444 | -20.537 | <.001[a] | +6.85 |
| Sedentary-Control | CK | 111.06 ± 18.68 | 113.23 ± 11.95 | +2.16000 | -.357 | .730 | +0.12 |
| | LDH | 257 ± 25.10 | 255.29 ± 28.62 | -1.72889 | 0.163 | .875 | -0.05 |
| | CRP | 2.09 ± 0.47 | 1.91 ± 0.58 | -0.18000 | 0.618 | .554 | -0.21 |
| | IL-6 | 2.81 ± 0.46 | 3.08 ± 0.70 | +0.26444 | -1.041 | .328 | +0.35 |

Values are given as mean ± SD; n = 9 in each group. V. CK, creatine kinase; LDH, lactate dehydrogenase; CRP, C-reactive protein; IL-6, interleukin-6; SD, standard deviation; t, paired t statistic; df, degrees of freedom; d, Cohen's d effect size.

[a] statistically significant pre–post change ($p < .05$).

Paired-samples t-tests revealed that only intervention groups had significant changes in muscle-damage and inflammatory biomarkers, and these changes were different depending on the activity status as presented in Table 2. Active-Intervention: Significant elevations in all four biomarkers (CK, LDH, CRP, IL-6; all $p < .001$) with large effect size suggesting a strong inflammatory and physiological response to fatigue.

Sedentary-Intervention demonstrated the greatest results compared to any group with significant increases in all biomarkers (all $p < .001$). CK increased from 113.70 ± 11.72 to 160.83 ± 6.79 U/L (Δ = +47.13) as shown in (Fig. 3), LDH from 262.06 ± 25.10 to 380.42 ± 21.94 U/L (Δ = +118.36) as shown in (Fig. 4), CRP from 1.96 ± 0.28 to 3.67 ± 0.40 mg/dL (Δ = +1.71) as shown in (Fig. 5), and IL-6 from 2.81 ± 0.47 to 5.97 ± 0.42 pg/mL (Δ = +3.16) as shown in (Fig. 6). This response was higher compared to Active-Intervention group for all markers (significantly higher for CK and IL-6), and had the largest effect sizes in the study (Cohen's $d$ for IL-6 = +6.85), suggesting that muscle-damage and inflammatory response was more pronounced among sedentary participants to fatigue. In Active-Control: No change was observed in any biomarker (all $p > .05$) and indicated a stable physiological state without the intervention. Sedentary-Control: no significant change in any biomarker (all $P > 0.05$), and slight non-significant decreases in LDH and CRP, indicative consistent with no meaningful fatigue response. Overall, these results suggest that biochemical and inflammatory responses to fatigue protocol differed according to activity status, with these responses being greater in sedentary participants than active participants, but no responses in either of the control groups.

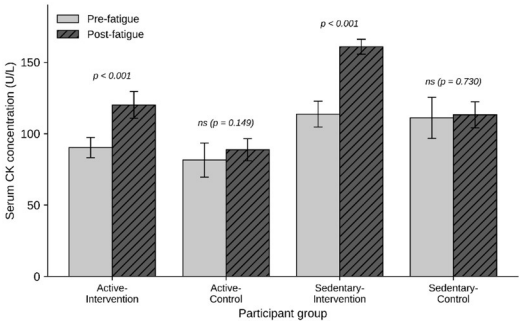



Fig. 3: The level of serum creatine kinase before and after the fatigue induction protocol for each group of participants. The means of each group are represented as bars; 95% CI for each group is represented by error bars, and p-values for within-group paired comparisons between the pre- and

post-fatigue values are shown above each pair. CK, creatine kinase; CI, confidence interval; ns, non-significant.

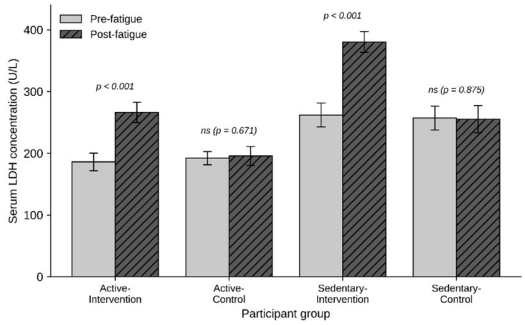



Fig. 4: Serum LDH levels before and after the fatigue induction protocol for the four groups of participants. Errors bars are 95% confidence intervals and p-values above each pair indicate within-group paired comparisons of pre versus post-fatigue values. In the following, LDH is lactate dehydrogenase, CI is confidence interval, and ns is not significant.

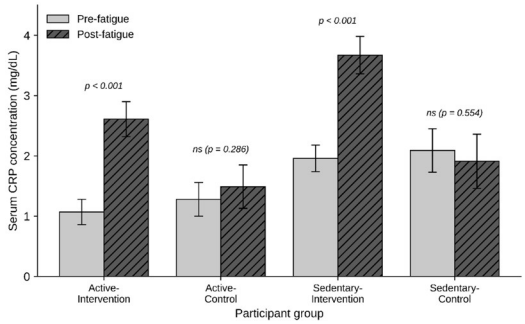



**Fig. 5**: Concentrations of serum C-reactive protein before and after the fatigue induction protocol among the four groups of participants. Bars represent group mean values; error bars represent 95% confidence intervals. *p*-values shown above each pair are from within-group paired comparisons of pre- versus post-fatigue values. ns = not significant; CI = confidence interval; CRP = C-reactive protein.

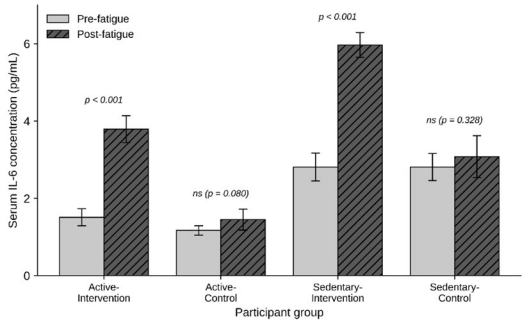



**Fig. 6**: Concentrations of serum interleukin-6 before and after the fatigue induction protocol in the four groups of participants. The *p*-Values above the groups of bars are based on pre- versus post-fatigue comparisons conducted within each group. The *p*-values shown above each pair of bars are based on within-group paired comparisons of pre- and post-fatigue values. IL-6, Interleukin-6; CI, Confidence interval; ns, Not significant.

Two-way ANOVA was used to assess the impact of activity level, intervention, and their interaction on the change (Δ) in each biomarker due to fatigue, as illustrated in Table 3. Main effect for intervention was significant for all four biomarkers (CK, LDH, CRP, IL-6; all $p < .001$), and had very large effect sizes (partial $\eta^2 = .587$–.831). This was the most consistent effect across, with the intervention being the main contributor to the increase in muscle-damage and inflammatory markers induced by fatigue, and being significant after Bonferroni correction for all four biomarkers ($\alpha = .0125$).

Table 3. Two-way analysis of variance (Activity × Intervention) for fatigue-induced change (Δ) in muscle-damage and inflammatory biomarkers.

| **Biomarker** | **Effect** | ***F* (df = 1, 32)** | ***p*** | **Partial η²** |
|---|---|---|---|---|
| CK | Activity | 1.47 | .235 | .044 |
| | Intervention | 45.49 | < .00[a] | .587 |
| | Activity × Intervention | 4.95 | .033[a] | .134 |
| LDH | Activity | 2.62 | .115 | .076 |
| | Intervention | 93.54 | < .001[a] | .745 |
| | Activity × Intervention | 4.58 | .040[a] | .125 |

| | | | | |
|---|---|---|---|---|
| CRP | Activity | 0.28 | .599 | .009 |
| | Intervention | 55.04 | < .001[a] | .632 |
| | Activity × Intervention | 1.61 | .214 | .048 |
| IL-6 | Activity | 4.90 | .034[a] | .133 |
| | Intervention | 157.43 | < .001[a] | .831 |
| | Activity × Intervention | 5.34 | .027[a] | .143 |

Values are the *F* ratio with significance and partial eta squared. CK, Creatine Kinase; LDH, Lactate dehydrogenase; CRP, C-reactive protein; IL-6, Interleukin-6, *F:* *F* ratio, df: degree of freedom, $\eta^2$, partial eta squared; Δ= post – pre.

[a] statistically significant ANOVA (Activity × Intervention) ($p < .05$).

Activity x Intervention interaction was significant for CK ($p = .033$), LDH ($p = .040$) and IL-6 ($p = .027$) suggesting intervention had a greater impact on fatigue in sedentary than in active participants. For CRP, it was not significant ($p = .214$) but it was followed the same numerical trend as the other biomarkers. Furthermore, the main effect of activity was only significant for IL-6 ($p = .034$, partial $\eta^2 = .133$), activity level was not independently significant for CK or LDH or CRP (all $p > .05$).

For the Sedentary-Intervention group, the significant interactions are associated with response rate. Simple-effects analysis indicated that the intervention effect was significant within each activity level (largest pre-to-post increase and effect size for sedentary participants, partial $\eta^2 = .56$ for CK, in comparison to .24 for active participants). The models accounted for a significant amount of variance in all the biomarkers ($R^2 = .619–.840$), and Levene's test was not significant for any of the biomarkers ($p = .251–.560$), thus providing evidence of homogeneity of error variances. The increase in all intervention biomarkers was generally strong, and the increase in biomarkers CK, LDH and IL-6 was large in sedentary participants compared to active participants. The interaction and activity effects were not maintained after Bonferroni correction ($p = .027–.040$) and interpreted as exploratory.

4. **Discussion**:

This study was aimed to compare serum creatine kinase and lactate dehydrogenase levels before and after a fatigue induction protocol in active and sedentary healthy male participants and to determine the levels of inflammatory biomarkers CRP and IL-6 before and after fatigue induction protocol in active and sedentary healthy male participants. The main results of this study were that fatigue elicits muscle damage markers (CK, LDH) and inflammatory markers (IL-6, CRP) in the intervention groups, with no substantial changes observed in the controls. Importantly, this response was not consistent among participants: the two-way analysis of variance showed that the change induced by fatigue was significantly greater in sedentary than in active participants for CK, LDH, and IL-6 (showing that the acute biochemical response to fatigue was differed accordingly to participant's activity status). 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 [32, 33]. Five participants were excluded and replaced following the baseline screening due to the pre-protocol values of their CK and CRP exceeding the defined inclusion criteria. A CRP value greater than the kit reference range >5 mg\dl was considered elevated and indicative of acute inflammation or infection. Increased CK was defined as values above the reference range > 171 U/L, which would indicate potential recent muscle stress, muscle injury, or inadequate rest prior to testing. Replacement was done before the treadmill induced fatigue protocol and before post-fatigue biomarker analysis. The baseline values of CK and CRP for the replaced

participants are presented in Supplementary Table S2.

4.1 Muscle damage markers:

The higher levels of CK and LDH prominent in the present study suggests increased muscle-cell membrane damage during fatigue, resulting in increased leakage of intracellular enzymes into the circulation. This response was only observed in intervention groups where all biomarkers significantly ($p < 0.001$) increased with large effect size, while the control groups did not significantly change, thus validating that the enzyme release was a specific effect of the fatigue stimulus. Importantly, the increase was most pronounced in the sedentary intervention group, which exhibited the largest rises in both CK (113.70 → 160.83 U/L) and LDH (262.06 → 380.42 U/L), and a significant Activity × Intervention interaction confirmed that the fatigue-induced enzyme release was significantly greater in sedentary than in active participants for both markers. The results indicate that the mechanical stress and microtrauma induced in the muscles of sedentary people was associated with greater disruption in the sarcolemma and leakage of sarcoplasmic enzymes into the blood stream during a comparable fatigue challenge. Overall, the high levels of elevation in both CK and LDH, combined with the large effect sizes, suggest that the fatigue response was more pronounced among sedentary subjects. A similar mechanism has been described in previous study investigated that exercise induced muscle damage higher levels of CK and LDH were observed in sedentary individuals compared active individuals [34]. It is important to note that the levels of CK and LDH were measured 24 h after fatigue induction protocol. CK has delayed dynamics, as it is not raised immediately after exercise, but rather is released into the blood as a result of the muscle cell membranes disruption and leakage of the enzymes. Therefore, the higher levels of CK seen in the present study are probably due to the direct effect of exercise-induced disruption of the muscle membrane as well as the early recovery phase [34]. Another study showed that high levels of creatine kinase 24 h after exercise are similar to previous findings that indicated that CK peaks later than lactate dehydrogenase, since it is released more slowly from damaged muscle fibers, and a continuous leak occurs during the recovery phase. Therefore, the higher CK response seen in the present study could be related with either a continuous muscle membrane disruption or an active tissue remodeling process in addition to acute metabolic stress [22]. However, study conducted by Barcelos et al and Marin et al found that muscle damage markers such as CK and LDH can be used as a sensitive tool to detect changes in muscle damage levels during the pre-session and post-session.

4.2 Inflammatory biomarkers:

In this study, inflammatory biomarkers (IL-6 and CRP) were measured. The results showed that IL-6 demonstrated the largest effect size observed (Cohen's $d = -6.85$ in the sedentary intervention group; partial $\eta^2 = 0.831$ for the intervention main effect, $p < 0.001$). It is consistent with the current perception of IL-6 as the prototype of exercise-responsive myokines which are released in massive amounts during contraction of exercising skeletal muscle and as an upstream signal that initiates the downstream acute phase response, which includes the production of CRP [10, 35]. In addition, the intervention also caused a significant increase in CRP (partial $\eta^2 = 0.632$, $p < 0.001$), but had a less pronounced response than IL-6, reflecting the different kinetics of the two markers; CRP is an acute-phase protein that is synthesized in the liver, its levels increase is delayed compared with those of IL-6 and which peak significantly later [10]. The Activity × Intervention interaction was significant for IL-6 ($F = 5.34$, $p = 0.027$, partial $\eta^2 = 0.143$), with the increase in IL-6 levels being significantly higher in sedentary than in active participants, but not for CRP ($F = 1.61$, $p = 0.214$, partial $\eta^2 = 0.048$). Notably, the activity main effect was also significant for IL-6 ($F = 4.90$, $p = 0.034$) but not for CRP ($F = 0.28$, $p = 0.599$). A lack of interaction for CRP may also be due to the single sampling point in this study after fatigue caused activity-dependent differences to have not yet been fully realized, as it may have a slower and more delayed time course than the other biomarkers measured. Importantly, the time course of cytokine release indicates that inflammation should not just be regarded as a response to tissue damage but rather a controlled physiological process that plays a role in muscle repair, regeneration and adaptation [36]. Dan Nash et al. describe that skeletal muscle contraction produces IL-6 during exercise, and affected by the intensity and duration of the exercise and the decrease in glycogen availability [37]. A systematic review also reported that a single bout of moderate-to-high intensity exercise can substantially increase IL-6, supporting our result post-fatigue IL-6 findings [38]. In another study, increased activity intensity leads to more pronounced response in inflammatory cytokines, particularly IL-6 [39]. These change outcomes

were compared between groups to show significant differences between the physiological response to the fatigue induction protocol in active and sedentary participants. Generally, these results suggest that biochemical and inflammatory responses to fatigue differed accordingly to activity status in terms of both magnitude and pattern, with a greater muscle damage and inflammatory activation response observed in sedentary individuals compared to active individuals. The sedentary subjects had greater increases in post-fatigue IL-6 and CRP, which may reflect a greater inflammatory and immune-metabolic response to the fatigue induced by the treadmill. IL-6 is an exercise-responsive myokine, produced when skeletal muscle is activated during strenuous exercise, where the duration, intensity, and muscle glycogen depletion play major role [37]. Thus, the higher IL-6 response in the sedentary might be reflecting a greater relative physiological stress in this group due to the fatigue protocol. A previous study demonstrated that acute moderate to high intensity exercise causes a cytokine response, specifically IL-6, and that the quantity of IL-6 released is related to amount of stress and recovery muscles [39].

4.3 Effect of training status:

The correlation between increases in IL-6 and CRP suggests that treadmill-exercise induced fatigue elicited a coordinated inflammatory response. On the other hand, the minor response in the active participants is possibly due to chronic training adaptation. Long-term exercise training has been reported to lower circulating concentrations of IL-6 and CRP, and regular exercise has been found to have a beneficial effect on antioxidant defence, on reducing the oxidative stress, and in improving inflammatory regulation. Therefore, active participants may undergo to same fatigue protocol without the same inflammatory response, while sedentary participants have exhibited larger metabolic disturbances, oxidative stress and strain on the muscles [35].

4.4 Methodological considerations on sampling timing:

Many factors that may explain some of the variations between study results include those described by others [40, 41]. An important point is that the timing of the last training session or competition in relation to the time for CK assessment. In the early post-exercise or post-competition period (first 78 h), CK levels are generally higher than normal levels. However, CK is not as reliable indicator for muscle damage after 78 h. Birdsey et al. reported that in professional athletes, peak blood CK levels typically occurred from 24 to 48 h after training and competitive events. In this context, creatine kinase is considered as a sensitive biomarker to recognize muscle damage in professional athletes [42]. It is important to note that the concentrations of CK are affected by the circadian rhythm and are generally higher in the morning. Therefore, it is important to standardize the timing of sample collection to obtain accurate results and interpretation. In addition, the level of the CK enzyme can rise after training or competition, and it has been recorded that it peaks between 24 and 48 h after strenuous exercise [41, 42, 43, 44]. Evaluating CK at these times, might give aware information about the individual's muscular status. Monitoring these biomarkers can assist coaches and athletes in optimizing training and recovery strategies, thereby helping to reduce injury risk and enhance athletic performance.

In the present study, IL-6 and CRP were assessed 24 h following fatigue induced protocol. IL-6 is known to be an early exercise-responsive cytokine, often increasing during exercise or immediately after exercise and declining during the subsequent recovery period. Thus, the increase in IL-6 at 24 h should not be considered the "peak" of the cytokine response, but rather as a sign of a persistent or ongoing inflammatory response after fatigue. In contrast, the acute-phase response of CRP is less rapid, and may peak later, typically at 24–72 h post-exercise of high intensity or muscle damaging exercises. Therefore, the 24 h sampling point was more useful to detect the elevation of CRP, while not suitable to fully characterize IL-6 kinetics. This exercise timing is corroborated by the findings of pervious study that have demonstrated rapid elevations of IL-6 in response to exercise and more sustained elevations of CRP [10].

4.5 Limitations of this study and suggests further research direction:

There is a certain limitations of the present study. Firstly, only male participants were included in the study, thus its results may not be generalizable to females and blood samples were only taken at baseline and 24 h after the treadmill-induced fatigue protocol. Thus, the time course and peak responses of CK, LDH, IL-6 and CRP were not able to be evaluated. This is especially for IL-6, which tends to rise immediately after exercise, and may drop during recovery, while CRP tends to have a slower acute-phase response. To better define the kinetics of the biomarkers, future studies should employ more than one post-

exercise time point, including immediately after exercise, 2 h, 6 h, 12 h, 24 h, and 48 h and further research should be address with different approach to emphasize the role of biomarkers following fatigue induction protocol. Secondly, The CRP and IL-6 were measured by a point-of-care immunoassay instead of a central reference-laboratory assay; this is convenient, but may be less sensitive and precise than the reference assay. Finally, research integrating biomarker data with other monitoring tools like Global Positioning System (GPS) metrics, subjective wellness measures, and performance indicators would provide a more comprehensive understanding of fatigue and recovery processes.

**5**.**Conclusion**:

In conclusion, the current study revealed that there was a significant muscle damage and inflammatory response following treadmill-induced fatigue in both active and sedentary individuals, but higher in sedentary individuals. There was an increase in both CK and LDH, which indicated increased muscle membrane permeability as well as muscle stress due to exertion. Furthermore, IL-6 and CRP were elevated following fatigue, indicative of an acute inflammatory and immune-metabolic response. The greater biomarker responses seen in sedentary subjects indicate that a higher degree of exposure to muscle stress, activation of inflammatory pathways and a delayed response to training could occur. Active participants, on the other hand, appeared to have a more regulated physiological response, which may have chronic adaptations such as enhanced muscle membrane stability, antioxidant activity, and more efficient recovery.

**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.

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Table S1: The Shapiro–Wilk W-statistics and p-values.

**Tests of Normality**

| | Participant Type | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | *df* | Sig. | Statistic | *df* | Sig. |
| pre fatigue CK (U/L) | Active - Intervention | .218 | 9 | .200* | .894 | 9 | .220 |
| | Active - Control | .275 | 9 | .049 | .908 | 9 | .303 |
| | Sedentary-Intervention | .334 | 9 | .005 | .831 | 9 | .046 |
| | Sedentary-Control | .169 | 9 | .200* | .931 | 9 | .495 |
| post fatigue CK (U/L) | Active - Intervention | .166 | 9 | .200* | .927 | 9 | .452 |
| | Active - Control | .239 | 9 | .145 | .918 | 9 | .377 |
| | Sedentary-Intervention | .225 | 9 | .200* | .928 | 9 | .462 |
| | Sedentary-Control | .213 | 9 | .200* | .938 | 9 | .561 |
| pre fatigue LD | Active - Intervention | .163 | 9 | .200* | .909 | 9 | .306 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| H (U/L) | Active - Control | .153 | 9 | .200* | .969 | 9 | .889 |
| | Sedentary-Intervention | .182 | 9 | .200* | .917 | 9 | .371 |
| | Sedentary-Control | .145 | 9 | .200* | .980 | 9 | .963 |
| post fatigue LDH (U/L) | Active - Intervention | .147 | 9 | .200* | .936 | 9 | .542 |
| | Active - Control | .193 | 9 | .200* | .913 | 9 | .338 |
| | Sedentary-Intervention | .201 | 9 | .200* | .954 | 9 | .733 |
| | Sedentary-Control | .183 | 9 | .200* | .939 | 9 | .573 |
| pre fatigue IL-6 (pg/ml) | Active - Intervention | .200 | 9 | .200* | .908 | 9 | .303 |
| | Active - Control | .167 | 9 | .200* | .955 | 9 | .740 |
| | Sedentary-Intervention | .197 | 9 | .200* | .883 | 9 | .169 |
| | Sedentary-Control | .160 | 9 | .200* | .970 | 9 | .895 |
| post fatigue IL-6 (pg/ml) | Active - Intervention | .153 | 9 | .200* | .957 | 9 | .766 |
| | Active - Control | .116 | 9 | .200* | .962 | 9 | .822 |
| | Sedentary-Intervention | .237 | 9 | .152 | .876 | 9 | .143 |
| | Sedentary-Control | .124 | 9 | .200* | .983 | 9 | .977 |
| pre fatigue CRP (mg/L) | Active - Intervention | .222 | 9 | .200* | .892 | 9 | .211 |
| | Active - Control | .314 | 9 | .011 | .798 | 9 | .020 |
| | Sedentary-Intervention | .232 | 9 | .178 | .881 | 9 | .160 |
| | Sedentary-Control | .165 | 9 | .200* | .965 | 9 | .853 |
| post fatigue CRP (mg/L) | Active - Intervention | .170 | 9 | .200* | .959 | 9 | .787 |
| | Active - Control | .232 | 9 | .178 | .910 | 9 | .318 |
| | Sedentary-Intervention | .251 | 9 | .107 | .928 | 9 | .467 |
| | Sedentary-Control | .170 | 9 | .200* | .956 | 9 | .757 |

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

a. Lilliefors Significance Correction.

Normality test for the study biomarkers. Normality was determined within each group of participants (n = 9) for pre- and post-fatigue creatine kinase (CK), lactate dehydrogenase (LDH), interleukin-6 (IL-6), and C-reactive protein (CRP). Lower limit of the true significance; df, degrees of freedom; Sig., significance (*p*-value); U/L, units per litre; pg/mL, picograms per millilitre; mg/dL, milligrams per decilitre.

Table S2: The baseline CRP and CK values of the replaced participants.

| Participant | Intended group | Baseline CK (U/L) | Baseline CRP (mg/L) | Reason for replacement |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| P1 | Sedentary group | 252 | 18 | Elevated CK and CRP above predefined reference range |
| P2 | Sedentary group | 220 | 12 | Elevated CK and CRP above predefined reference range |
| P3 | Active group | 188 | 8 | Elevated CK and CRP above predefined reference range |
| P4 | Active group | 426 | 24 | Elevated CK and CRP above predefined reference range |
| P5 | Active group | 333 | 16 | Elevated CK and CRP above predefined reference range |

Baseline creatine kinase (CK) and C-reactive protein (CRP) values of the replaced participants. Five participants were excluded before analysis due to the high baseline level of CK and CRP above the pre-defined reference range. Values reported are those by intended group allocation. Participant (P); Units per liter (U/L); milligrams per deciliter (mg/dL); CK, creatine kinase; CRP, C-reactive protein.

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