Title: Muscle damage, physiological changes and energy balance in ultra-endurance mountain event athletes

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Abstract:

The biological response to ultra-endurance mountain race events is not yet well understood. The aim of this study was to determine the biochemical and physiological changes after performing an ultra-endurance mountain race in runners. We recruited 11 amateur runners (age: 29.7±10.2 years; height: 179.7±5.4 cm; body mass: 76.7±10.3 kg). Muscle damage, lactate concentration, energy balance, rating of perceived exertion (RPE), heart rate (HR), heart rate variability (HRV), body composition changes and jump performance were analyzed before, during (only lactate, HR, and HRV) and after the race. Athletes completed 54 km in 6 h 44 min±28 min. After the race, myoglobin and creatine kinase concentration increased from 14.9±5.2 to 1419.9±1292.1 μg/l and from 820.0±2087.3 to 2421.1±2336.2 UI/l, respectively ($p<0.01$). In addition, lactate dehydrogenase and troponin I significantly increased after the race ($p<0.01$). Leukocyte and platelet count increased by 180.6±68.9% and 23.7±11.2%, respectively ($p<0.001$). Moreover, after the competition, athletes presented a 3704 kcal negative energy balance; a significant increase in RPE values; a decrease in Countermovement and Squat Jump height and a decrease in body mass and lower limb girths. During the event, lactate concentration did not change, subjects presented a mean HR of 158.8±17.7 bpm, a significant decrement in vagal modulation, and a significant increase in sympathetic modulation. Despite the relative "low" intensity achieved, ultra-endurance mountain race was a stressful stimulus that produces high level of muscle damage in the athletes. These findings may help coaches to design specific training programs that may improve nutritional intake strategies and prevent muscle damage.

Keywords: autonomic modulation, creatine kinase, energy intake, lactic dehydrogenase, myoglobin.

Introduction

Endurance events are becoming increasingly popular (Black et al. 2012) both in amateur and recreational athletes. However, while regular physical activity exerts a range of beneficial

physiological effects on cardiovascular health, a long-duration ultra-endurance exercise might produce substantial changes in biochemical parameters (Fallon et al. 1996; Kim et al. 2007) and muscle damage (Del Coso et al. 2013).

Physical demand maintained at a high level during these events induces a wide range of metabolic changes, causes micro-injuries to the muscles and other tissues, which in turn increases the migration of white blood cells to injury sites and induces acute phase inflammatory reactions (Kim et al. 2007). In addition, mountain running events include increased amount of eccentric muscle contractions that may generate more exercise-induced muscle damage (Friden et al. 1983). Further, eccentric exercise has been shown to elevate serum levels of myoglobin and plasma levels of creatine phosphokinase (CK) activity, indicating protein leakage from skeletal muscle (Del Coso et al. 2013).

Many studies have examined energy balance of athletes in numerous ultra-endurance events; however, less investigation has been focused on an ultra-endurance mountain running event. It has previously been demonstrated that the extreme caloric demand during ultra-endurance competition requires an adequate supply of metabolic fuel (Kreider 1991). Prolonged, sustained endurance training sessions exert significant metabolic demands that include the depletion of endogenous fuel stores (e.g., liver and muscle glycogen), loss of body fluid and electrolytes, hormonal perturbations, among others (Jeukendrup and Jentjens 2000). Because carbohydrate stores in the body are limited, the long duration of muscle activity raises the need for carbohydrates not only to fuel muscles but also to rapidly metabolize fats for energy and provide glucose for normal functioning of the central nervous system (Clemente-Suarez 2015). It is known that in endurance and ultra-endurance events athletes presented a large caloric expenditure to complete these prolonged exercises. O'Hara et al estimated the energy expenditure (EE) in a 160 km ultra-endurance running event around 10000-13000 Kcal (O'Hara et al. 1977). In accordance, some studies have reported a significant decrease in body weight mainly due to decrements in body fat (Clemente et al. 2011).

It is well known that ultra-endurance race events have a major impact on fat metabolism, particularly the activation of fat oxidation and increasing consumption of triglycerides as energy substrate (Fallon et al. 1996). This suggests that race intensity was generally maintained below the onset blood lactate concentration (OBLA). However, Laursen and Jenkins (2002) showed a 4.8 ± 0.5 mmol /L of blood lactate concentration in triathletes who swum 3 km and cycled 3 h. Physical performance can also become altered as a result of damage to the muscle. Following exercise-induced muscle damage, there is a reduction in the ability of the muscle to contract with maximal force (Kirby et al. 2012). Explosive muscle actions, such as those used during a vertical jump, can also become impaired as a result of muscle damage (Kirby et al. 2012).

Heart Rate Variability (HRV) is becoming one of the most useful variables for training, as it is a marker of cardiovascular autonomic function and because reduced HRV is a direct predictor of cardiovascular risk (Penttila et al. 2001). Gratze et al. (2005) analyzed post-race heart rate variability (HRV) in Ironman participants and found an increase in the sympathetic modulation of autonomous nervous system (i.e., a decrease in high frequency (HF) values and an increase in low frequency (LF) values of the HRV temporal domain). Moreover, heart rate response of athletes during an ultra-endurance event have been, shown to be at 71% of maximum heart rate (HRmax) (Neumayr et al. 2003).

Although some studies have described the physiological response of athletes in different ultra-endurance events (Del Coso et al. 2013; Fallon et al. 1996; Kim et al. 2007; Laursen et al. 2000), only one (Clemente-Suarez 2015) examined the psycho-physiological changes (but not changes in muscle damage) in ultra-endurance mountain amateur runners. Therefore, the present study aimed to determine changes in biochemical and hematological parameters, blood lactate concentration, rating of perceived exertion, heart rate, heart rate variability, energy balance, anthropometric parameters and vertical jump before, during and after the performance of a 54 km ultra-endurance mountain running event. The study was designed to further examine whether a 54 km ultra-endurance mountain running event: a) increases the muscle damage and white blood cells; b) produces blood lactates concentrations below the OBLA; c) increases the

RPE, and sympathetic modulation; d) rises the energy expenditure; and e) decreases the body mass, and jump height.

METHODS:

Participants:

Eleven healthy, ultra-endurance runners volunteered to participate in this study (Age: 29.7 ± 10.2; Height: 179.7 ± 5.4 cm; Body mass: 76.7 ± 10.3 Kg). All participants were amateur athletes with at least five years of experience in ultra-endurance events. The subjects were recruited by a phone call according to the following inclusion criteria: 18-40 years of age; the subjects had at least four years endurance training experience, and performed exercise five times per week; <7.5 hours in the previous edition of Castle of Cartagena race; none of the subjects had any musculoskeletal disorder within six months before the study. Prior to the race, the experimental procedures and risk and discomforts associated with the study were explained to all subjects and they provided signed informed consent, approved by the University's Institutional Review Board and in accordance with the Declaration of Helsinki. Furthermore, runners completed a questionnaire on training status according to Smith et al. (2004).

Experimental procedures

One day before the race, hematological tests were conducted in a laboratory on the athletes who were fasted overnight. The blood sample (6.5 mL) was withdrawn from an antecubital vein using a sterile technique to analyze hematological variables. Subsequently, three hours after consuming a standardized breakfast, each subject underwent a 10 min warm-up period consisting of 5 min of running followed by supervised active stretching of the lower limbs. Then, the jumping ability of the athletes was evaluated with a force platform (Kistler 9286AA Portable, Kistler, Switzerland) with the sampling rate set at 1000 Hz. The subjects performed two different jumps, a squat jump (SJ) and a countermovement jump (CMJ). The arms were kept at the waist at all the times to minimize any extra contribution to the jump impulse by the upper body. All athletes were already familiar with these types of actions. Three attempts were

carried out for each type of jump, and the best result was used. A 2 min rest was allowed between jumps to minimize the effect of fatigue on jump performance. The SJs were performed starting from a 90º knee angle position, and no drop or countermovement was permitted. If any countermovement was detected on the force-time display, the subject was required to repeat that trial. For the CMJs, the subjects were instructed to perform the jump as fast as possible with the aim to activate the stretch-shortening cycle Jump height (cm) was determined in SJ and CMJ. Jump heights (h) were calculated from the take-off vertical velocity using the following equation: $h = vi^2 \cdot 2g^{-1}$. Power was calculated as follows: vertical force multiplied by the instantaneous vertical velocity of the system's center of mass.

Thirty minutes before the onset of the race, participants arrived to the start line after their habitual warm-up and anthropometric evaluation was performed. All variables (listed in Table 3) were measured by a Level 3 anthropometrist certified by the International Society for the Advancement of Kinanthropometry (ISAK), in accordance with the ISAK guidelines (Stewart et al. 2011). Variables were taken twice, or three times (if the difference between the first two measures was greater than 5% for skinfolds and 1% for the rest of the dimensions), with the mean or median values, respectively, used for data analysis. The technical error of measurement scores was required to be within 5% for skinfolds and within 1% for the remaining variables. Body mass was measured using a SECA 862 (SECA, Germany); stretch stature with a GPM anthropometer (Siber-Hegner, Switzerland), 7 girths with a metallic non-extensible tape Lufkin W606PM (Lufkin, USA) and 8 skinfolds with a Harpenden skinfold caliper (British Indicators, UK). Body mass index (BMI) and sums of six and eight skinfolds were calculated. Girths were corrected for the skinfold at the corresponding site using the following formula: corrected girth = girth – ($\pi \cdot$ skinfold thickness).

After this, participants performed 54 km across the Castles of Cartagena race (Spain) with 5391 m of accumulative altitude. Moreover, 2726 m were uphill and 2665 m were downhill. Heart rate (HR) and heart rate variability (HRV) were recorded (Polar RS800; Polar Electro Oy; Kempele, Finland) during the entire race and lactate concentration, muscle soreness for lower

limb and rating of perceived exertion (RPE) were measured before and after the competition and at the halfway point (~27.5 km) of the race.

RPE was analyzed using a 6-20 RPE scale (Borg 1970) and muscle soreness for lower limb was measured using a 100 mm visual analog scale (VAS). Blood lactate concentration was measured using a Lactate Pro 2 portable lactate analyzer (Arkray Inc., Kyoto, Japan), while the subjects stood with their arms flexed. The finger prick extractions were made on a right index finger to fill the capillary tube.

The ratio HRmean/HRmax was calculated according to Padilla et al's (2001) protocol. Additionally, HR ranges used were defined by previous studies (Clemente-Suarez 2015). Long term endurance range (HRlte) (<50% Heart rate reserve; HRR), extensive aerobic range (HRea) (50–70% HRR), intensive aerobic range (HRia) (70–90% HRR) and high intensity range (HRhi) (>90% HRR) (Gilman 1996) were evaluated.

Two days before the event, HRV was analyzed during five hours of sleeping to determine basal values. All R-R interval data were exported from Polar Pro-Training v5 software (Polar Electro, Kempele, Finland) and analyzed using Kubios HRV analysis software (version 2.0 beta 4, University of Kuopio, Kuopio, Finland). Time-domain parameters during the race were analyzed. Average of NN intervals (ms), standard deviation of all normal NN intervals (SDNN) (ms), standard deviation of the difference between consecutive NN intervals (SDSD) (ms), square root of the mean of the sum of the squared differences between adjacent normal NN intervals (RMSSD) (ms) and percentage of the number of differences between adjacent normal NN intervals higher than 50 ms (PNN50) (%) were analyzed.

Participants hydrated themselves ad libitum in various refreshment points during the race. In addition, the food items and total amount of water consumed during the race by the subjects was accounted for by an individualized log book used for the nutrition recall. Moreover, the food items consumed during the race were known in advance because nutritional planning was a part

of athlete's preparation. Thus, food items were evaluated for energy, carbohydrate, fat and protein content using the nutritional information provided by the manufacturer of the food.

Energy expenditure was estimated using an equation between oxygen uptake and heart rate (Linderman and Laubach 2004). Heart rate was recorded and averaged every 5 min during the entire race to allow for the determination of VO_2using the same time interval, which was later converted to calories, assuming a caloric equivalent of 4.875 kcal/L O_2.

Within 3 min after finishing the race, participants performed vertical jumps as previously described at a designated area. Then, participants rested for five minutes followed by anthropometric testing and a venous blood sample was obtained.

Blood Samples

Blood extraction was performed while the subject seated. A portion of each blood sample (3 mL) was introduced into a tube with EDTA to determine hemoglobin concentration and haematocrit, erythrocytes, white blood cell and platelet counts using a hematology analyzer (Sysmex XS-1000i, Kobe, Kansai, Japan).The remaining blood (3.5 mL) was allowed to clot and centrifuged (10 min at 5000 g) to separate out the serum. Using the serum,blood markers of muscle damage and biochemical parameters (creatine phosphokinase, myoglobin, lactate dehydrogenase, ferritin, glucose, glycated haemoglobin, creatinine, uric acid, bilirubin, cholesterol tryglicerides, high-density lipoprotein, low-density lipoprotein, troponin I and C-Reactive Protein) and hepatic enzymes (glutamic-oxaloacetic acid transaminase and glutamic-pyruvic acid transaminase) were analyzed by automated chemical analysis (IL ILAB 600 Chemistry Analyzer of Instrumentation Laboratory, Holliston, MA, USA).

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 21.0 for Windows. Descriptive statistics (mean and standard deviation) were calculated. Before using parametric tests, the assumption of normality and homoscedasticity were verified using the Shapiro-Wilks test. A dependent t-test was used to investigate differences in physiological and hematological

variables between, before and after ultra-endurance event. A one-way ANOVA with post hoc Bonferroni correction was carried-out to determine differences among the three evaluations of lactate, RPE, VAS and HR values. All data are reported as mean (± SD) with the statistical significance set at $p<0.05$.

RESULTS

The training status of the subjects, according to the Smith et al. (2004) questionnaire was 2.1 ± 0.5 points. All the participants reported that they aerobically trained for 5.2 ± 1.5 days of training per week, 10.8 ± 2.4 weekly training hours and 88.9 ± 16.4 minutes of daily training. On average, athletes completed the race in 6 h 44 min 12 s (± 28 min 14 s). Their average speed during the competition was 7.9 ± 0.5 km/h. No changes were observed in blood lactate concentration during the race (Table 1). The RPE and VAS values significantly increased from the start of the race to the end ($p<0.001$; Table 1).

Table 1 near here

Prior to the race, HR value was 57 ± 4.4 bpm. At the 27.5 km of the competition, HR significantly increased (171 ± 23.6 bpm; $p<0.001$) and remained steady until the end of the race (175.2± 21.2 bpm). Overall, subjects presented a HRmax of 193.3 ± 9.3 bpm, a HRmean of 158.8 ± 17.7 bpm and HR minimum of 57.0 ± 14.0 bpm. The calculated HRmean/HRmax ratio was 0.82. The relative time performed at the different HR intensity zones was for HRLte 8.5 ±4.4 %, HRea 27.5 ± 14.3%, HRia 51.7± 7 % and HRhi 12.5± 11.2 %. However, mean NN, SDNN, SDSD, RMSSD and pNN50 variables significantly decreased ($p<0.05$) after the race (Table 2).

Table 2 near here

Furthermore, CMJ height (before: 30.6 ± 5.3 cm, after: 27.2 ± 7.3 cm) and SJ height (before: 27.4± 5.9 cm, after: 24.2 ± 7.9 cm) were significantly reduced following the race ($p<0.05$).

Anthropometric changes after the ultra-endurance race are presented in Table 3. Participants lost an average of 1.82 kg of body mass ($p<0.001$). However, there was no significant difference in skinfold changes except in the triceps site ($p<0.001$). Girths and corrected girths of the thigh (1 cm from gluteal line; $p<0.01$), calf ($p<0.001$), ankle ($p<0.05$) and corrected calf ($p<0.01$) significantly decreased after the race.

Table 3 near here

Pre and post-ultra-endurance race data for the blood variables are presented in Table 4. Briefly, no changes were observed in erythrocyte counts, haemoglobin and haematocrit. However, post-race leukocyte counts significantly increased by 180.6 ± 68.9 %, while platelet counts only increased by 23.7 ± 11.2 %. In addition, serum markers of muscle damage (e.g., myoglobin, CK, lactate dehydrogenase (LDH) and troponin I) significantly increased at the end of the race ($p<0.01$).

Table 4 near here

The estimated energy expenditure was 5197.1± 488.8 kcal, averaging 793.8± 83.9 Kcal/h. The total energy intake was 1493.1 ± 491.5 kcal, and a negative energy balance of 3704 kcal was measured. The nutrient distribution from energy bars, glucose tablets, energy drinks and fruits was: 0.0257 kg protein, 0.242 kg carbohydrate (of which 0.108 kg was sugar), 0.0199 kg fat and 0.0017 kg sodium.

DISCUSSION

The aim of this study was to investigate the acute changes in: blood lactate concentration, rating of perceived exertion, muscle soreness for lower limb, heart rate and heart rate variability, vertical jump performance, body composition values, hematological and muscle damage markers and energy balance during an ultra-endurance mountain running race.

The main findings of this study were: (a) race intensity was below the OBLA; (b) sympathetic modulation was increased after the race; (c) post-race jump height significantly decreased; (d)

only triceps skinfold increased their size meanwhile girths and body mass decreased; (e) post-race muscle damage markers significantly increased; and (f) energy intake did not compensate for the high energy expenditure.

The athletes completed the race with an average speed of 7.9 ± 0.5 km/h. This speed is considerably higher than those shown by Clemente-Suarez (2015) study (3.8 km/h) with similar characteristics (a 54 Km run with 6441 m of altitude change) and suggests that the level of our participants was higher in comparison. In addition, the lack of change in blood lactate concentration indicated that the intensity of the mountain run was not high enough to accumulate high amounts of blood lactate (Linderman and Laubach 2004). Additionally, the lactate concentration was below the maximum equilibrium point between lactate production and clearance and the OBLA (Sjodin and Jacobs 1981). These values are in agreement with studies of similar characteristics (Clemente-Suarez 2015), but lower than other ultra-endurance events studies (Clemente et al. 2011; Laursen et al. 2000; Linderman and Laubach 2004).

Although the low level of lactate concentrations did not increase significantly, the RPE significantly increased from 6.0 to 14.7 points at the end of the event, suggesting that the increase in perceived exertion may be explained by the aerobic component of the run. These values are similar to those presented by other ultra-endurance studies. For example, Laursen et al. (2000) analyzed triathletes after 3 km of swimming and 3 h of cycling obtaining values close to 14.6 points after swimming and 14.0 points after cycling; or Jeukendrup et al. (2006) who studied the RPE changes after 5 h on a cycloergometer at 58% maximum O_2 consumption obtaining 13.1 points after the exercise. On the contrary, Clemente-Suarez (2015) observed that athletes felt the event to be hard and high stressful (19.5 ± 1.5 points). These controversial results could be explained by the fact that our athletes performed better (twice the speed) than those by Clemente-Suarez (2015). VAS values presented a similar tendency, with a significant increase ($p<0.001$) from 0.6 ± 0.2 cm before the race to 5.6 ± 3.2 cm at the 27.5 Km and to 6.6 ± 3.0 cm after the race. These values are similar to those presented by Sauge et al. (2013) after a

mountain ultra-marathon and were shown to be related with markers of inflammation and muscle damage.

The HRV changes showed a decrease in parasympathetic activity and an increase in sympathetic nervous system activation because pNN50, RMSSD, SDNN, SDSD and average NN decrease (Penttila et al. 2001). These changes in autonomic modulation of athletes have been reported by other studies examining long-distance events (Clemente-Suarez 2015; Linderman and Laubach 2004; Penttila et al. 2001) and may be the factor that produces cardiac adaptations in ultra-endurance efforts. Moreover, we observed a decrease in vagal modulation and an increase in sympathetic modulation showing that this type of effort is very stressful for athletes (Clemente-Suarez 2015). Therefore, HRV analysis could inform and help the athlete in stress event to determine the fatigue level. Furthermore, HRmean/HRmax ratio in ultra-endurance runners was higher than observed in amateur athletes (0.64 vs 0.82) (Clemente-Suarez 2015) or in cyclists (Padilla et al. 2001) (0.77 vs 0.82). These controversial results could be explained by the fact that our athletes performed the race at higher intensity (more quickly). Moreover, the reduced value found in previous studies could be explained by the long duration of the event and the lower performance level of participants.

The ultra-endurance race affected the vertical jump performance of athletes, producing a decrease in jump height, indicating of the presence of muscle fatigue in reducing leg power production (Del Coso et al. 2012; Suzuki et al. 2006). According to previous studies, jump height change may also be related to damage markers such as higher blood myoglobin concentration or blood CK concentration (Del Coso et al. 2012).This relationship suggests that the continuous foot strikes during running request concentric and eccentric actions of the legs muscles and can damage muscle fibers (Friden et al. 1983). Thus, the training program and the race strategies should include muscle damage prevention to diminish the muscle fatigue in ultra-endurance athletes.

The loss in body mass following the event is consistent with data obtained in 100 km ultra-marathoners (Knechtle et al. 2012), ultra-endurance cyclists (Bischof et al. 2013) and ultra-endurance open water swimmers (Knechtle et al. 2012). Bischof et al. (2013) showed a larger decrease in individual skinfold thicknesses (from -6.4 to -14.9%) and in the sum of skinfolds (-10.7%) in cyclists. These results support previous research in swimmers (Knechtle et al. 2009). However, the observed difference between the skinfold thickness before and after the race in this study was only observed in the triceps site. These results are consistent with those of Knechtle et al. (2012) who found small decreases in individual skinfolds (from 0.0 to -2.8%) but an increase of 4.8% in the triceps skinfold. In addition, the significant decrease in the calf girth, higher than in the rest of girths could be associated with a large strain of running in this part of the lower limb (Knechtle et al. 2012).

The continuous foot strikes during ultra-endurance running event can damage muscle fibers by the high-force eccentric actions (Friden et al. 1983), producing the release of muscle proteins into the blood stream. CK, LDH and hepatic enzymes levels are the most common markers to indicate skeletal muscle damage (Skenderi et al. 2006). We reported an increase of 107.6 % in LDH and 195.3 % in CK, which are in accordance with Kim et al. (2007) in an extreme ultramarathon. LDH changes were similar to those observed in others studies of ultra-endurance running (Fallon et al. 1996; Millet et al. 2011). However, other studies that examined longer distance races have reported CK levels of 4,500 and 5000 and 13600 UI/L after races of 1600-km (Fallon et al. 1996), 100 km (Overgaard et al. 2002) and 166 km (Millet et al. 2011) respectively. These high CK reported values were likely obtained when patients were undergoing severe rhabdomyolysis. In addition, we observed an elevation in myoglobin concentration, indicating the presence of muscle damage, which may have contributed to the decrease in their performance (Del Coso et al. 2012). Previous studies (Del Coso et al. 2012; Schiff et al. 1978) reported a similar increase in myoglobin concentration before completing an endurance running event. Thus, the degree of muscle fiber damage could be an essential factor that affects performance in ultra-endurance events (Del Coso et al. 2013). Another important

factor affecting ultra-endurance performance is the increase in platelets and white blood cell counts. We reported an increase of 180.6 % in leukocyte and 23.7 % in platelet count, suggesting the occurrence of temporary inflammation. Previous studies (Hikida et al. 1983; Skenderi et al. 2006) showed a similar response after a long continuous exercise and observed that the degree of inflammation was comparable to many clinical situations such as surgery or sepsis. Inflammation produces disorientation, degeneration of myofibrils and disruption of the sarcolemma, muscle fiber necrosis and release of mitochondria in the extracellular space (Hikida et al. 1983; Skenderi et al. 2006), which negatively affects the physical performance of ultra-endurance athletes.

Interestingly, we observed an increase in troponin I values (49 %), indicating some form of ischemia. Troponin I is the most sensitive and specific marker for the detection of cardiomyocyte necrosis, even in the presence of skeletal muscle damage (Neumayr et al. 2001). Previous studies have also shown significant increases in Troponin I concentrations following ultra-endurance exercise competitions (Lucia et al. 1999). An increase in troponin I could be an indicator for subclinical cardiovascular disease that is being unmasked by strenuous exercise (Roth et al. 2007), as this marker is an important factor in predicting short and long term cardiac events (Vidotto et al. 2005). Although aerobic exercise in general has many health benefits, it has been shown that ultra-endurance exercise may induce transient subclinical myocardial damage, which may trigger physiological reparative or adaptive process (Vidotto et al. 2005). Therefore, monitoring and assessing biochemical and performance parameters could be an effective method to prevent pathologies and injuries. Thus, a medical evaluation of the participant should be included in all ultra-endurance events to avoid unexpected health problems.

The post-race creatinine (35%) and uric acid (44%) levels were higher than pre-race values. The intensification in creatinine production may be related to the rise in catabolic metabolites of muscle damage as shown in previous studies (Neumayr et al. 2003). In addition, the change of uric acid may be explained by the enhanced protein catabolism during long endurance exercise

when glycogen depots become depleted (Kreider 1991).The final concentration of triglycerides showed a significant increase of 44.8 %, similar to what was shown at the end of a 24-hour ultra-endurance relay race (Clemente et al. 2011). These changes were likely due to the discharge of catecholamines induced by the exercise, which stimulated lipolysis in the adipose tissue and led to a release of lipid substrates that include triglycerides in the blood stream (Coggan et al. 2000). On the other hand, glucose values did not changed and is in accordance with similar findings from other studies (Clemente et al. 2011; Fallon et al. 1996). Thus, the ingestion of foodstuffs during the race could affect triglyceride concentration and could produce no change in blood glucose concentration. This normoglucemia is achieved by means of the effect of hormones and the consumption of sports drinks and food during the competition (Willmore and Costill 1999). Therefore, ultra-endurance athletes should consider including in their training program energy consumption strategies to provide rapid absorption of carbohydrates that meets the demands of the exercise.

Having a correct energy balance may be advantageous to the athlete when it comes to endurance performance. Moreover, inadequate nutrition could generate symptoms such as hypoglycemia, weakness, fainting, decreased urinary output, glycogen depletion, loss of electrolytes, minerals and lean tissue and increased injury rates (Benson et al. 1985). We reported a high rate of energy expenditure in comparison with energy intake (EB= -3704 kcal), which indicates that energy intake provides approximately 30% of total energy expenditure. Thus, a large part of the energy consumption during the event came from endogenous fuel stores. Furthermore, this finding illustrates the importance of consuming a high carbohydrate diet prior to ultra-endurance race to maximize endogenous fuel stores (Black et al. 2012). The carbohydrate intake of 0.6 g/min is slightly below the optimal amount to sustain a high carbohydrate oxidation rate (1-1.5 g/min) (Jeukendrup and Jentjens 2000) that corresponds to an intake of 3.2 g/kg body mass, which is much less than the recommended range (10-12 g/kg body mass) for extremely prolonged and intense exercise (Hawley and Burke 1998). This response was in accordance with previous studies in mountain running (Clemente et al. 2011) or ultra-endurance cycling (Black

et al. 2012). Thus, endogenous fat and CHO contribution to EE may assist in validating the energy deficit results reported in this study. Moreover, carbohydrate intake was positively correlated with race performance (Havemann and Goedecke 2008). An adequately high carbohydrate intake during race could be the best tool to conserve muscle and hepatic glycogen store and to maintain blood glucose concentration. The intake of other macronutrients, such as protein or fat, could also serve as a source to meet the energy demands of this type of race and may spare carbohydrate stores, thus delaying the onset of fatigue (Black et al. 2012). The energy deficit was correlated with performance, which suggests that reducing the energy deficit may be an advantage for these athletes. Therefore, a correct dietary strategy to increase energy intakes may be an important factor affecting ultra-endurance performance and intake planning should be considered as a part of the athlete's training program.

The principal limitation of the present study was the low number of participant evaluated. On the other hand, the present research offers an important contribution to understand the physiological consequences ofan ultra-endurance mountain race. In this sense, the main strength of the study is the direct application to the specific training for preparing the peak performance in the race.

In summary, an ultra-endurance mountain race caused muscle damage and showed an increase in markers for myocardial damage and white cell response. Furthermore, this type of event produced a negative energy balance and an increase in sympathetic nervous system modulation. Despite an increase in reported RPE values, blood lactate concentration was unchanged below the OBLA. Body mass and calf girths decreased significantly. Moreover, jump performance decreased after the race. Therefore, these findings indicate that the ultra-endurance mountainrace has a profound impact not only at the muscular level but also biochemically and physiologically, which may impede performance during the competition.

The major finding of the current study could be useful for coaches in designing specific training programs that can help improve nutritional intake strategies and prevent muscle damage.

Furthermore, the results obtained in this study showed that this type of race was very stressful and may not be recommendable without a proper training program prior to the event. It is also advisable to do a medical health check before undergoing these competitions due to possible complications (rhabdomyolysis or myocardial damage) that may be produced by this type of exercise.

ACKNOWLEDGMENTS

The authors would like to thank all athletes who participated in this study, the organization of the Castle of Cartagena race, and to the Escuela de Infantería de Marina General Albacete y Fuster (Cartagena, Spain) for the support before, during and after the race.

CONFLICTS OF INTEREST:

The authors declare that there are no conflicts of interest.

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TABLES:

Table 1: Differences in blood lactate concentration, rating perceived exertion (RPE) and VAS (Visual-analogical scale) before, at 27.5 Km and after ultra-endurance event (mean ± SD).

Variable	Before	At 27.5 Km	After	p-value
Blood lactate concentration (mmol/L)	2.9 ± 1.6	2.5 ± 1.1	3.0 ± 0.9	0.858
RPE	6.0 ± 0.0	12.5 ± 2.9***	14.7 ± 3.9***	<0.001
VAS (cm)	0.6 ± 0.2	5.6 ± 3.2***	6.6 ± 3.0***	<0.001

*Differences among before, at 27.5 Km and after competition values (*** $p<0.001$)

Table 2: Anthropometric measures before and after an ultra-endurance event (mean ± SD).

	Pre-race	Post-race
Body mass (kg)	76.7 ± 10.3	74.9 ±10.2 ***
Triceps skinfold (mm)	9.4 ± 2.9	10.1 ± 3.1***
Subscapular skinfold (mm)	9.3 ± 3.2	9.3 ± 2.6
Biceps skinfold (mm)	4.3 ± 1.5	4.2 ± 0.8
Iliac crest skinfold (mm)	12.6 ± 4.7	12.6 ± 4.9
Supraspinal skinfold (mm)	7.8 ± 4.9	8.2 ± 4.6
Abdominal skinfold(mm)	13.6 ± 7.2	14.4 ± 6.8
Front thigh skinfold (mm)	10.8 ± 2.7	11.0 ± 3.0
Medial calf skinfold (mm)	6.5 ± 2.5	6.5 ± 2.4
Sum of 6 skinfolds (mm)	57.4 ± 17.9	59.5 ± 15.9
Sum of 8 skinfolds (mm)	74.2 ± 23.1	76.4 ± 20.4
Arm girth (cm)	31.0 ± 3.4	31.0 ± 3.2
Arm flexed and tensed girth (cm)	33.5 ± 3.1	33.5 ± 3.1
Forearm girth (cm)	26.4 ± 4.8	26.3 ± 4.7
Thigh girth (1 cm from gluteal line (cm)	58.1 ± 4.7	56.8 ± 4.4**
Mid thigh girth (cm)	54.4 ± 4.2	53.9 ± 4.4
Calf girth (cm)	37.9 ± 3.1	37.4 ± 3.1***
Ankle girth (cm)	22.5 ± 1.6	22.3 ± 1.6 *
Corrected arm girth (cm)	28.0 ± 3.2	27.8 ± 3.1
Corrected mid thigh girth (cm)	51.0 ± 3.9	50.5 ± 4.2
Corrected calf girth (cm)	35.9 ± 2.9	35.4 ± 3.0**
BMI (kg/m2)	23.7 ± 2.3	23.1 ± 2.3***

*Differences between before and after competition values (*p<0.05; **p<0.01; *** p<0.001)

Table 3: Heart rate variability values in basal and race conditions (mean ± SD).

Variable	Basal	Race
Average NN (ms)	1103.7 ± 59.7	394.4 ± 43.7*
SDNN (ms)	96.5 ± 16.9	72.2 ± 29.8*
SDSD (ms)	34.1 ± 1.6	19.4 ± 2.3*
RMSSD (ms)	31.6 ± 6.1	7.3 ± 2.7*
pNN50 (%)	12.4 ± 5.2	0.1 ± 0.1*

SDNN: standard deviation of all normal NN intervals; SDSD: standard deviation of the difference between consecutive NN intervals; RMSSD: square root of the mean of the sum of the squared differences between adjacent normal NN intervals; PNN50: percentage of the number of differences between adjacent normal NN intervals higher than 50 ms. *$p<0.05$ vs basal values

Table 4: Differences in haematological values before and after the race (mean ± SD).

	Pre	Post
Erythrocytes (x 10^6/µl)	4.9 ± 0.4	5.0 ± 0.4
Haemoglobin (g/dl)	14.8 ± 1.2	14.9 ± 1.0
Haematocrit (%)	43.1 ± 3.5	44.0 ± 3.1
MCV (fl)	87.5 ± 2.5	88.6 ± 2.7*
MCH (pg)	30.1 ± 1.1	30.1 ± 1.1
MCHC (g/dl)	34.4 ± 1.0	33.9 ± 0.7**
Leukocytes ($10^{\wedge 3}$/mmc)	5.8 ± 1.3	16.1 ± 4.2***
Lymphocytes ($10^{\wedge 3}$/mmc)	2.0 ± 0.4	1.3 ± 0.5**
Monocytes ($10^{\wedge 3}$/mmc)	0.4 ± 0.1	1.0 ± 0.3***
Eosinophils ($10^{\wedge 3}$/mmc)	0.2 ± 0.1	0.01 ± 0.0**
Basophils ($10^{\wedge 3}$/mmc)	0.03 ± 0.0	0.1 ± 0.1**
Neutrophils ($10^{\wedge 3}$/mmc)	3.2 ± 1.1	13.7 ± 4.1***
Platelets ($10^{\wedge 3}$/mmc)	214.2 ± 32.3	264.6 ± 42.9***
Ferritin (mcg/l)	101.1 ± 38.3	103.8 ± 44.2
Glucose (mg/dl)	91.8 ± 8.3	104.7 ± 25.2
HbA1c (%)	5.3 ± 0.2	5.1 ± 0.2 **
Creatinine (mg/dl)	1.1 ± 0.1	1.4 ± 0.3***
Uric Acid(mg/dl)	5.0 ± 1.1	7.2 ± 1.0 ***
GOT (UI/l)	34.2 ± 31.0	78.4 ± 44.7**
GPT(UI/l)	27.6 ± 15.3	34.9 ± 20.6**
LDH(UI/l)	383.0 ± 178.6	795.0 ± 260.7**
CK(UI/l)	820.0 ± 2087.3	2421.1 ± 2336.2**
Bilirubin(mg/dl)	1.1 ± 0.6	1.2 ± 0.5
Cholesterol(mg/dl)	159.4 ± 22.8	150.3 ± 41.7**
Tryglicerides(mg/dl)	49.5 ± 18.1	71.7 ± 16.3**
HDL(mg/dl)	61.6 ± 8.4	65.2 ± 8.6**
LDL(mg/dl)	87.8 ± 15.8	89.2 ± 17.4
Myoglobin (µg/l)	14.9 ± 5.2	1419.9 ± 1292.1**
Troponin I (µg/l)	0.01 ± 0.03	0.05 ± 0.04**
C-Reactive Protein(mg/l)	2.1 ± 2.0	2.5 ± 1.5

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular haemoglobin concentration; HbA1c: glycated haemoglobin; GOT: glutamic-oxaloacetic acid transaminase; GPT: glutamic-pyruvic acid transaminase; LDH: lactic dehydrogenase; CK: creatine kinase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; *Differences between before and after competition values (*$p<0.05$; **$p<0.01$; *** $p<0.001$)