European Journal of Human Movement

OPEN ACCESS

Original Research

Clustering classification of cyclists according to acute fatigue outcomes produced by an ultra-endurance event

José Luis Sánchez-Jiménez[1], Alexis Gandia-Soriano[1], Pedro Pérez-Soriano[1,2], José Ignacio Priego-Quesada[1,2*], Alberto Encarnación-Martínez[1,2]

1 Research Group in Sports Biomechanics (GIBD), Department of Physical Education and Sports, University of Valencia, Valencia, Spain.
2 Research Network in Cycling and Woman (REDICYM), St: Avenida Conde de Torrefiel, 22, 46870, Ontinyent, Valencia, Spain.

*Correspondence: (JPQ) j.ignacio.priego@uv.es 0000-0002-0375-1454

Received: 11/05/2023; Accepted: 26/06/2023; Published: 30/06/2023

Abstract: This study aimed to analyze the differences between clusters obtained by the acute fatigue effect following an ultra-endurance event on the internal and external load of cyclists. 26 volunteers participated in the study, divided into the experimental group (N = 18; height: 177 ± 8 cm; body mass: 78.6 ± 10.3 kg) and the control group (N = 8; height: 176 ± 10 cm; body mass: 78.0 ± 15.7 kg). The experimental group completed a 12 h non-stop cycling event. Jump height, lactate, plasma antioxidant capacity, pain perception and fatigue perception were measured before and after the event. Cyclists of the experimental group were classified taking into account their training characteristics (recreational vs. competitive) and conducting non-supervised K-means clustering. The differentiation of cyclists according to training characteristics resulted in a lower distance covered by recreational cyclists than competitive cyclists (279.4 ± 39.7 km vs. 371.0 ± 71.7 km; ES ≥ 0.8; $p < 0.01$), although no differences were observed in the other variables between groups ($p > 0.05$). The clustering analysis resulted in two clusters. Cluster 2 suffered a greater jump height decrease (-3.3 ± 1.6 vs. 1.2 ± 0.8; ES ≥ 0.8; $p < 0.001$) and increased pain and fatigue perception (ES ≥ 0.5; $p < 0.05$) after the race than Cluster 1. In conclusion, counter-movement jump and fatigue/pain perception can differentiate the fatigue produced by a cycling ultra-endurance event and therefore, these non-invasive measurements are useful in fatigue monitoring and recovery planning.

Keywords: cycling performance; cyclist profile; road cycling; fatigability

1. Introduction

Interest and participation in ultra-endurance events (endurance races with a duration of at least 6 hours) have increased in recent years (Scheer, 2019; Shoak et al., 2013), now including a wide variety of events performed during one day, consecutive days or multiple sporting modalities (Turner et al., 2014). Elite and recreational age group athletes take part together in these events, the participation in the age groups of between 30 and 60 years having increased over recent decades (Nikolaidis et al., 2021). More specifically, this participation increase has also been observed in ultra-endurance cycling events (Scheer, 2019). However, goal setting in ultra-endurance events differs between participants, in that the race includes some participants who only want to finish the race and others who compete and are focused on sporting performance, so providing different participants profiles (Vitti et al., 2020).

Performance in endurance exercise across the different types of events is closely related to the delay in physical capacity decrease associated with the onset of fatigue (Maunder et al., 2021). The physical demands of cycling depend on the type of race, and the mechanisms involved in fatigue onset will vary with the specific task performed (Carins et al., 2005). Hence, determining the outcomes related to cycling performance in each type of race will allow us to accurately monitor fatigue. The tools employed by previous studies to assess fatigue status in ultra-endurance events gathered information on different outcomes such as internal load (e.g., oxidative stress or blood lactate) (Dantas et al., 2014; Stelzer et al., 2015), external load (e.g., training impulse or jump performance) (Bescós et al., 2011; Truppa et al., 2020), and subjective load variables (e.g., fatigue perception) (Smith et al., 2020). For example, a previous study observed that an ultra-endurance cycling event results in a decrease of hemoglobin levels, decrease of body mass, jump performance, and peak torque during an isokinetic test (Clemente-Suarez, 2014).

Although there is lack of research into the effect of cycling profile (e.g., professional vs. recreational) on physiological responses during and after a ultra-endurance cycling event, it is known that cycling profile can affect pedaling kinematics, muscle recruitment, pedal forces and physiological outcomes (Bini et al., 2016; Chapman et al., 2008; Coyle et al., 1991; García-López et al., 2016). Due to the differences in profiles at ultra-endurance events, it would also be interesting to know the variability of the internal load response to these competitions, with the aim of optimizing recovery planning. Although in recent years cluster analysis has been used by some cycling studies to determine different physiological responses depending on the type of terrain and specialization (Gandia et al., 2020) or the anthropometric characteristics associated with sprint or endurance performance (van der Zwaard et al., 2019), to the author's knowledge this technique has not been used in cycling ultra-endurance competitions for assessing different fatigue profiles. Nevertheless, one previous study did reveal the potential of clustering for evaluating the effect of fatigue on endurance exercise by assessing the effect of a marathon on running biomechanics (Clermont et al., 2019).

The aim of this study was to analyze the differences between clusters obtained by the acute fatigue effect following an ultra-endurance event on the internal and external load of cyclists. Moreover, a classification based on the profile (recreational vs. competitive cyclists) was performed to compare how these profiles differ in their fatigue compared with non-supervised clustering.

2. Materials and Methods

Sample —26 volunteers were divided into either the experimental group (n = 18) or the control group (n = 8). The control group remained awake during the event but did no exercise. Table 1 shows the characteristics of the sample. Participants were recruited from individuals enrolled in a cycling event and who had to be (1) a physically active cyclist, (2) have experience with ultra-endurance races, (3) have participated in at least one ultra-endurance event, (4) signed up in the individual category of the event and (5) to have not suffered an illness or an injury in the last two months. Exclusion criteria included voluntary withdrawal from the study or inability to complete the entire cycling event. All participants signed a written informed consent agreeing with the protocols and voluntary participation. The study was performed in agreement with the Declaration of Helsinki and approved by the ethics committee of the local institution (University of Valencia, [ref. 1877154]).

Table 1. Characteristics of the participants in the sample.

Characteristics	Experimental Group (n = 18)	Control Group (n = 8)	P Value
Age (years)	47 ± 10	31 ± 8	0.001
Height (cm)	177 ± 8	176 ± 10	0.691
Body Mass (kg)	78.6 ± 10.3	78.0 ± 15.7	0.914
Body Mass Index (kg/m^2)	25 ± 3	25 ± 4	0.617
Adipose Tissue (%)	18.4 ± 7.5	18.06 ± 9.3	0.913
Skeletal Muscle (%)	60.6 ± 7.9	57.02 ± 9.60	0.334
Weekly Training (km)	289.7 ± 126.3	124.0 ± 126.0	0.017

P Value was calculated through Mann-Whitney test for non-parametric variables ($p < 0.05$) and t-test for parametric variables ($p > 0.05$).

Experimental Design —The experimental approach consisted in observational research with the incorporation of a control group. Participants completed a nocturnal 12 h non-stop cycling event (from 20:00 to 08:00) on the Ricardo Tormo racing circuit, Valencia, Spain. The aim of the race was to cover the maximum number of kilometers going around the circuit over a period of 12h (Figure 1), being free to stop to rest or eat whenever they wanted. Drafting during the race was allowed. Conditions during the competition were a mean environmental temperature of 19°C and an altitude of 154 m.

Participants were requested to avoid intense exercise the day before the race and

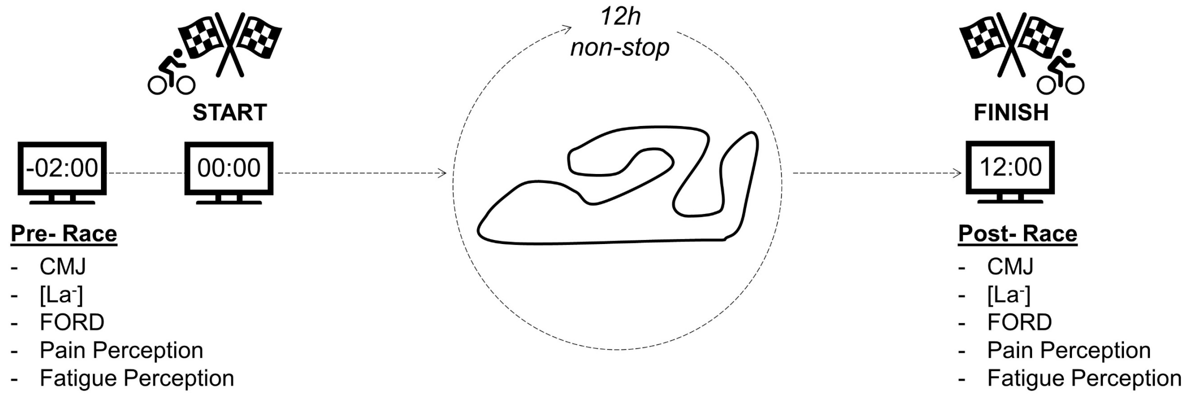

Figure 1. Design of the study. CMJ: Counter Movement Jump; [La-]: Lactate; FORD: Free Oxygen Radical Defence Test.

to maintain their usual hydration and nutrition habits, including stimulant drinks (e.g., coffee), but avoid alcohol, and sleep at least seven hours the night before the race. Participants were allowed to self-select their nutritional and hydration strategies to avoid detrimental effects on their sport performance.

Methodology — All the measurements [countermovement jump (CMJ), lactate concentration, pain and fatigue perception and antioxidant response] were performed at two moments: pre-race (over the two hours before starting the race) and post-race (immediately after the race ends).

Participants performed the CMJ using a contact mat (model DIN-A3, Chronojump Bosco-System, Barcelona, Spain). They were encouraged to jump as high as possible and they were provided with information about the following technique: to hold a start position from a standing posture with the hands placed at the hips to minimize the influence of arm movements and to perform the fastest possible upward movement by jumping as high as possible and landing on their toes (Petrigna et al., 2019). Knee flexion-extension range was self-selected by the participants for achieving the highest jump height. Prior to the test, participants were allowed to perform several jumps at low intensity to familiarize themselves with the protocol. Then, each participant completed three repetitions of the CMJ performed with a rest interval of 30 s between repetitions. The mean of the three jumps was used for analyzing jump height (Claudino et al., 2017).

Blood lactate [La^-] was measured employing a portable Lactate Scout + system (SensLab CmbH, Leipzig, Germany and EKF Diagnostics GmbH, Barleben, Germany) from 5 μl blood samples collected from the right hand (Tanner et al., 2010).

Plasma Antioxidant Capacity was determined using the FORD test (Callegari, Catellani, Italy) (Lewis et al., 2020; Pavlatou et al., 2009), a colorimetric essay that relates the discoloration of the sample with antioxidants concentration, according to Lambert-Beer law (Pavlatou et al., 2009). A single drop of finger capillary blood (20 μL) was extracted. The chromogen that contains 4-amino-*N,N*-diethylaniline sulfate in presence of an acidic buffer (pH = 5.2) and a suitable oxidant ($FeCl_3$) form a stable radical cation photometrically detectable at 505 nm (Pavlatou et al., 2009). Antioxidant compounds present in the sample reduce the radical cation producing a discoloration of the solution (Pavlatou et al., 2009). The absorbance values are compared with standard curves of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and FORD values are ranged from 0.25 to 3.0 mmol•L^{-1} (Pavlatou et al., 2009).

Perception of fatigue and pain was measured using a 150-mm visual analogue scale (VAS) (Mündermann et al., 2002). The scales were labelled from the left as “absence of fatigue/pain” (0 mm) to the right as “highest fatigue/pain imaginable” (150 mm). Fatigue and pain were measured taking into account the following regions: overall, legs and arm. Participants marked their perception on the scale of each region before and after the race. The values were calculated measuring from 0 to the marks in cm with a sensitivity of one millimeter.

Post-race measurements were performed immediately after the race finished (specially blood samples, that were measured less than 1 minute after stopping cycling). It should be borne in mind that not all participants finished at the same time. Once the 12 hours were up, the leaders stopped when the last ones were just starting the last lap, or in the middle of it. The distance of the circuit meant that there was a margin of about 15 minutes between the fastest and the slowest, allowing everyone's blood samples to be measured as soon as they finished. After measuring the entire experimental group, the measurements of the control group were performed.

Time per lap and the number of laps completed by each participant were provided by the organizers of the event. Speed Lost was calculated as the percentage of speed reduction obtained between the fastest lap achieved during the first and the last 10 laps of the event. The fastest lap of the event of each participant was also recorded.

Two classifications of cyclists were undertaken, one according to established criteria based on demographic characteristics (Priego et al., 2018), and the second one by clustering statistical methods taking into account the outcomes measured in the competition (Hartigan & Wong, 1979). The classification provided by (Priego et al., 2018) for amateur cyclists was employed, recreational cyclists being considered as those who trained weekly but covered less than 260 km and competitive cyclists as those who trained weekly and covered more than 260 km. Non-supervised K-means clustering (Hartigan & Wong, 1979) was conducted using R Studio software (version 2022.02.3, package "cluster", Posit company, Boston, USA) to divide cyclists (experimental group) according to the variation (Δ: post-race result – pre-race result) of three of the internal load outcomes obtained: jump height, lactate and FORD. Before clustering, the silhouette method was used to determine the optimal number of clusters (Rousseeuw, 1987) for introducing this number of dimensions into the K-means algorithm.

Statistical Analyses— Statistical analyses of the data were performed with R Studio Software (version 2022.02.3). Results are reported as mean ± SD. The normality of the variables was checked using the Kolmogorov-Smirnov test. Student t-test for paired data was used to identify differences between pre-race and post-race in parametric variables and the Wilcoxon test for non-parametric variables for each group (experimental and control). Kruskal-Wallis rank sum test with Mann–Whitney U post-hocs were applied to assess the differences in outcomes measured and demographic characteristics between profiles (recreational vs. Competitive vs. Control) and the clusters obtained with the K-means clustering in non-parametric variables, while a Student t-test for independent samples was applied in parametric variables. Effect size (ES) was reported in those variables with a significance $p < 0.05$. ES for parametric variables was calculated through Cohen's D and R Wilcoxon for non-parametric variables (Cohen, 1988; Tomczak & Tomczak, 2014). ES were classified as small (ES 0.2-0.5), moderate (ES 0.5-0.8) or large ($ES > 0.8$) (Cohen, 1988). The level of significance was set at $p < 0.05$.

3. Results

Ultra-endurance event response of the experimental and control group - The experimental group completed 78 ± 19 laps during the 12-h of the event and averaged 36.9 ± 4.6 Km/h during the fastest lap of the race. The average speed decreased 15.1 ±

8.4% in the last 10 laps in comparison with the first 10 laps (36.7 ± 4.6 vs. 31.0 ± 3.9; ES = 1.3; p < 0.001). Jump height in the experimental group was lower in post-race than at the pre-race moment (ES = 0.3; p = 0.009) (Table 2). However, no statistical differences were observed between pre-race and post-race moments in the experimental group in lactate and FORD (p > 0.05). Concerning perceptive measurements, all the measurements showed higher values in post-race moment than pre-race (ES ≥ 0.8; p < 0.001). No statistical differences were observed in the comparison between pre-race and post-race values in any of the variables assessed in the control group (p > 0.05).

No differences between the experimental and control group were obtained in pre-race measurements for any variable (p > 0.05). Regarding post-race assessments, the control group obtained lower values than the experimental group in lactate (ES = 1.2; p < 0.05) and perceptive measurements (ES > 2.5; p < 0.01), but no differences were observed in the remaining variables between the groups in post-race measurements (p > 0.05).

Ultra-endurance event response depending on training characteristics - The groups classified, depending on training characteristics, resulted in ten recreational cyclists and eight competitive cyclists (Table 3). For the non-supervised clustering, the silhouette method reported an ideal number of two clusters (Figure 2.A.), and the K-means clustering provided two clusters of 6 and 12 participants (Figure 2.B).

Table 2. Differences between pre- and post-race measurements in experimental and control group.

	Experimental Group (N = 18)		**Control Group (N = 8)**	
Characteristic	**Pre**	**Post**	**Pre**	**Post**
Jump Height (cm)	21.6 ± 6.1	19.8 ± 5.8$^{\dagger S}$	24 ± 9	24 ± 8
Lactate (mmol/L)	1.1 ± 1.5	1.0 ± 0.5	0.7 ± 0.4	0.5 ± 0.3^{*L}
FORD (mmol/L)	0.62 ± 0.38	0.57 ± 0.28	0.61 ± 0.33	0.75 ± 0.38
Overall Pain (cm)	1.1 ± 1.5	9.0 ± 4.4$^{\dagger L}$	1.1 ± 1.8	1.7 ± 1.9^{**L}
Legs Pain (cm)	1.4 ± 1.7	10.0 ± 4.2$^{\dagger L}$	1.2 ± 1.5	2.5 ± 3.2^{**L}
Arms Pain (cm)	1.2 ± 1.7	8.8 ± 4.4$^{\dagger L}$	1.1 ± 1.5	1.3 ± 1.5^{**L}
Overall Fatigue (cm)	1.8 ± 1.4	10.4 ± 3.8$^{\dagger L}$	1.5 ± 1.5	3.7 ± 3.5^{**L}
Legs Fatigue (cm)	1.7 ± 1.6	10.5 ± 4.3$^{\dagger L}$	1.4 ± 1.2	3.3 ± 4.1^{**L}
Arms Fatigue (cm)	1.3 ± 1.7	9.1 ± 4.6$^{\dagger L}$	0.6 ± 0.8	0.8 ± 0.9^{**L}

Mean ± SD. Differences between pre and post measurements († p < 0.01). Differences between experimental and control group in post-race moment (p < 0.05; ** p < 0.01). S = Small ES; L = Large ES.*

Table 3. Characteristics of recreational and competitive cyclists.

Characteristics	**Recreational (N = 10)**	**Competitive (N = 8)**	**P Value**
Age (years)	48 ± 8	43 ± 12	0.289
Height (cm)	174 ± 6	181 ± 8	0.052
Body Mass (kg)	77.8 ± 10.0	79.4 ± 11.3	0.754
Body Mass Index (kg/m^2)	26 ± 4	24 ± 2	0.272
Adipose Tissue (%)	20.1 ± 6.4	16.4 ± 8.6	0.308
Skeletal Muscle (%)	58.7 ± 6.9	62.9 ± 9.0	0.282

Mean ± SD.

Ultra-endurance event response according to profile and cluster - No statistical differences were obtained in any of the variables assessed between recreational and competitive cyclists (Table 4) (p > 0.05). Distance performed is the only variable which presented statistical differences between groups, showing a lower distance performance in recreational than in competitive cyclists (279.4 ± 39.7 km vs. 371.0 ± 71.7 km; ES = 1.6; p < 0.01).

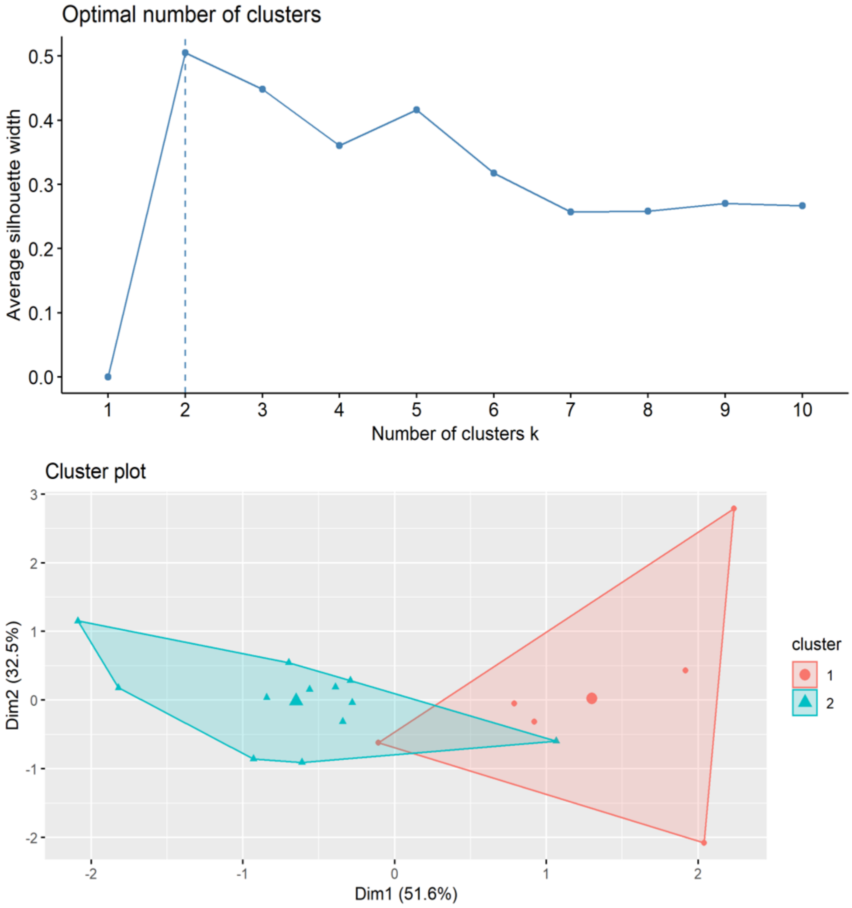

Figure 2. Clustering of participants using k-means. (A) Optimal number of clusters determination using silhouette method for the data and (B) participants distribution where points in the same color correspond to the same cluster.

No differences were observed in ΔJump Height between the recreational and control groups (p > 0.05), but a greater Jump Height decrease was observed in competitive cyclists than in the control group (p < 0.05). Regarding perceptive variables, recreational and competitive cyclists presented greater variations in all the variables than the control group (p < 0.01). In addition, recreational cyclists presented more years (age) than the control group (p < 0.001). No statistical differences (p > 0.05) were observed between recreational, competitive and control group in the remaining variables: ΔLactate, ΔFORD, Height, Body Mass, Adipose Tissue, Muscle Mass and BMI.

The differences between clusters are presented in Table 5. Generally, Cluster 1 presented the greater pain perceived after the competition, and Cluster 2 was the group with the greatest jump height decrease, and greatest fatigue perceived after the competition (ES > 0.5; p < 0.05). No differences were observed in distance performed, lactate, FORD, and anthropometric characteristics (height, body mass, adipose tissue, muscular mass, body mass index) between groups ($p > 0.05$). Moreover, no differences were observed between clusters ($p = 0.6$) in the distance performed during the competition.

Table 4. Differences between Recreational and Competitive cyclists.

Characteristic	Recreational	Competitive	Control
Δ Jump Height (cm)	-1.0 ± 2.2	-2.8 ± 2.8	$0.6 \pm 1.9^{\dagger L}$
Δ Lactate (mmol/L)	0.6 ± 1.8	0.5 ± 0.8	-0.3 ± 0.7
Δ FORD (mmol/L)	-0.2 ± 0.6	0.2 ± 0.4	0.02 ± 0.67
Δ Overall Pain	8.03 ± 4.10	7.8 ± 4.5	$0.6 \pm 1.1^{\#\#\#\dagger\dagger L}$
Δ Legs Pain	8.5 ± 4.1	8.8 ± 4.6	$1.4 \pm 3.2^{\#\#\dagger\dagger L}$
Δ Arms Pain	7.4 ± 4.4	7.8 ± 4.2	$0.2 \pm 0.3^{\#\#\dagger\dagger\dagger L}$
Δ Overall Fatigue	8.8 ± 4.1	8.3 ± 4.5	$2.3 \pm 3.2^{\#\#\dagger\dagger L}$
Δ Legs Fatigue	8.7 ± 4.3	9.1 ± 4.9	$1.9 \pm 3.8^{\#\#\dagger\dagger L}$
Δ Arms Fatigue	8.2 ± 4.2	7.3 ± 4.6	$0.1 \pm 0.2^{\#\#\#\dagger\dagger L}$
Age (years)	47.9 ± 8.2	42.8 ± 11.7	$31 \pm 8^{\#\#\# L}$
Height (cm)	173.9 ± 6.4	181.0 ± 8.0	176 ± 10
Body Mass (kg)	77.8 ± 10.0	79.4 ± 11.3	78 ± 16
Adipose Tissue (%)	20.1 ± 6.4	16.4 ± 8.6	18 ± 9
Muscular Mass (kg)	58.7 ± 6.9	62.9 ± 9.0	57 ± 10
BMI (kg/m²)	25.8 ± 3.5	24.2 ± 2.3	25.2 ± 4.5
Distance Performed (km)	279.4 ± 39.7	$371.0 \pm 71.7^{**L}$	0 ± 0
Speed Lost (%)	14.7 ± 8.8	15.6 ± 7.4	0 ± 0
Fast Lap (Km/h)	35.0 ± 4.3	39.3 ± 3.3	0 ± 0

*Mean ± SD. Δ = Variation (post-pre). Differences between Recreational and Competitive in each variable (** $p < 0.01$). Differences between Recreational and Competitive in each variable (** $p < 0.01$). Differences between Recreational and Control Group in each variable (# $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$). Differences between Competitive and Control Group in each variable († $p < 0.05$; †† $p < 0.01$; ††† $p < 0.001$). L = Large ES.*

Table 5. Differences between the cluster groups.

Characteristic	Cluster 1	Cluster 2	Control	P Value
Δ Jump Height (cm)	1.2 ± 0.8	$-3.3 \pm 1.6^{***L}$	$0.6 \pm 1.9^{\dagger\dagger L}$	**<0.001**
Δ Lactate (mmol/L)	-0.7 ± 2.5	0.2 ± 0.7	-0.3± 0.7	0.4
Δ FORD (mmol/L)	-0.3 ± 0.5	0.1 ± 0.5	0.02 ± 0.67	0.2
Δ Overall Pain	$5.3 \pm 2.9^{\#\# L}$	9.3 ± 4.2	$0.6 \pm 1.1^{\dagger\dagger\dagger L}$	**<0.001**
Δ Legs Pain	$6.6 \pm 4.1^{\# M}$	9.6 ± 4.0	$1.4 \pm 3.2^{\dagger\dagger M}$	**0.004**
Δ Arms Pain	$6.8 \pm 3.4^{\#\# L}$	8.0 ± 4.6	$0.2 \pm 0.3^{\dagger\dagger\dagger L}$	**0.001**
Δ Overall Fatigue	5.5 ± 3.6	$10.2 \pm 3.6^{*M}$	$2.3 \pm 3.2^{\dagger\dagger L}$	**0.002**
Δ Legs Fatigue	6.5 ± 3.9	10.1 ± 4.3	$1.9 \pm 3.8^{\dagger\dagger M}$	**0.006**
Δ Arms Fatigue	$6.8 \pm 3.4^{\# L}$	8.3 ± 4.7	$0.1 \pm 0.2^{\dagger\dagger\dagger L}$	**0.001**
Age (years)	$50 \pm 11^{\# L}$	43 ± 9	$31 \pm 8^{\dagger L}$	**0.007**
Height (cm)	175.7 ± 7.9	177.8 ± 8.1	176 ± 10	0.8
Body Mass (kg)	79 ± 11	78 ± 10	78 ± 16	>0.9
Adipose Tissue (%)	23 ± 8	16 ± 7	18 ± 9	0.3
Muscular Mass (kg)	58 ± 9	62 ± 7	57 ± 10	0.4
BMI (kg/m²)	25.76 ± 4.09	24.73 ± 2.51	25.2 ± 4.5	0.8
Distance Performed (km)	302 ± 67	329 ± 75	0 ± 0	0.6
Speed Lost (%)	13.9 ± 8.1	15.7 ± 8.2	0 ± 0	0.7
Fast Lap (Km/h)	35.5 ± 4.9	37.7 ± 4.0	0 ± 0	0.3

Mean ± SD. Δ = Variation (post-pre). Differences between Group 1 and Group 2 in each variable ($p < 0.05$). Differences between Group 1 and Control Group in each variable (# $p < 0.05$; ## $p < 0.01$). Differences between Group 2 and Control Group in each variable († $p < 0.05$; †† $p < 0.01$; ††† $p < 0.001$). S = Small ES; M = Moderate ES; L = Large ES.*

4. Discussion

The aim of this study was to assess the differences between clusters obtained by the acute fatigue effect after a cycling ultra-endurance event. The main results of this study were that 1) 12-h of cycling did not alter FORD and lactate values but jump height and fatigue/pain perception were the main outcomes altered, 2) recreational and competitive cyclists did not differ in their response to the event, and 3) non-supervised clustering provided two groups depending mainly on CMJ decrease, these groups also differing in pain and fatigue variation.

Ultra-endurance events are highly demanding activities, and the decrease in jump height, together with increased values of pain and fatigue perception, support this insight. However, the physiological markers measured (lactate and FORD) did not show any statistical difference. Previous studies of ultra-marathon events observed greater values of lactate in athletes following the race (Mrakic et al., 2015; Wolff et al., 2022), contrasting with our results. These studies were performed during trial running and their lactate value suggested a high contribution of anaerobic metabolism (Mrakic et al., 2015). However, Suárez et al. (2011) did not obtain variations on lactate values after completing a 20-hour ultra-endurance event of kayak and cycling. One possible explanation may be that these long-duration events are performed under low intensities due to the difficulty of maintaining high intensities, so reducing the accumulation of lactate (Suárez et al., 2011) and lactate production (Yang et al., 2020). It could also be due to a depletion of intramyofibrillar glycogen produced by a long-duration exercise (Ørtenblad et al., 2013), a decrease in available glucose, a precursor of lactate, which would also lead to this decrease in lactate during exercise (Yang et al., 2020). This discrepancy between results suggests the need for further research to clarify lactate behavior after ultra-endurance exercise. Future studies should measure muscle glycogen following ultra-endurance events in order to understand why lactate does not increase in this type of event, either because of glycogen depletion or because of the low intensity which does not demand these energy pathways. Moreover, the changes observed in plasma antioxidant capacity in other studies were produced in high-intensity activities (Lewis et al., 2016), as opposed to the present study. In this sense, the stress hormones released in response to high intensity exercise increase plasma vitamin C from the adrenal glands and that has an effect on plasma antioxidant capacity (Lewis et al., 2016).

We classified the cyclists of the study according to their weekly training (Priego et al., 2018) in order to study whether the acute effect of an ultra-endurance event was related with training characteristics. However, no statistical differences were observed in any variables between the profiles established ($p > 0.05$). The classification applied only considered the number of kilometers accumulated in a week by amateur cyclists but did not consider other aspects related to training (e.g., intensity) or cycling performance (e.g., FTP). It seems, therefore, that this classification is not enough to distinguish physiological differences between the cyclists included in the study. However, it is important to take into account that this classification was performed only to help in the recruitment of participants based on demographic data (Priego et al., 2018).

With the aim of assessing whether there are different groups according to the outcomes of an ultra-endurance event, we conducted a K-means clustering model. This process divided the cyclists into two groups, and the statistical differences suggest that they were classified according to Δ Jump Height. The groups, however, also differed in Δ of perceived pain and fatigue. So, Cluster 1 was associated with the greatest pain perceived after the competition, and Cluster 2 with the greatest jump height decrease, and higher fatigue perceived following the competition. However, no differences were obtained in the performance markers (speed loss and fastest lap) between groups ($p > 0.05$). Our results suggest that an ultra-endurance event may trigger fatigue mechanisms related with muscle damage or overall fatigue. Previous studies in ultra-trail events have reported the presence of muscle damage in one day (Schenk et al., 2021) and in stage races (Lecina et al., 2022), obtaining greater values of muscle damage in the races of greater durations and elevation-gain due to the greater presence of eccentric contractions (Lecina et al., 2022; Schenk et al., 2021). However, cycling time-trials produce more muscle damage than mountain stages during the Tour de France despite being of shorter durations (Gómez et al., 2003). Therefore, these studies suggest that muscle damage after ultra-endurance events not only depends on duration but is also related to intensity, specifically in cycling activities. Hence, the results suggest that participants of Cluster 2 may have ridden the event at greater intensity due to the greater fatigue reflected in jump decrease and subjective measurements. However, the absence of data about heart rate or power output intensity zones makes it difficult to fully support this statement.

One of the limitations of this study is the lack of information about internal and external load data during the race (e.g., power output and heart rate) and training data. Furthermore, the self-selected nutritional strategies may affect plasma antioxidants and lactate assessment, due to the presence of antioxidant compounds in sports supplements and the postprandial release of lactate. Moreover, the absence of data about oxidative stress biomarkers makes it difficult to reach conclusions from FORD results. Finally, as the control groups were attendees at the competition and not participants, and we did not have the possibility to do a proper selection that took into account the characteristics of the experimental group, they presented a lower age and weekly training volume than the experimental group. Although we believe that this did not interfere with our results, it can be considered as a limitation of the study. Further research is necessary to establish nutritional strategies, including oxidative stress biomarkers, antioxidant capacity, and more continuous data on internal and external load during the competition.

5. Practical Applications.

The increase of fatigue and pain perception and decrease in jump eight suffered by the cyclists in comparison with control group after completing a 12-h non-stop cycling event confirms that the assessment of jump height with CMJ and perception outcomes are good non-invasive markers to detect fatigue. CMJ has been applied previously to predict fatigue and the studies have concluded that CMJ is a strong predictor of muscle fatigue (Dawson et al., 2015; Wu et al., 2019). CMJ presents some benefits such as being a simple, effective and popular performance-monitoring test for individual and team sport athletes (Claudino et al., 2017). Fatigue and pain perception have been highlighted as important outcomes to measure during cycling, as they can have an effect on power output and be the factor limiting the time before exhaustion (Salam et al., 2018; Staiano et al., 2018).

6. Conclusions

Cluster analysis reveals that CMJ and fatigue/pain perception can differentiate the fatigue produced by an ultra-endurance event, so making these non-invasive measurements useful in fatigue monitoring and recovery planning. Nevertheless, internal load markers such as lactate and plasma antioxidant capacity do not seem to be adequate markers in fatigue assessment during ultra-endurance events. Cyclists' profiles, based on demographic and training characteristics, failed to predict fatigue following an ultra-endurance event, suggesting that new classifications related with other variables should be considered in future studies.

Funding: Consejo Superior de Deportes, Ministerio de Cultura y Deporte, Gobierno de España" (2083210_2021 - Ayudas para Investigación 37/UPB/21..

Acknowledgments: We would like to thank the participants of the study for their important contribution.

Conflicts of Interest: The authors declare no conflict of interest.

References

Bescós, R., Rodríguez, F. A., Iglesias, X., Knechtle, B., Benítez, A., Marina, M., Padullés, J. M., Vázquez, J., & Torrado, P. (2011). Physiological demands of cyclists during an ultra-endurance relay race: A field study report. Chinese Journal of Physiology, 54(5), 339–346. https://doi.org/10.4077/CJP.2011.AMM065

Bini, R. R., Dagnese, F., Rocha, E., Silveira, M. C., Carpes, F. P., & Mota, C. B. (2016). Three-dimensional kinematics of competitive and recreational cyclists across different workloads during cycling. European Journal of Sport Science, 16(5), 553–559. https://doi.org/10.1080/17461391.2015.1135984

Carins, S., Knicker, A. J., Thompson, M. W., & Sjøgaard, G. (2005). Evaluation of Models Used to Study Neuromuscular Fatigue: Exercise and Sport Sciences Reviews. Exercise and Sport Sciences Reviews, 33(1), 9–16.

Chapman, A. R., Vicenzino, B., Blanch, P., & Hodges, P. W. (2008). Patterns of leg muscle recruitment vary between novice and highly trained cyclists. J Electromyogr Kinesiol, 18(3), 359–371. https://doi.org/10.1016/j.jelekin.2005.12.007

Claudino, J. G., Cronin, J., Mezêncio, B., McMaster, D. T., McGuigan, M., Tricoli, V., Amadio, A. C., & Serrão, J. C. (2017). The countermovement jump to monitor neuromuscular status: A meta-analysis. Journal of Science and Medicine in Sport, 20(4), 397–402. https://doi.org/10.1016/J.JSAMS.2016.08.011

Clemente-Suarez, V. J. (2014). Changes in Biochemical, Strength, Flexibility, and Aerobic Capacity Parameters after a 1700 km Ultraendurance Cycling Race. BioMed Research International, 2014, 602620. https://doi.org/10.1155/2014/602620

Clermont, C. A., Benson, L. C., Edwards, W. B., Hettinga, B. A., & Ferber, R. (2019). New Considerations for Wearable Technology Data: Changes in Running Biomechanics During a Marathon. Journal of Applied Biomechanics, 35(6), 401–409. https://doi.org/10.1123/JAB.2018-0453

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences (Second). Lawrance Eribaum Associates.

Coyle, E. F., Feltner, M. E., Kautz, S. A., Hamilton, M. T., Montain, S. J., Baylor, A. M., Abraham, L. D., & Petrek, G. W. (1991). Physiological and biomechanical factors associated with elite endurance cycling performance. Medicine and Science in Sports and Exercise, 23(1), 93–107.

Dantas, R., Caputo, F., Mendes, K., Sigwalt, A. R., Ghisoni, K., Lock, P. C., Remor, A. P., da Luz, D., Antonacci, L. G., & Latini, A. (2014). Increased platelet oxidative metabolism, blood oxidative stress and neopterin levels after ultra-endurance exercise. Journal of Sports Sciences, 32(1), 22–30. https://doi.org/10.1080/02640414.2013.797098

Dawson, D., Fletcher, A., & Hussey, F. (2015). Fatigue and Transportation Report To the Neville Committee. 29(1), 37–46.

Gandia, A., Carpes, F. P., Rodríguez, A., & Priego, J. I. (2020). Effect of cycling specialization on effort and physiological responses to uphill and flat cycling at similar intensity. European Journal of Sport Science, 1–7. https://doi.org/10.1080/17461391.2020.1785016

García-López, J., Díez-Leal, S., Ogueta-Alday, A., Larrazabal, J., & Rodríguez-Marroyo, J. A. (2016). Differences in pedalling technique between road cyclists of different competitive levels. Journal of Sports Sciences, 34(17), 1619–1626. https://doi.org/10.1080/02640414.2015.1127987

Gómez, M. C., Pallardó, F. V., Sastre, J., Viña, J., & Garcia, L. (2003). Allopurinol and Markers of Muscle Damage Among Participants in the Tour de France. JAMA, 289(19), 2503–2504. https://doi.org/10.1001/JAMA.289.19.2503-B

Hartigan, J. A. ., & Wong, M. . A. . (1979). Algorithm AS 136: A K-Means Clustering Algorithm Author (s): J Published by: Wiley for the Royal Statistical Society Stable URL : http://www.jstor.org/stable/2346830. Journal of the Royal Statistical Society: Series C (Applied Statistics), 28(1), 100–108.

Lecina, M., Castellar, C., Pradas, F., & López, I. (2022). 768-km Multi-Stage Ultra-Trail Case Study-Muscle Damage, Biochemical Alterations and Strength Loss on Lower Limbs. International Journal of Environmental Research and Public Health, 19(2). https://doi.org/10.3390/ijerph19020876

Lewis, N. A., Simpkin, A. J., Moseley, S., Turner, G., Homer, M., Redgrave, A., Pedlar, C. R., & Burden, R. (2020). Increased Oxidative Stress in Injured and Ill Elite International Olympic Rowers. International Journal of Sports Physiology and Performance, 15(5), 625–631. https://doi.org/10.1123/IJSPP.2019-0425

Lewis, N. A., Towey, C., Bruinvels, G., Howatson, G., & Pedlar, C. R. (2016). Effects of exercise on alterations in redox homeostasis in elite male and female endurance athletes using a clinical point-of-care test. Https://Doi.Org/10.1139/Apnm-2016-0208, 41(10), 1026–1032. https://doi.org/10.1139/APNM-2016-0208

Maunder, E., Seiler, S., Mildenhall, M. J., Kilding, A. E., & Plews, D. J. (2021). The Importance of 'Durability' in the Physiological Profiling of Endurance Athletes. Sports Medicine, 51(8), 1619–1628. https://doi.org/10.1007/S40279-021-01459-0/FIGURES/3

Mrakic, S., Gussoni, M., Moretti, S., Pratali, L., Giardini, G., Tacchini, P., Dellanoce, C., Tonacci, A., Mastorci, F., Borghini, A., Montorsi, M., & Vezzoli, A. (2015). Effects of mountain ultra-marathon running on ROS production and oxidative damage by micro-invasive analytic techniques. PLoS ONE, 10(11), 1–19. https://doi.org/10.1371/journal.pone.0141780

Mündermann, A., Nigg, B. M., Stefanyshyn, D. J., & Humble, R. N. (2002). Development of a reliable method to assess footwear comfort during running. Gait & Posture, 16(1), 38–45. https://doi.org/10.1016/S0966-6362(01)00197-7

Nikolaidis, P. T., Knechtle, B., Vancini, R., Gomes, M., & Sousa, C. (2021). Participation and Performance in the Oldest Ultramarathon-Comrades Marathon 1921-2019. International Journal of Sports Medicine, 42(7), 638–644. https://doi.org/10.1055/a-1303-4255

Ørtenblad, N., Westerblad, H., & Nielsen, J. (2013). Muscle glycogen stores and fatigue. The Journal of Physiology, 591(18), 4405–4413. https://doi.org/10.1113/JPHYSIOL.2013.251629

Pavlatou, M. G., Papastamataki, M., Apostolakou, F., Papassotiriou, I., & Tentolouris, N. (2009). FORT and FORD: two simple and rapid assays in the evaluation of oxidative stress in patients with type 2 diabetes mellitus. Metabolism, 58(11), 1657–1662. https://doi.org/10.1016/J.METABOL.2009.05.022

Petrigna, L., Karsten, B., Marcolin, G., Paoli, A., D'Antona, G., Palma, A., & Bianco, A. (2019). A Review of Countermovement and Squat Jump Testing Methods in the Context of Public Health Examination in Adolescence: Reliability and Feasibility of Current Testing Procedures. Frontiers in Physiology, 10. https://www.frontiersin.org/articles/10.3389/fphys.2019.01384

Priego, J. I., Kerr, Z. Y., Bertucci, W. M., & Carpes, F. P. (2018). The categorization of amateur cyclists as research participants: Findings from an observational study. Journal of Sports Sciences, 36(17), 2018–2024.

https://doi.org/10.1080/02640414.2018.1432239

Rousseeuw, P. J. (1987). Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. Journal of Computational and Applied Mathematics, 20(C), 53–65. https://doi.org/10.1016/0377-0427(87)90125-7

Salam, H., Marcora, S. M., & Hopker, J. G. (2018). The effect of mental fatigue on critical power during cycling exercise. Eur. J. Appl. Physiol., 118(1), 85–92. https://doi.org/10.1007/s00421-017-3747-1

Scheer, V. (2019). Participation trends of ultra endurance events. Sports Medicine and Arthroscopy Review, 27(1), 3–7. https://doi.org/10.1097/JSA.0000000000000198

Schenk, K., Rauch, S., Procter, E., Grasegger, K., Mrakic-Sposta, S., & Gatterer, H. (2021). Changes in Factors Regulating Serum Sodium Homeostasis During Two Ultra-Endurance Mountain Races of Different Distances: 69km vs. 121km. Frontiers in Physiology, 12(November). https://doi.org/10.3389/fphys.2021.764694

Shoak, M., Knechtle, B., Knechtle, P., Rüst, C., Rosemann, T., & Lepers, R. (2013). Participation and performance trends in ultracycling. Open Access Journal of Sports Medicine, 4, 41. https://doi.org/10.2147/OAJSM.S40142

Smith, K. A., Kisiolek, J. N., Willingham, B. D., Morrissey, M. C., Leyh, S. M., Saracino, P. G., Baur, D. A., Cook, M. D., & Ormsbee, M. J. (2020). Ultra-endurance triathlon performance and markers of whole-body and gut-specific inflammation. European Journal of Applied Physiology, 120(2), 349–357. https://doi.org/10.1007/S00421-019-04279-3

Staiano, W., Bosio, A., de Morree, H. M., Rampinini, E., & Marcora, S. (2018). Chapter 11 - The cardinal exercise stopper: Muscle fatigue, muscle pain or perception of effort? In S. Marcora & M. Sarkar (Eds.), Progress in Brain Research (Vol. 240, pp. 175–200). Elsevier. https://doi.org/10.1016/bs.pbr.2018.09.012

Stelzer, I., Kröpfl, J. M., Fuchs, R., Pekovits, K., Mangge, H., Raggam, R. B., Gruber, H. J., Prüller, F., Hofmann, P., Truschnig-Wilders, M., Obermayer-Pietsch, B., Haushofer, A. C., Kessler, H. H., & Mächler, P. (2015). Ultra-endurance exercise induces stress and inflammation and affects circulating hematopoietic progenitor cell function. Scandinavian Journal of Medicine and Science in Sports, 25(5), e442–e450. https://doi.org/10.1111/SMS.12347

Suárez, V. C., Valdivielso, F. N., & Ravé, J. M. G. (2011). Changes in biochemical parameters after a 20-hour ultra-endurance kayak and cycling event. International SportMed Journal, 12(1), 1–6.

Tanner, R. K., Fuller, K. L., & Ross, M. L. R. (2010). Evaluation of three portable blood lactate analysers: Lactate Pro, Lactate Scout and Lactate Plus. European Journal of Applied Physiology, 109(3), 551–559. https://doi.org/10.1007/S00421-010-1379-9/FIGURES/5

Tomczak, M., & Tomczak, E. (2014). The need to report effect size estimates revisited. An overview of some recommended measures of effect size. Trends in Sport Sciences, 1(21), 19–25.

Truppa, L., Guaitolini, M., Garofalo, P., Castagna, C., & Mannini, A. (2020). Assessment of Biomechanical Response to Fatigue through Wearable Sensors in Semi-Professional Football Referees. Sensors (Basel, Switzerland), 21(1), 1–15. https://doi.org/10.3390/S21010066

Turner, J. E., Bennett, S. J., Bosch, J. A., Griffiths, H. R., & Aldred, S. (2014). Ultra-endurance exercise: Unanswered questions in redox biology and immunology. Biochemical Society Transactions, 42(4), 989–995. https://doi.org/10.1042/BST20140120

van der Zwaard, S., de Ruiter, C. J., Jaspers, R. T., & de Koning, J. J. (2019). Anthropometric Clusters of Competitive Cyclists and Their Sprint and Endurance Performance. Frontiers in Physiology, 10, 1276. https://doi.org/10.3389/FPHYS.2019.01276/BIBTEX

Vitti, A., Nikolaidis, P. T., Villiger, E., Onywera, V., & Knechtle, B. (2020). The 'New York City Marathon': Participation and performance trends of 1.2M runners during half-century. Research in Sports Medicine (Print), 28(1), 121–137. https://doi.org/10.1080/15438627.2019.1586705

Wolff, S., Picco, J. M., Díaz, L., Valenzuela, P. L., Gonzalez, E., Santos, A., Matile, P., Wolff, D., Boraita, A., & Lucia, A. (2022). Exercise-Induced Cardiac Fatigue in Recreational Ultramarathon Runners at Moderate Altitude: Insights From Myocardial Deformation Analysis. Frontiers in Cardiovascular Medicine, 8(January), 1–8. https://doi.org/10.3389/fcvm.2021.744393

Wu, P. P. Y., Sterkenburg, N., Everett, K., Chapman, D. W., White, N., & Mengersen, K. (2019). Predicting fatigue using countermovement jump force-time signatures: PCA can distinguish neuromuscular versus metabolic fatigue. PLoS ONE, 14(7), 1–16. https://doi.org/10.1371/journal.pone.0219295

Yang, W. H., Park, H., Grau, M., & Heine, O. (2020). Decreased Blood Glucose and Lactate: Is a Useful Indicator of Recovery Ability in Athletes? International Journal of Environmental Research and Public Health, 17(15), 1–16. https://doi.org/10.3390/IJERPH17155470