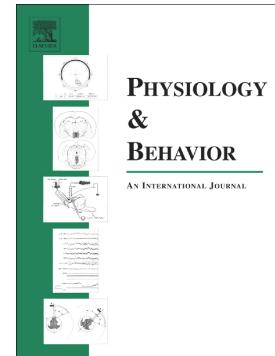


Accepted Manuscript

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PII: S0031-9384(18)30849-7
DOI: doi:[10.1016/j.physbeh.2018.10.002](https://doi.org/10.1016/j.physbeh.2018.10.002)
Reference: PHB 12331
To appear in: *Physiology & Behavior*
Received date: 26 November 2017
Revised date: 27 September 2018
Accepted date: 2 October 2018

Please cite this article as: Jacobo Á. Rubio-Arias, Vicente Ávila-Gandía, Javier López Román, Fulgencio Soto-Méndez, Pedro E. Alcaraz, Domingo J. Ramos-Campo, Muscle damage and inflammation biomarkers after two ultra-endurance mountain races of different distances: 54 km vs 111 km. *Phb* (2018), doi:[10.1016/j.physbeh.2018.10.002](https://doi.org/10.1016/j.physbeh.2018.10.002)

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Muscle damage and inflammation biomarkers after two ultra-endurance mountain races of different distances: 54km vs 111km

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ABSTRACT

The aims of this study were 1) to describe the effects of a 54km and 111km ultra-endurance mountain race on the biomarkers of muscle damage and inflammation, 2) to

compare the effects between the two races regarding the biomarkers of muscle damage and inflammation.

Sixteen ultra-endurance amateur runners volunteered to participate in this study. Ten runners completed a 54km race (Group 1; age: 27.0 ± 5.7 ; height: 179.5 ± 5.8 cm; and body mass: 77.3 ± 10.7 Kg) and six completed a 111km race (Group 2; age: 30.5 ± 8.0 ; height: 179.4 ± 5.5 cm; and body mass: 76.2 ± 9.4 Kg). Blood samples were taken at five different points during the investigation, 24hrs before the race, immediately post-race, and again at 24, 48, and 72hrs after the race.

There were increases in leukocyte (Group 1: $p = < 0.001$, ES = 2.8; Group 2: $p = 0.001$, ES = 3.5) and platelet concentrations (Group 1: $p = < 0.001$, ES = 2.3; Group 2: $p = 0.02$, ES = 1.7) post-races. Significant inter-race differences were also observed in leukocyte at 72hrs (Group 1: 5.5 ± 0.9 , Group 2: 4.2 ± 0.9 , $p = 0.012$, ES = 1.5). Erythrocytes, hematocrit and hemoglobin concentration decreased after 54km and 111km races at 24, 48 and 72hrs ($p = < 0.001$, ES = 2.0 – 3.18). Serum uric acid concentration increased after the 54km race (pre = 4.9 ± 1.2 – post = 7.3 ± 1.0 mg/dl; $p = < 0.001$, ES = 2.4), and also the 111km race (pre = 5.3 ± 0.9 – post = 6.7 ± 0.8 mg/dl; $p < 0.008$, ES = 2.2). GPT, GOT and LDH had changed by the end of the races ($p < 0.05$) and differences between the groups were observed in GOT post-race ($p = 0.008$, ES = 1.7) 24hrs ($p = 0.004$, ES = 1.8), 48hrs ($p = 0.007$, ES = 1.6), and 72hrs ($p = 0.02$, ES = 1.4) and also in LDH at 24, 48, 72hrs. Serum creatinine decreased post-race in Group 1 (pre = 1.1 ± 0.1 – post = 1.4 ± 0.2 mg/dl; $p = 0.001$, ES = 1.5) and Group 2 (pre = 1.2 ± 0.1 , post = 1.5 ± 0.2 ; $p = 0.002$, ES = 3.3) along with CK and myoglobin. In addition, values did not return to baseline levels after 72hrs in Group 2 for C-reactive protein, myoglobin, and CK. Differences between the races were also observed post-race in Troponin I (Group 1 = 0.06 ± 0.05 , 111km = 0.02 ± 0.01 μ g/l, $p = 0.047$, ES = 1.1) and C-reactive protein post-race (Group 2 = 2.5 ± 1.6 , 111km = 18.2 ± 6.4 mg/l, $p = < 0.001$, ES = 4.4) at 24 and 48hrs.

The athletes had increased concentrations of markers associated with damage, inflammation, muscle injury and cardiac damage after the races. Furthermore, athletes who completed the greater distance (111km) had higher concentrations of the markers associated with muscle damage and muscle inflammation which remained changed for a period of 72 hours. However, the participants of the 'shorter race' showed higher values associated with cardiac damage. Consequently, athletes who take part in these kinds of races should wait at least 72 hours before training with high load.

Key words: Hematocrit and hemoglobin, CK, LDH, C-Reactive Protein, Transaminases.

1. Introduction

Regular physical activity is beneficial for cardiovascular health, enhances parts of the innate immune system, and reduces the risk of infection [1, 2]. Aerobic training has been promoted as healthy physical activity that provides positive effects on the cardiovascular system. Therefore, in recent times, endurance and ultra-endurance races have begun to become very popular and the participation of athletes has increased [3]. These ultra-endurance races or ultra-marathon races can be classified by distance, elevation and the environment, there are different types of ultra-endurance races and ultra-marathon mountain races. The athletes can perform a mountain race over great elevation, alternating walking and running according to distance and modality of the event [4].

Several studies have investigated the acute changes in muscle injury related biomarkers along with inflammation and muscle damage after ultra-endurance races [5, 6]. The exercise-induced muscle damage is accompanied by the presence of a systemic inflammatory response, which protects the athlete's body from further damage [7, 8]. Several studies have used blood marks to evaluate the inflammation damage and cardiac / muscle injury after participation in ultra-endurance races. The ultra-endurance races can induce hemolysis (loss of erythrocytes, decreases in hematocrit) [6] or a process of inflammation induced by exercise stress [9]. Furthermore, exercise-induced inflammation appears to modulate homeostasis of the bone marrow, leading to increased leukocyte turnover and decreased erythroid compartment[10]. The creatine kinase (CK) concentration, as well as inflammatory and infection markers such as C-Reactive Protein (CRP) [11], liver injury lactate dehydrogenase (LDH) or cardiac pathology markers (i.e. cardiac troponin I) may be high for several days after strenuous ultra-endurance exercise [8]. Therefore, well established muscle damage after ultra-endurance exercise, promotes higher values of plasma enzymes such as myoglobin or CK concentrations and liver

damage indicators such LDH.

In addition, these biomarkers can be elevated for a few days and can impair athlete's health due to the appearance of rhabdomyolysis [12]. Moreover, there are few studies that have analysed the modulation of biochemical markers during the hours following the end of the race [13]. Neubauer, Konig [7] analysed the response of the inflammatory markers after an Ironman triathlon race, and concluded that muscle inflammation markers returned to baseline levels after 5-19 days. Marklund, Mattsson [14] concluded that although ultra-endurance races were performed with low-intensity, these races were very prolonged and induced an extensive infiltration of inflammatory cells into the skeletal muscles of well-trained athletes even 28hrs after exercise cessation. Previously published studies have been limited to analysing just the acute effect of different ultra-endurance races on muscle damage biomarkers and the inflammatory response. However, few studies have analysed the evolution and recovery of these parameters over the subsequent days.

Running over longer distances could cause minor muscle damage [6], but the distances which also include higher elevations might lead to increased levels of induced muscle damage[6, 15]. In some instances, ultra-endurance races are considered extreme exercise events because induce cardiac dysfunctions [16] increase inflammatory markers, muscle and myocardial damage [11, 13], mainly, with over very long-distance races [17]. For example, Waskiewicz, Klapcinska [17] found that CK increased 3.1, 17.1 and 70 times after the first 42km, 12 and 24hrs respectively, during a 168km ultra-endurance race. Therefore, the distance covered by the athletes seems to influence and affect the muscle damage and inflammation biomarkers, as well as the recovery process. However, there are no studies that have compared the acute biochemical effects of a "short" endurance mountain race (~50km) vs. a long ultra-endurance mountain race (~100km). Thus, the study provides an important opportunity to advance the understanding of the acute effects

according to the two different distances of ultra-endurance races on muscle damage and inflammation biomarkers as well as recovery time. Furthermore, the data could help to understand the importance of recovery before returning to training to optimize the training load. Therefore, the aims of this study were: 1) to determine the alterations in muscle damage and inflammation biomarkers of amateur runners immediately after a race, and again 24hrs, 48hrs and 72hrs after finishing either a 54km or 111km ultra-endurance mountain race; 2) to compare the differences in the acute response in serum concentration of muscle damage and inflammatory response between the two distances of the ultra-endurance mountain race events (54 vs 111km)

2. Material and Methods

2.1 Design

A comparative description (cross-sectional study) was conducted to analyse the effects of two different ultra-marathons (54km and 111km) on muscle damage, inflammation and muscle injury markers.

2.2 Participants

Sixteen ultra-endurance male runners volunteered to participate in this study (age: 30.5 ± 8.0 years; height: 179.4 ± 5.5 cm; body mass: 76.2 ± 9.4 Kg). Participants enrolled in a short-distance (Group 1) or long-distance (Group 2) race depending on their previous experience. Finally, ten runners enrolled in the 54km race (short-distance; Age: 27.0 ± 5.7 years; Height: 179.5 ± 5.8 cm; Body mass: 77.3 ± 10.7 Kg; BMI: 23.92 ± 2.4 kg/m²; Body fat: 11.9 ± 3.2 %; Fat free mass: 64.5 ± 7.6 kg) and six completed the 111km race (long-distance; age: 30.5 ± 8.0 years; height: 179.4 ± 5.5 cm; body mass: 76.2 ± 9.4 Kg; BMI: 22.98 ± 2.0 kg/m²; Body fat: 11.4 ± 2.0 %; Fat free mass: 60.9 ± 5.9 kg). All participants were amateur athletes who had participated in ultra-endurance events for at least four

years. The participants were recruited by phone according to the following inclusion criteria: aged 18-40 years old; at least four years of endurance training experience, and who exercised five times per week; participation in the previous edition of the Castle of Cartagena race with a race time of <7.5hrs; and no musculoskeletal disorders in the previous six months before the study. Prior to the race, the experimental procedures and risk and discomforts associated with the study were explained to all participants and they provided a signed informed consent. The study was approved by the University's Institutional Review Board and was in accordance with the Declaration of Helsinki. Lastly, the runners completed a questionnaire on training status according to Smith et al. [18]

2.3 Methodology

The data collection was performed on five different days, hematological tests were conducted on the athletes in a laboratory setting, blood samples were taken 24hrs before the race (pre-race), immediately post-race, and at 24, 48 and 72 hrs after the race. The blood sample (6.5 ml) was withdrawn from an antecubital vein using a sterile technique to analyse hematological variables. Lastly, 10 participants took part in a 54km race, with 2726m of ascent and 2665m of descent, and 6 participants completed a 111km race that included 4474m of ascent and 4420m of descent. Furthermore, on the first visit, height (cm) and body mass (kg) were assessed with a digital stadiometer Seca 700 (Seca® Ltd, Germany). Additionally, a body composition analysis using the direct Segmental Multi-Frequency Bioelectrical Impedance Analysis Method (model type: BC-601, Tanita, Japan) was determined following the manufacturer's guidelines.

The extraction of blood was performed while the subject was seated. Three millilitres of the sample were placed into a tube containing EDTA to determine hemoglobin concentration and Hematocrit, erythrocytes, white blood cell and platelet counts using a hematology analyser (System XS-1000i. Kobe. Kansai. Japan). The remaining 3.5ml

sample was allowed to clot and was centrifuged for 10mins at 5000 x g to separate out the serum. Blood markers of muscle damage and biochemical parameters (leukocytes, platelets, erythrocytes, hemoglobin, hematocrit, Mean Corpuscular Volume; MCV, Mean Corpuscular Hemoglobin; MCH, Uric Acid, LDH, Creatinine, CK and myoglobin) and hepatic enzymes (GOT, GPT) were then analysed by automated chemical analysis (ILAB 600 Chemistry Analyzer of Instrumentation Laboratory, Holliston, MA, USA) using the serum.

2.4 Statistical Analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS, version 21, SPSS Inc, Chicago, IL, USA). Standard descriptive statistics were performed (mean and standard deviations). For the inferential analysis, a Shapiro-Wilk Test was performed to establish the normality of sampling distribution. A General Lineal Model (repeated measures ANOVA) was used to investigate differences in hematological variables between, pre-race, at the finish, 24hrs, 48hrs and 72hrs post-race and inter-race.

All data is reported as mean (\pm SD) with the statistical significance set at $p \leq 0.05$. The effect size (ES) was calculated using Cohen's guidelines [19]: $ES = \frac{M_{pre} - M_{post}}{SD_{pre}} \left(1 - \frac{3}{4n - 5}\right)$ for

related samples and $ES = \frac{(M_{g1} - M_{g2})}{SD_{pool}}$ for independent samples, where M is the mean (M_{pre} =

mean before the exercise program; M_{post} = mean after the exercise program; M_{g1} = mean of the first group after the exercise program and M_{g2} = mean of second group after the

exercise program; $SD_{pool} = \sqrt{\frac{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}{n_1 + n_2 - 2}}$, is the standard deviation, and n is the

sample size. The effect size (ES, 95% confidence limit) in the selected variables was calculated using the SD. Threshold values for Cohen ES statistics were >0.2 (small), >0.6 (moderate), >1.2 (large), and >2.0 (very large)[20]

3. Results

No significant differences were observed between the 111km race runners and the 54km race runners in anthropometrics data (Height: $p = 0.576$, ES = 0.34 [-0.83 - 1.5]; Body mass: $p = 0.419$, ES = 0.49 [-0.68 - 1.7]; Body fat: $p = 0.770$, ES = 0.189 [-1.0 - 1.3]; Fat free mass: $p = 0.410$, ES = 0.506 [-0.7 - 1.7]). All runners completed the races, with the 54km race runners averaging 6hrs 41min and 43s. On the other hand, the 111km race runners completed the race in an average of 18hrs 16min and 38s (Figure 1).

---Insert figure 1---

Complete Blood Count basic measures

Significant increases in leukocyte concentration post-race were observed (Table 1). Exclusively, there were significant inter-race differences (favours 111km, Figure 2) at 72hrs in leukocyte concentration ($p = 0.012$, ES = 1.5 [2.6 - 0.3]) (Figure 2). Immediately after the races, there was an increase in platelet concentrates (Group 1: $p = <0.001$, ES = 2.3 [1.1 - 3.5] Group 2: $p = 0.02$, ES = 1.7 [0.2 - 3.1]). Nevertheless, in Group 2, the platelet concentrates had decreased to baseline levels after 24hrs ($p = 0.043$; ES = 1.1 [0.03 - 2.1]) and 48hrs ($p = 0.003$, ES = 2.2 [0.6 - 3.6]). Erythrocytes, hemoglobin and hematocrit concentration decreased in Group 1 and Group 2 at 24, 48 and 72hrs (Table 1).

---Insert table 1 and Figure 2---

Muscle damage, inflammation and muscle injury markers

Serum uric acid concentration was higher after race in Group 1 ($p < 0.001$, ES = 2.4 [1.1 - 3.6]) and Group 2 ($p = 0.008$, ES = 2.2 [0.5 - 3.9]). Moreover, GPT and GOT had increased post-race (Table 2). In Group 1 and Group 2, increases of GPT remained elevated after 72hrs. Besides, GOT levels increased during race in Group 1, but decreased

to basal levels in the following 24 and 72hrs in group 2, although in Group 2, the increase in GOT levels remained unchanged even after 72hrs. There were differences in GOT values between races (favours Group 2, Figure 3), post-race ($p = 0.008$, ES = 1.7 [0.4 – 3.0]) as well as at 24hrs ($p = 0.004$, ES = 1.7 [0.5 – 2.9]), 48hrs ($p = 0.007$, ES = 1.6 [0.4 – 2.8]) and 72hrs ($p = 0.003$, ES = 1.8 [0.5 – 3.0]). Moreover, an increase in LDH concentration were observed post-races (Group 1 and Group 2), and by 72hrs an increase as compared to baseline was also shown in the Group 2. Moreover, higher values of LDH concentration were observed in Group 2 than in Group 1 at 24hrs ($p = 0.02$, ES = 1.3 [0.2 – 2.9]), 48hrs ($p = 0.006$, ES = 1.7 [0.5 – 2.8]) and 72hrs ($p = 0.006$, ES = 1.7 [0.5 – 2.9]) (Figure 3).

---Insert Table 2 and Figure 3---

Serum creatinine increased post-race in Group 1 ($p = 0.001$, ES = 1.4 [0.5 – 2.3]) and Group 2 ($p = 0.002$, ES = 3.3 [1.0 – 5.7]) (Table 3) although in Group 2, it was reduced from 48 to 72hrs. In addition, serum markers of muscle damage (CK, myoglobin, troponin I, C-Reactive Protein) increased at the end of the race after and 24, 48 and 72hrs (Table 3). Furthermore, greater increases were found in muscle damage in participants who completed the 111km (Group 2) in CK post-race ($p = 0.003$, ES = 2.0 [0.7 – 3.3]) and at 24hrs ($p = 0.053$, ES = 1.1 [0.0 – 2.2]), in C-Reactive Protein after race ($p < 0.001$, ES = 4.2 [2.2 – 6.0]), at 24hrs ($p = 0.033$, ES = 1.2 [0.1 – 2.3]) and 48 hrs ($p = 0.022$, ES = 1.3 [0.2 – 2.4]) after race. However, higher values in participants who completed the 54km (Group 1) in Troponin I after race ($p = 0.047$, ES = 1.1 [0.0 – 2.2]) (Figure 4).

---Insert table 3 and Figure 4---

4. Discussion

The aims of this study were: a) To investigate hematological variations-related changes in participants in a 54km or 111km ultra-endurance mountain running event b) to determine what type of run/distance produced greater changes on hematological variations in participants. The main findings of this study were: a) running an ultra-endurance mountain race resulted in highly significant increases in muscle damage and inflammation markers; b), The baseline values of muscle damage remained significantly elevated after 72 hours post-race, especially in the 111km runners, with statistically significant differences being observed between groups.

Complete Blood Count basic measures

After analysing the red blood cell series, erythrocytes, hemoglobin and hematocrit, we observed that hemoglobin and hematocrit values trended toward below normal in the pre-competition period in both shorter and longer-distance runners. This could be because they are well-trained athletes in endurance sports [5]. The trend in both tests right after competing was of maintenance compared to baseline values, by physiological hemoconcentration caused by decreased plasma volume [21] and dehydration [22].

The ultra-marathon mountain races (54km and 111km) led to hemolysis in the participants with a loss of erythrocytes, hemoglobin and hematocrit at 24hrs, 48hrs and 72hrs after finishing the race. These results agree with previous works, Yusof et al. [23] observed a loss erythrocyte (6 - 9%) in male runner after 216km. Robach et al. [24] found that after a 166km ultra-marathon mountain race, hemolysis occurred but it was mainly a response after competition due to hemodilution that was maintained for several days depending on the duration of the race [25]. This blood cell lysis could be due to factors such as

mechanical haemolytic anemia involving microtraumatism from running impact [11, 26]. In addition, one of the hemolytic functions is to maintain higher temperatures. However, the longer this high temperature is maintained, the more muscle damage is generated. [22, 27].

In addition, after race we observed an increase of leukocytes (Group 1 = 176.3%, ES: 2.8; Group 2 = 159.2%, ES: 3.5) and platelets (Group 1 = 24.1%, ES: 2.3; Group 2 = 23.9, ES: 1.7) in both races. Furthermore, a decrease of platelets from baseline was found (Group 1 = 24.1%, ES: 2.3; Group 2 = 23.9, ES: 1.7) after race and at 24hrs (Group 1 = 9.5%, ES: 0.5; Group 2 = 6.1%, ES: 1.1). These results agree with a previous work carried out by Zakovska, Knechtle [9] who concluded that after 100km ultra-marathon under cold conditions that the leukocytes increased post-race by 185.3%. Shin and Lee [28] conclude that prolonged endurance ultra-endurance running was associated with perturbation in leukocyte subsets. A possible explanation for this might be that the increased leucocytes and platelets seem to be due to the stress on the immune system and acute inflammation of the organism caused by tissue injury [9, 28].

Muscle damage, inflammation and muscle injury markers

The results of this study showed an increase of uric acid concentration after races in 54km (49%, ES: 2.4) and in 111km (26.4%, ES: 2.2). These results were in agreement with Waring, Convery [29], which showed that an increase of the concentration of uric acid increased serum antioxidant capacity and reduced exercise-induced oxidative stress in a young healthy population. On the other hand, there were increases in GPT (Group 1: 24.8%, ES: 1.1; Group 2: 120%, ES: 3.2) and GOT (Group 1: 108%, ES: 2.0; Group 2: 672%, ES: 2.1) activity and these values did not return to baseline level after 72hrs. Previous studies have pointed out that an increase in CK, myoglobin and GOT after an

ultra-endurance race indicates exertional rhabdomyolysis and skeletal muscle leakage, while GPT is a hepatic injury marker [12]. Therefore, the high production of GOT could lead hepatic injuries, mainly in long distances races.

The level of CK increased after both races (Group 1: 150%, ES: 1.5; Group 2: 5059%, ES: 2.0) with differences between races (ES: 2.0). These results are consistent with data obtained from a 200km ultra-marathon, where mean CK values increased 19-fold at 100km, and 90-fold at the end of the race as compared to pre-race values [30]. This difference has been previously described by Waskiewicz, Klapcinska [17], who found that serum activity of CK increased 3.1, 17.1 and 70 times after the first 42km, 12hrs and at the end of a 24hrs (168 km) ultra-endurance race, respectively. In our study, maximum levels of CK were reached immediately after the races, and decreased over the next 72hrs, without reaching basal values in the 111km race. This result corroborates the ideas of previous studies where 5 or more days are needed for CK to return to basal values after an ultra-endurance race (100-166km) [31, 32]. The differences in damage and inflammation biomarkers between groups may be explained by the greater elevation gains and losses in the 111km race [3], the greater number of eccentric contractions, and the duration of the race. Thus, the increase of CK activity after the race depends on the type and duration of the exercise [33]. Thus, this result may be explained by the fact that in mountain ultra-endurance races, the eccentric component and the continuous foot strikes gain importance in increasing the values of CK [34].

In the same way, increases in myoglobin (Group 1: 6671%, ES: 1.7; Group 2: 8470%, ES: 2.2) and LDH (Group 1: 80%, ES: 4.4; Group 2: 124%, ES: 5.8) were observed after both races. These markers decreased from 48 to 72hrs after the races, but still remained significantly above pre-exercise values, except for LDH in the 54km ultra-marathon. These results are consistent with data obtained by Millet, Tomazin [31] who pointed out that

more than 5 to 9 days were needed to return to pre-exercise values of LDH and myoglobin, respectively. A possible explanation for this might be that ultra-endurance exercise induces stress, which is associated with an activation of the sympathetic system and the activation of inflammation and muscle damage biomarkers [35]. All these results provide us with information on the effect of ultra-endurance events on muscle damage, mainly when the distance is greater.

Several studies have shown the acute effect of biomarkers associated with cardiac injury, which showed significant increase after long-term activities with an eccentric component such as ultra-marathon mountain racing. [11, 36]. Interestingly, this study has been unable to demonstrate an increase of Troponine I only in 54km runners, post-race (460%, ES: 1.4), which was higher than in 111km runners (ES: 1.1). Thus, these results are likely to be related to intensity and length of time of the races. These results suggest that the greater distances completed could increase cardiovascular diseases, as an elevated concentration of these two compounds are biomarkers of cardiac injury [8]. Additionally, increases were observed after the race in 54km runners at 24 and 48hrs and 111km athletes, at 24, 48 and 72hrs. Moreover, greater increases in C-reactive protein were observed in athletes that ran the 111km race, who showed a great concentration immediately after the race (ES: 4.1) at 24hrs (ES: 1.2) and 48hrs (ES: 1.3). These findings suggest that ultra-endurance leads to inflammatory reaction [6] and the distance can be associated with the severity of inflammation.

The principal limitation of the present study was the low number of participants evaluated and inter-individual variability in response to the competitions of ultra-endurance mountain races. Additionally, several factors in the characteristics of the participants could be a bias factor of the results such as the age of the participants [37] or the body composition [38]. On the other hand, the present research offers an important contribution

to understand that ultra-endurance races can lead to muscle damage, inflammation and other pathologies that can put the runner's health at risk. The findings of the current study do support the recent comprehensive review [6] which concluded that ultra-endurance events could be harmful to health but the effects on biomarkers which are associated with damage, inflammation, injury muscle and cardiac are reversible in a few days. In addition, an increase in the race distance (volume: km) seems to lead to more muscle damage and muscle inflammation injury.

However, the intensity (running speed) of the race could have a greater effect on markers of cardiac damage. Consequently, the authors recommend that before executing an ultra-endurance mountain race, a training program under expert supervision should be carried out, as well as an exhaustive medical examination. Moreover, after ultra-endurance mountain races, do not perform high training loads until at least 72 hours after the race.

5. Conclusions

From the current data it is possible to conclude that the athletes who completed ultra-endurance mountain races had increased concentrations of markers associated with damage, inflammation, muscle injury and cardiac damage. Furthermore, athletes who completed a 111km had higher concentrations the biomarker associated with muscle damage and inflammatory processes and the 54km participants had higher concentrations of the markers associate with cardiac damage. The research has also shown that in 111km runners, the biomarkers analysed for damage, inflammation, muscle injury did not return to baseline values after 72hours (Erythrocytes, Hemoglobin, Hematocrit, GPT, CK, Myoglobin, C-reactive protein). However, in 54km runners the markers analysed returned to baseline values between 24 and 48 hours. Following the evidence shown in a recent review, it is important to carry out training specific to the competition [6]. Therefore, high

load training is not recommended until 48 hours after for ~50km and 72 hours for ~100km runners.

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Figure1. Elevation profile of the races.

Figures 2. Differences between races in effect size (IC 95%) on levels of white and red blood cells.
ES: Effect size. Differences between races: §p<0.05.

Figures 3. Differences between races in effect size (IC 95%) on biomarkers associated with muscle damage and inflammation cells.
ES: Effect size. Differences between races: §p<0.05; §§p<0.01.

Figures 4. Differences between races in effect size (IC 95%) on levels of biomarkers associated with muscle injury.
ES: Effect size. Differences between races: §p<0.05; §§p<0.01; §§§p<0.01.

Dist anc e	Pre- race		Post-race				24 hours				48 hours				72 hours				
	Rac es	M ea n	± S D	Me an	± S D	Δ(%)	ES	M ea n	± S D	Δ(%)	ES	M ea n	± S D	Δ(%)	ES	M ea n	± S D	Δ(%)	ES
Leuko cytes (10 ³ / mmc)	54 Km	5.9	1.3	16.3	4.3	17.6	2.8**	5.50	1.0	-6.8	0.5	5.20	1.0	-11.9	1.0*	5.59	0.9	-6.8	0.6
	111 km	4.9	0.5	12.7	2.6	15.9	3.5**	5.00	0.7	2.0	0.2	4.46	0.6	-10.2	1.0	4.26	0.6	-14.3	2.2**;
Platele ts (10 ³ / mmc)	54 Km	22.1	2.9	27.4	2.8	24.1	2.3**	20.3	5.5	-9.5	0.5	20.5	5.9	-7.2	0.3	22.0	46.9	1.0	0.1
	111 km	20.1	3.8	24.9	4.0	23.9	1.7*	18.9	4.0	-6.1	1.1*	18.4	3.9	-8.6	2.1**	19.0	42.5	-2.8	0.5
Erythr ocytes (x 10 ⁶ /μl)	54 Km	4.9	0.5	5.0	0.4	2.0	0.1	4.7	0.3	-4.1	1.3*	4.7	0.4	-4.1	1.3**	4.8	0.4	-2.0	1.1**
	111 km	5.0	0.3	5.1	0.3	2.0	0.0	4.6	0.2	-8.0	2.0*	4.5	0.2	-10.0	2.5**	4.7	0.3	-8.0	2.9***
Haem oglobi n (g/dl)	54 Km	14.8	1.3	14.9	1.0	0.7	0.1	14.1	0.9	-4.7	1.3*	14.2	1.1	-4.1	1.1**	14.50	1.1	-2.0	0.9**
	111 km	15.0	0.7	14.8	0.5	0.5	0.3	13.7	0.6	-8.7	2.1*	13.5	0.4	-10.0	2.5**	13.90	0.5	-7.3	2.6*
Haema tocrit (%)	54 Km	43.1	3.7	43.9	3.2	1.9	0.6	41.3	2.5	-4.2	1.2*	41.4	3.1	-3.9	0.2	42.1	3.2	-2.3	1.0**
	111 km	43.4	0.6	43.3	1.0	-0.2	0.0	40.0	1.6	-7.8	2.1*	39.4	0.9	-9.2	3.8**	40.4	1.3	-6.9	2.1**
MVC (fl)	54 Km	87.3	2.6	88.5	2.8	1.4	1.0	88.1	2.7	0.9	1.6	87.70	2.0	0.5	0.3	87.50	2.60	0.2	0.3
	111 km	86.4	4.8	85.3	4.4	-1.3	0.0	87.0	4.6	0.7	0.2	87.20	4.7	0.9	0.3	87.00	4.70	0.7	0.2
MCH (pg)	54 Km	30.0	1.1	30.0	1.2	0.0	0.0	30.1	1.1	0.3	0.4	30.1	1.1	0.3	0.3	30.1	1.0	0.3	0.5

111	29	2.	29.	1.	-	1.	29	2.	0.	0.	29.	2.	0.	0.	29.	2.	0.	0.2
km	.9	0	1	9	2.	7	.9	0	0	1	9	1	0	0	9	0	0	

Table 1. **Differences in levels of white and red blood cells.**

MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; ES: effect size.

Differences between before and immediately after and by 24, 48 and 72 h: * p<0.05 vs basal; ** p<0.01 vs basal; *** p<0.001 vs basal.

Differences between race: §p<0.05

Table 2. Differences in levels of biomarkers associated with muscle damage and inflammation.

Distance	Pre-race		Post-race				24 hours				48 hours				72 hours				
	Races	Mean	±SD	Mean	±SD	Δ(%)	ES	Mean	±SD	Δ(%)	ES	Mean	±SD	Δ(%)	ES	Mean	±SD	Δ(%)	ES
Uric Acid (mg/dl)	54 Km	4.9	1.2	7.3	1.0	49.0	2.4***	5.3	1.0	8.2	0.9*	5.2	1.1	6.1	0.6	5.1	1.1	4.1	0.5
	111 km	5.3	0.9	6.7	0.8	26.4	2.2**	5.4	0.7	1.9	0.2	4.8	0.7	-9.4	1.0	5.0	0.8	-5.7	0.7
GOT (U/l)	54 Km	34.7	32.7	72.0	41.6	107.5	2.0***	62.8	21.1	81.0	0.9*	43.1	11.6	24.2	0.3	34.5	6.9	-0.6	0.0
	111 km	21.3	5.1	159.2	66.8	672.2	2.1***;§	109.3	34.2	413.1	2.7***;§	74.3	27.7	248.8	2.1***;§	54.7	19.0	156.8	1.9***;§
GPT (U/l)	54 Km	26.6	15.7	33.2	20.8	24.8	1.1**	38.4	15.9	44.4	1.2**	36.8	13.9	38.3	1.0*	34.7	12.2	30.5	0.7*
	111 km	17.3	5.3	38.0	10.5	119.7	3.2**	44.2	10.7	155.5	3.9***	42.8	11.7	147.4	3.4***	40.0	12.1	131.2	2.9***
LDH (U/l)	54 Km	406.9	135.0	731.3	161.1	79.7	4.4***	449.8	66.9	10.5	0.3	430.9	72.1	5.9	0.1	409.9	59.8	0.7	0.0
	111 km	335.1	35.2	751.0	57.2	124.1	5.8***	588.7	90.7	75.7	2.6***;§	551.1	72.7	64.5	2.5***;§	509.1	56.7	51.9	2.2***;§

GOT: glutamicoxaloacetic acid transaminase; GPT: glutamic-pyruvic acid transaminase; LDH: lactic dehydrogenase.

Differences between before and immediately after and by 24, 48 and 72 h: * p<0.05 vs basal; ** p<0.01 vs basal; *** p<0.001 vs basal.

Differences between race: § p<0.05; §§ p<0.01.

Table 3. Differences in levels of biomarkers associated with muscle injury.

	Dist- ance			Pre- race				Post-race				24 hours				48 hours				72 hours			
	Rac- es	M ea- n	±S D	Me- an	±S D	Δ(%)	ES	Me- an	±S D	Δ(%)	ES	Me- an	±S D	Δ(%)	ES	Me- an	±S D	Δ(%)	ES	M ea- n	±S D	Δ(%)	ES
Creat- inine (mg/d l)	54 Km	1. 06	0.1 0	1.4 1	0.2 5	27. 3	1.4* *	1.0 4	0.0 9	- 9.1	0.3	1.0 3	0.1 0	- 9.1	0.5	1.0 09	0.1 07	- 0	0.3	1. 0	0. 07	0. 0	0. 3
	111 km	1. 15	0.0 9	1.5 0	0.1 2	25. 0	3.3* *	1.0 9	0.1 4	- 8.3	0.9	1.0 0	0.0 7	- 16. 7	4.7* **	1. 10	0. 07	- 8. 3	1. 1*	1. 10	0. 07	- 8. 3	1. 1*
CK(UI/l)	54 Km	88 6.	21 87. 9	22 13. 8	23 54. 5	14 9.7	1.5* **	10 14. 9	73 2.6	14. 5	0.1	49 5.8	35 8.0	- 44. 1	0.2	30 9. 4	19 1. 6	- 65. 1	0. 3	30 9. 4	19 1. 6	- 65. 1	0. 3
	111 km	17 4.	19 7.4	89 76. 0	43 27. 1	50 58. 6	2.0*; §§	21 32. 5	13 99. 6	11 25. 6	1.6*; §	12 77. 7	13 68. 2	63 4.3	0.9	60 4. 2	87 8. 3	24 7. 2	0. 6	60 4. 2	87 8. 3	24 7. 2	0. 6
Myog- lobin (µg/l)	54 Km	14 .0	4.7	94 8.0	53 8.0	66 71. 4	1.7* *	29. 8	20. 1	11 2.9	0.7	27. 3	18. 6	95. 0	0.6	29 .2	15 .0	10 8. 6	0. 9*	29 .2	15 .0	10 8. 6	0. 9*
	111 km	17 .8	8.6	15 25. 4	69 4.1	84 69. 7	2.2* *	74. 3	70. 5	31 7.4	0.9	36. 2	24. 6	10 3.4	1.1*	30 .8	16 .6	73 .0	1. 1*	30 .8	16 .6	73 .0	1. 1*
Trop- onin I (µg/l)	54 Km	0. 01	0	0.0 56	0.0 45	46 0.0	1.4* *	0.0 1	0	0.0 NA	NA	0.0 1	0	0.0 NA	NA	0. 01	0	- 10 .0	0. 5	0. 01	0	- 10 .0	0. 5
	111 km	0. 01	0	0.0 15	0.0 12	50. 0	0.5§ §	0.0 1	0	0.0 NA	NA	0.0 1	0	0.0 NA	NA	0. 01	0	0. 0	NA	0. 01	0	0. 0	NA
C- React- ive Protei- n (mg/l)	54 Km	2. 2	2.1	2.5	1.6	13. 6	0.2	9.4	5.2	32 7.3	1.7* *	5.2	3.1	13 6.4	1.7	2. 7	1. 2	22 .7	0. 3	2. 7	1. 2	22 .7	0. 3
	111 km	0. 4	0.3 §§	18. 2	6.4	44 50. 0	3.6* **;§§§§	18. 0	9.5	44 00. 0	2.4* **;§§§§	8.8	1.9	21 00. 0	2.4* **;§§§§	3. 6	2. 8	80 0.	1. 5** *	3. 6	2. 8	80 0.	1. 5** *

CK: creatine kinase. NA: Not Available

Differences between before and immediately after and by 24, 48 and 72 h: * p<0.05 vs basal; ** p<0.01 vs basal; *** p<0.001 vs basal.

Differences between race: §p<0.05; §§p<0.01; §§§p<0.001.

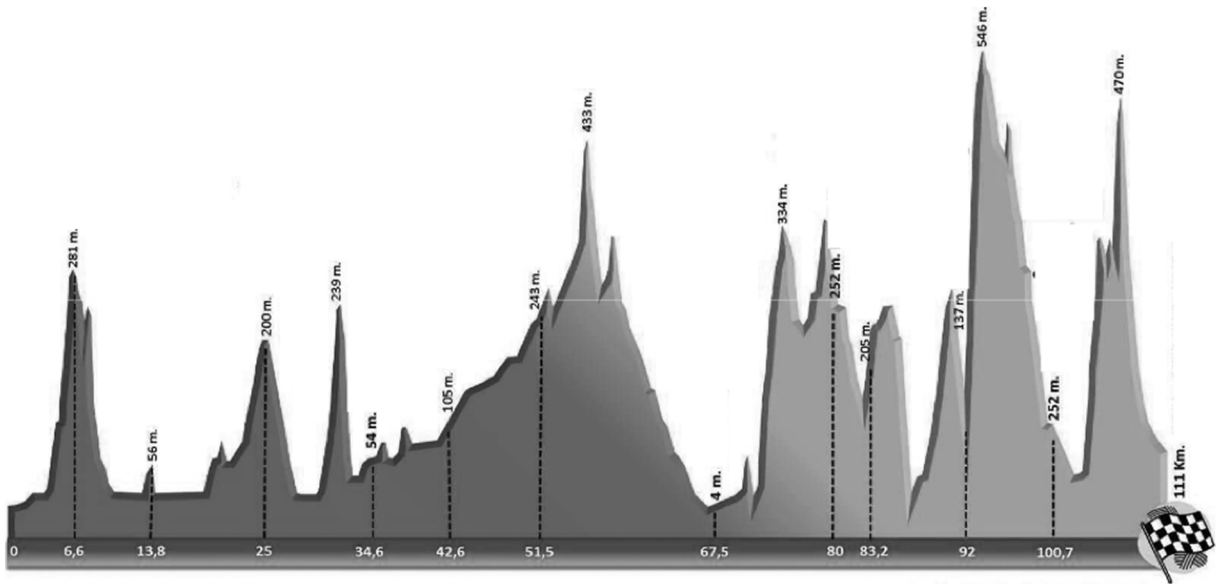


Figure 1

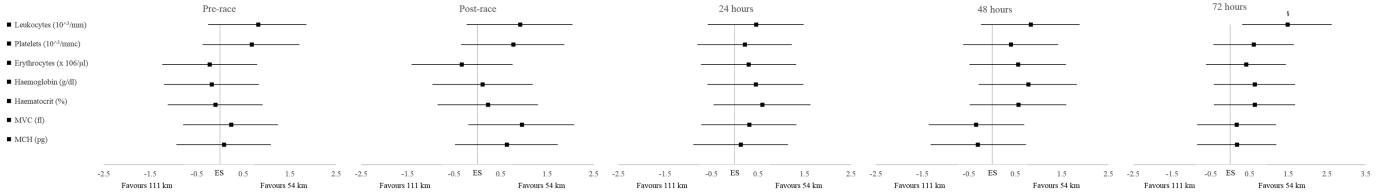


Figure 2

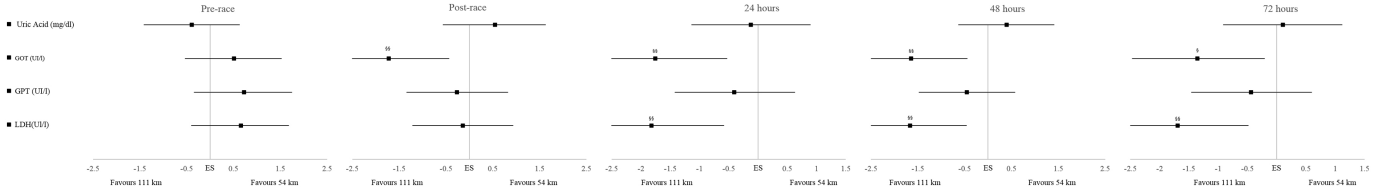


Figure 3

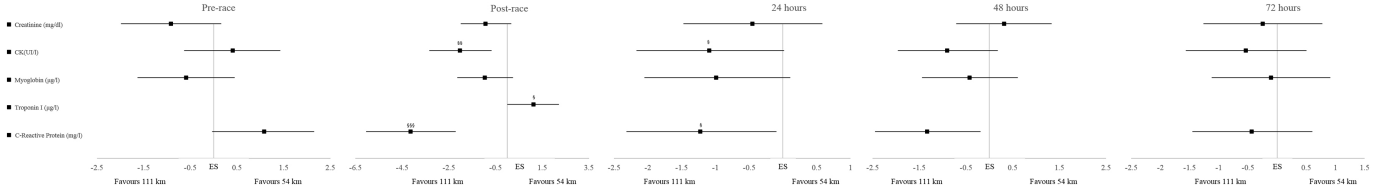


Figure 4