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Fatigue effects on angular kinematics in male recreational runners grouped by functional response

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Abstract

Background While current evidence on injury risk factors remains limited, this study aims to provide insights into how fatigue-induced changes in biomechanical risk factors (BRF) differ between runners, potentially offering a new approach to understanding the development of running-related injuries. Thirty-nine ($N=39$) male recreational runners underwent analysis of lower leg angular kinematics, heart rate, blood lactate levels, and perceived effort before and after a 30-minute exhaustive continuous treadmill running test.

Results Three functional groups (FG) were identified using the K-means algorithm, which grouped participants based on changes in lower limb angular kinematics between pre- and post-fatigue. While FG1 and FG2 exhibited similar behaviours to maintain their usual running dynamics (e.g. no significant changes in hip flexion at touchdown and toe-off, and similar reductions in leg stiffness after fatigue), FG3 showed more pronounced changes, including increased hip flexion (7.4%) and knee flexion (21%) at touch-down, as well as increased knee flexion at maximal knee flexion (6%) and at the toe-off instant (9%) during the running cycle.

Conclusions Fatigue-induced alterations in the considered biomechanical risk factors allow for the functional grouping of recreational athletes. Changes in FG3 impact running patterns and alter running economy-related variables, which may be associated with an increased injury risk and could guide future research into tailored training and preventive strategies.

Key Points

- This study successfully identified three distinct functional groups among healthy recreational runners based on fatigue-related changes in biomechanical risk factors (BRF).
- While two functional groups (FG1 and FG2) displayed similar behaviours to maintain their usual running dynamics, a third group (FG3) exhibited more pronounced changes in hip and knee angular displacement during the running cycle, significantly impacting running economy and altering running patterns.
- The findings suggest that FG3 may be at a higher risk of injury due to the observed biomechanical alterations, highlighting the importance of considering fatigue-related changes in BRF when assessing injury risk in runners.

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Keywords Fatigue, Running, Clustering, Biomechanics, Risk factors

Background

Running is increasingly recognized as a simple yet effective means to enhance health, physical fitness, and psychological well-being, including improvements in aerobic power, body composition, cardiovascular health, muscle strength, mobility, self-confidence, and reductions in health risk factors and substance intake [1].

However, there is a high incidence rate of running-related injuries (RRI) [2], primarily focused on the lower extremities, with the knee, along with the ankle and foot, being the anatomical locations most affected by injuries [3, 4]. Researchers have long studied RRI factors, classifying them into intrinsic (e.g., sex, body mass) and extrinsic (e.g., training volume, intensity) risk factors categories [4]. Despite advances in correlating risk factors with the occurrence of RRI, the evidence level remains low [5]. The lack of homogeneity in protocols (e.g. intensities, duration, variables, etc.), more narrowed approaches (e.g. sample features, performance level, etc.), or methodological shortcomings are some of the barriers to determining risk factors with high evidential value [3, 6].

The influence of fatigue has been suggested as a potential contributor to changes leading to the occurrence of injuries [7, 8]. It affects RRI by altering running mechanics, muscle activation, ground reaction forces, joint kinematics, and plantar pressure distribution, potentially increasing the risk and changing the nature of injuries [6, 7]. Many of these fatigue-related changes are observed at the knee joint, affecting movement in the sagittal plane [8] as well as other axes [7].

Despite advancements in understanding these factors, preventing RRI remains a complex challenge, necessitating ongoing research and multidisciplinary approaches. A recent strategy involves identifying running-related biomechanical risk factors (BRF) to differentiate between athletes who are at risk of injury and those who have already sustained injuries. BRF has been presented in the literature as changes in biomechanical responses, typically in discrete variables, that differ between healthy and injured runners and could be linked to the appearance of new or concomitant injuries. Identifying these risk factors helps in designing interventions or training programs to prevent injuries and optimize performance by improving the biomechanics of movement [9, 10]. Recent studies [9, 10] have identified BRF that may predispose athletes to injuries. Additionally, recent research suggests the existence of functional groups (FG) among runners who share similarities in their technique, offering a more effective approach to grouping and analysing athletes [11]. Research on FG can help identify individuals who respond similarly to interventions like specific footwear

[12], aiding in the identification of specific BRF for particular groups. In the past, classification was often done using basic grouping variables, such as age, level and/or sex [12]. However, it has been shown that BRFs are only partially affected by this basic pre-grouping [9, 10]. Consequently, a more sophisticated framework is required to identify FG.

In this regard, there are many data analysis techniques that allow athletes to be grouped based on their functional responses [13]. Unsupervised learning techniques for clustering can utilize multivariate datasets instead of isolating a single variable and condition [11], allowing for a more comprehensive description of an athlete's running technique and profile. A machine learning technique, k-means clustering, has been utilized in previous studies within the field of sports biomechanics to analyse the effect of different footwear interventions depending on FG, showing varying behaviours depending on the group classification and identifying one group with a higher risk of injury [12].

The specific factors contributing to RRI can vary significantly among individuals. Although the catalytic effect of fatigue on running biomechanics is well-documented, few studies have examined its impact on groups of healthy recreational runners classified by their biomechanical response to fatigue in specific BRF-related kinematic variables. Understanding how fatigue impacts movement patterns could offer valuable insights into the prevention of injuries. Given the lack of success with approaches over the last 40 years [13], exploring fatigue's role may help develop more effective treatments and preventive interventions in the future. This could lead to improved strategies for managing athlete performance and reducing injury rates.

The aim of this study is to investigate the effects of fatigue on lower limb angular kinematics during running in recreational athletes, grouped by functional response, to better understand how fatigue affects running biomechanics. We hypothesize that fatigue will lead to significant changes in angular kinematics, particularly in variables related to knee joint movement. Additionally, we anticipate that athletes grouped into different categories will demonstrate varying degrees of alteration in angular kinematics, with those exhibiting a more adverse response likely to experience greater changes.

Methods

Participants

Based on previous studies [8], sample size calculation was performed a priori using G-Power software (version 3.1.9.7, Düsseldorf, Germany). The analysis showed

that a repeated-measures ANOVA analysis would require a minimum of 39 participants to detect a significant within-between interaction with a large effect size ($f=0.77$; power=0.953, $\alpha=0.05$, and $\beta=0.15$). The sample was recruited using an informational poster and an online registration form, shared across various sports and academic spaces, social media, and running-related establishments and centers. The poster featured a QR code linking to the online form, where participants provided necessary details for the study (injuries, medications, medical conditions, availability) along with sports-related information (weekly running distance, skill level, training frequency, etc.). Finally, the study involved a sample of 39 recreational male runners (age: 29.6 ± 10.7 years, height: 1.7 ± 0.1 m, weight: 73.3 ± 8.6 kg, BMI: 24 ± 2.6 kg/m², weekly running distance: 38.4 ± 17.9 km/week, Maximal Aerobic Speed (MAS): 16.6 ± 1.8 km/h). All participants met the study inclusion criteria: they were recreational runners aged between 18 and 50 years and were required to have a regular practice level of at least 2–3 days of training per week, with a weekly volume of between 15 and 20 km, have not suffered injuries in the past 6 months or presented any ailments that could impede the study at the time of testing, not be taking medication that could influence their physical or mental capacities, not be undergoing any medical treatment, have no cardiac ailments or diseases, and runners accustomed to running with neutral cushioned shoes to avoid variability due to the type of footwear. Informed written consent was obtained after each participant was given a verbal and a written explanation of the experimental protocol and fully understood the possible risks involved in taking part in the study. Following the ethical principles established in the Declaration of Helsinki, the study protocol was reviewed and approved by the local Ethics Committee of the University of Valencia (No. 2588640).

Procedures

Participants attended the laboratory on two occasions for data collection, with at least 48 h of rest between each session, and after a period of 48 h without any high-intensity exercise. During the first session, the sample was characterized, while during the second session, a 30-minute exhaustive continuous running test was conducted on the treadmill, during which study variables were recorded (Fig. 1). After registering pre-post fatigue data, different functional groups were identified using the unsupervised clustering algorithm K-means, based on specific BRFs [10], and comparisons were made between the identified functional groups [12]. All participants wore their own shoes during both sessions to avoid potential interference from using standardized footwear different from their usual.

During the first session, participants' weight was recorded using a scale (Tanita BIA Technology, Amsterdam, The Netherlands), and height was measured using a mechanical scale (Asimed Barys Plus, Sibel, S.A.U, Barcelona, Spain). Body Mass Index (BMI) was calculated using the formula: BMI = Weight (kg) / Height (m)². Additionally, foot posture was assessed using the Foot Postural Index (FPI) [14]. All measurements were performed by the same experienced observer. Intra-observer reliability was previously assessed using a reliability analysis (Cronbach's $\alpha=0.927$) and Intra-Class Correlation Coefficient (ICC = 0.927, 95%CI = 0.790–0.974), demonstrating values above 0.8, ensuring data quality.

After anthropometric variables were taken, Maximal Aerobic Speed (MAS) was determined at least 48 h before laboratory measurements by a maximal effort 5-minute running test on a 400 m track [15], to establish the individual speed for each participant during the 30-minute continuous run test.

In the second session, with a minimum 48-hour interval [8], the biomechanical response during a fatiguing

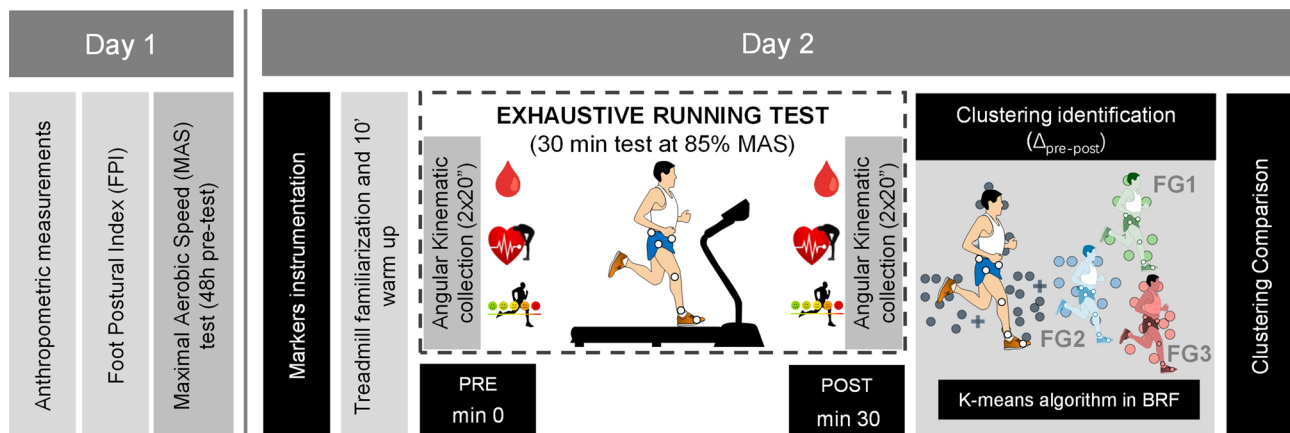


Fig. 1 Procedure employed in the study. PRE: Pre fatigue condition (min 0–5'); POST: post-fatigue condition (min 29–30'); FG: functional group. BRF: biomechanic risk factor, $\Delta_{pre-post}$: differences in each variable between pre and post condition

30-minute continuous running test was recorded, specifically the lower limb angular kinematics, along with heart rate, blood lactate, and subjective rate of perceived exertion (RPE). Prior to the test, participants were instrumented with a set of retro-reflective markers placed on the right leg at the lateral aspects of the greater trochanter, femoral condyle, lateral malleolus, and 5th metatarsal head [8]. Additionally, four posterior markers were placed on the shoe and lower leg for ankle inversion-eversion evaluation (Fig. 2).

After instrumentation, participants completed a 10-minute warm-up during which the speed was progressively increased to allow participants to adapt to running on the treadmill (h/p/cosmos pulsar[®]3p, h/p/cosmos, Nußdorf, Germany). The treadmill used in this study provided a stiff running surface without an integrated impact cushioning system, enabling observation of running mechanics on a firm surface. This setup more closely replicates outdoor running technique on variable terrain, where surface cushioning is inconsistent [16]. All participants had prior experience running on a treadmill. Subsequently, the test consisted of 30 min of continuous running at 80% of each participant's individual MAS, with a 1% treadmill gradient [8] to replicate the metabolic cost of running outdoors [17]. Additionally, studies have shown that treadmill running can produce reliable and comparable physiological responses to outdoor running, making it a suitable alternative for controlled laboratory settings.

During the test, the biomechanical response was recorded twice: at the beginning of the test (minute 0–1'), without fatigue; and at the end of the test (minute

29–30'), with fatigue. Two consecutive 20-second recordings were made at each instant (pre and post fatigue), capturing over 50 complete cycles per instant and condition per participant. Kinematic variables were captured using a 3D infrared motion camera system (Optitrack V120:Trio, NaturalPoint, Inc., Corvallis, OR, USA) operating at 120 Hz. After the kinematic recording, participants' RPE was collected using the Borg 6–20 RPE scale, and heart rate (HR) was recorded using a heart rate monitor (Polar V800, Polar Electro, UK). Blood lactate concentration was measured before and after the 30-minutes running test using a lactate analyser (Lactate Pro 2, Akay Europe BV, Amstelveen, Netherlands). For this purpose, the finger skin was punctured using a lancet (Unistik3, 1.8 mm, Owen Mumford, Woodstock, UK), and the first droplet of blood was wiped away, extracting the sample from the second blood droplet.

Data analysis

Before recording, a standing calibration trial was conducted, and the angular positions of analysed body segments were defined as the anatomical position [8]. Motive software (NaturalPoint, Inc., Corvallis, OR, USA) was utilized to process the initial kinematic data. Marker data underwent filtering with a fourth-order low-pass Butterworth filter with a cutoff frequency of 6 Hz [8, 18]. Once the data were processed, angular variables and spatiotemporal parameters were computed using a custom programme (MatLab R2022b, Mathworks Inc, Natick, MA, USA).

For each running cycle, events such as touch-down (TD) (initial contact), the instance of maximum knee

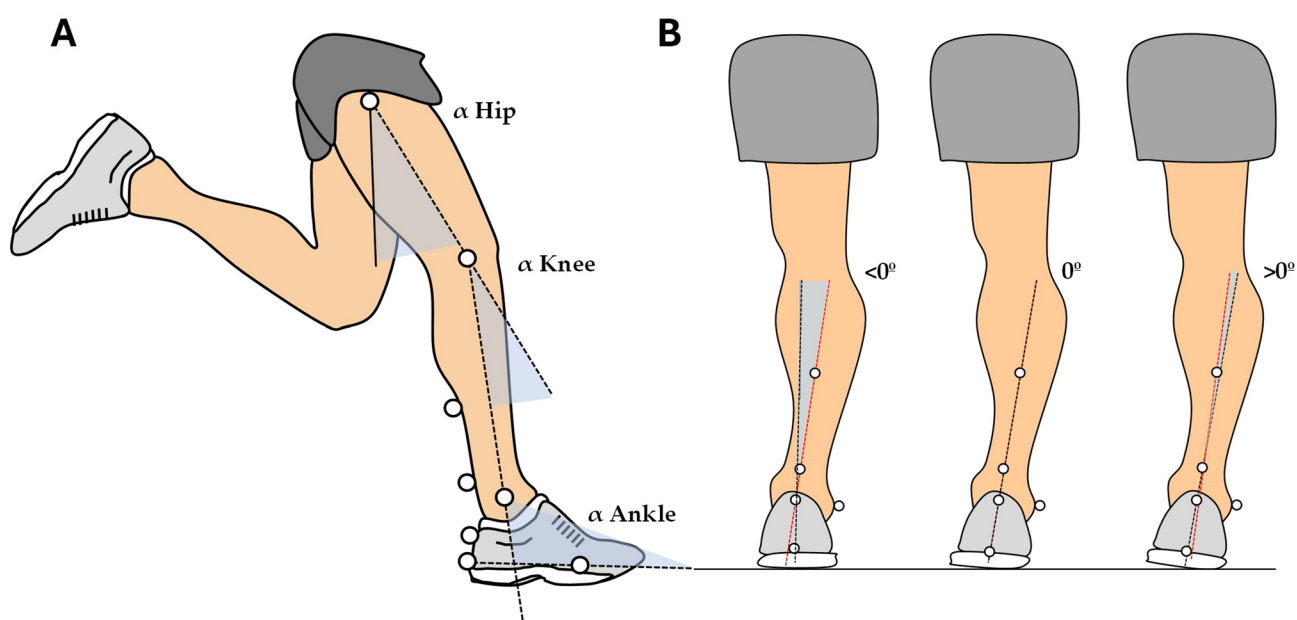


Fig. 2 Schematic representation of the marker set employed in the study. A: sagittal view, B: posterior view

flexion (MKF) (as the transition between absorption and propulsion phases), toe-off (TO), and the mid-swing phase (SW) preceding the next initial contact were detected following the previously employed methodology [8] (Fig. 3) Touch-down (TD) was detected by the point of maximum downward velocity of the trochanter [19]. Maximum knee flexion in stance was identified as the peak between two knee extensions, and toe-off aligned with the second knee extension peak [20]. Maximum swing oscillation was the knee flexion peak between toe-off and initial contact [8].

Utilizing the extracted kinematic variables, spatiotemporal parameters such as stride frequency and length were computed [8]. Additionally, leg stiffness and vertical stiffness were estimated using the method proposed by Morin et al. [21]. Pre-fatigue, post-fatigue values, and the pre-post fatigue difference (Δ_{pre_post}) were extracted for all study variables.

Once the variables were obtained, the participants were segmented into FG based on the variation (Δ_{pre_post} : outcome after fatigue - outcome before fatigue protocol) of BRF, as described in previous studies [10, 12]. For the present study, we selected the BRF of knee angle that presented limited and very limited evidence [9, 10, 22], and leg stiffness that presented moderate evidence with changes related to fatigue [6]. This was achieved by grouping participants using the unsupervised algorithm known as K-means [23] with Matlab software. K-means clustering is a widely used technique in data analysis and machine learning for partitioning datasets into functionally relevant groups [23]. Its popularity stems from its simplicity, efficiency, and effectiveness in handling large datasets [24]. Prior to grouping, the silhouette method was used to determine the optimal number of clusters [25]. The silhouette method assesses the quality of clusters by comparing the similarity of each object to its own cluster versus others. To achieve this, the method

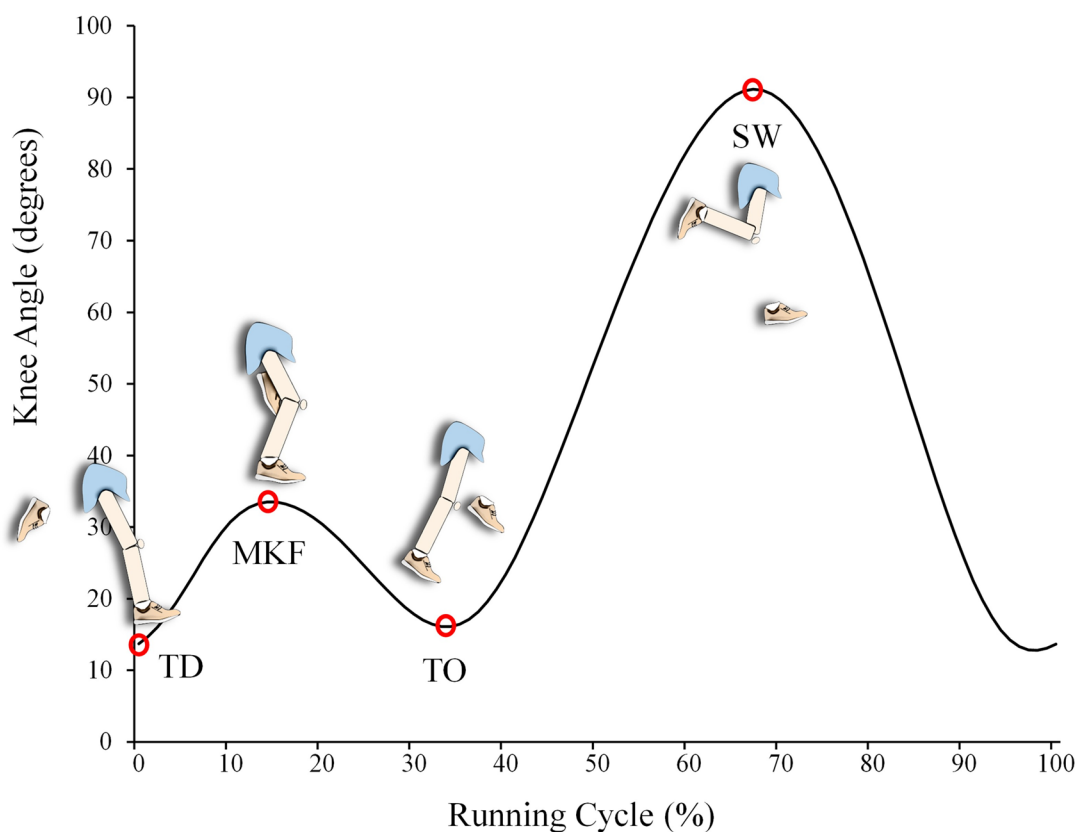


Fig. 3 Running cycle events detected during the 30 min of continuous running test

Note: TD = touch-down, MKF = maximum knee flexion, TO = toe-off, SW = mid-swing phase. Red circles indicate the instant of every event

Table 1 Descriptive values for functional groups

	FG1 (n=22)	FG2 (n=9)	FG3 (n=8)
Age (years)	30±11	33±12	26±8
Height (m)	1.77±0.1*†	1.72±0.1†	1.72±0*
Body mass (kg)	75.7±9*	70.8±6.8	69.5±7.8*
Body mass index (BMI) (kg/m ²)	24.3±2.9	23.9±2.2	23.4±2
Weekly training distance (Km)	34±16	41±18	48±20
MAS (km/h)	16.3±1.9	16.9±1.8	17±1.6

Data showed as mean±standard deviation. FG: Functional group, MAS: maximal aerobic speed. *: differences ($p<0.05$) between FG1 and FG3, †differences ($p<0.05$) between FG1 and FG2.

requires running the K-means algorithm across different values of k and calculating the silhouette score each time. This iterative process highlights the optimal k by maximizing the silhouette score, which identifies the best cluster count to fit into the K-means model accurately [26].

Statistical analysis

The data were analysed using SPSS software v28 (IBM Corp., Armonk, NY). Firstly, the outliers were removed. Next, the normality of the data was checked using the Shapiro-Wilk test (adequate due to its suitability for small to moderate sample sizes, typically up to 50), with all variables being normally distributed. The relationship between foot type (FPI) and FG of the sample was analysed using a chi-square test. To analyse the effects of fatigue (within-subject factor) and membership in FG based on BRF (between-subject factor) on each of the dependent variables (a) Kinematic variables: hip, knee, ankle, and inversion-eversion angle at TD, MKE, TO, and SW, b) heart rate, c) Blood lactate concentration, and d) RPE, a repeated measures analysis of variance (ANOVA) was conducted, adjusting significance levels in pairwise comparisons using Bonferroni correction. Additionally, sphericity assumption was checked using Mauchly's test, and Huynh-Feldt adjustment was used in cases where this assumption was not met. Additionally, change magnitudes associated with fatigue for each FG and kinematic variable were calculated (Δ_{pre_post}) and differences based on FG were tested using a one-way ANOVA, with significance levels in pairwise comparisons adjusted using Bonferroni correction. Moreover, effect size was calculated using partial eta squared [27], interpreted as: 0.01 = small, 0.06 = moderate, > 0.14 = large. The magnitude of changes found was expressed through mean differences and 95% confidence intervals. The significance level was set at $p<0.05$.

Equity, diversity, and inclusion statement

All the recreational runners included in the study were male Caucasians. The established inclusion criteria limited the selection of women and individuals from other

Table 2 FPI distribution among functional groups

	FG1	FG2	FG3
Normal (n=25)	59%	78%	63%
Pronated (n=13)	36%	22%	38%
Supinated (n=1)	5%	0%	0%

FPI: Foot Posture Index, FG: functional group.

Table 3 Descriptive values of perceptual and physiologic variables

		FG1	FG2	FG3
HR (bpm)	PRE	149.1±24.5*	152.9±14.4*	158.3±9.6*
	POST	173.5±15	169.3±15.2	179.6±11
RPE	PRE	10.3±2.3*	10.2±1.9*	8.5±2.4*
	POST	15.6±2.6	16.3±2.9	15±3
Lactate (mmol/L)	PRE	1.4±0.5*	2.1±0.8*	1.5±0.5*
	POST	4.2±2.3	5.5±3.6	3.7±1.7

Results showed as mean±standard deviation. HR: heart rate, RPE: Rating of Perceived Exertion, PRE: Pre fatigue condition (min 0–5'); POST: post-fatigue condition (min 29–30'); FG: functional group. *: differences ($p<0.05$) between pre and post condition ($p<0.05$).

communities, which may have affected the generalizability of our results.

Results

Group classification

After applying the silhouette method, it was determined that at least three functional groups could be defined. This criterion was then input into the K-means algorithm, which subsequently divided the sample into three functional groups based on variations in BRF, specifically focusing on knee flexion and leg stiffness (Table 1).

Athletes in FG1 were significantly taller ($p=0.014$, $ES=0.108$) than those in FG2 ($p=0.012$, mean diff.= 0.04, 95%CI=0.01 to 0.08 m) and FG3 ($p=0.010$, mean diff.= 0.05 m, 95%CI=0.01 to 0.08 m). Additionally, athletes in FG1 were significantly heavier ($p=0.002$, $ES=0.155$) than athletes in FG3 ($p=0.032$, mean diff.= 6.2 kg, 95%CI=0.4 to 12.1 kg). However, no differences were observed ($p>0.05$) in BMI or in the rest of the descriptive variables.

Regarding foot type (FPI), 64.1% had normal feet, 33.3% were pronators, and 2.6% were supinators. No dependency was observed between foot type and grouping (Chi-square, $p=0.816$).

Perceptual and physiologic response

Table 3 presents the descriptive statistics for HR, RPE, and blood lactate (Pre/Post fatigue).

Regarding the effect of prolonged running, the results showed a significant increase in HR ($p<0.001$, $ES=0.491$; mean diff. = 21.0 bpm, 95%CI=16.7 to 28.3 bpm), RPE ($p<0.001$, $ES=0.748$; mean diff. = 6.1, 95%CI=4.9 to 7.3), and blood lactate concentration ($p<0.001$, $ES=0.584$; mean diff. = 2.8 mmol/L, 95%CI=2.0 to 3.6 mmol/L) after the prolonged running test.

However, concerning differences between FGs, no significant differences were found in HR ($p=0.419$), RPE ($p=0.249$), and blood lactate concentration ($p=0.176$) among the 3 FG. No interactions were found between fatigue and FG in perceptual and physiological variables.

Spatiotemporal response

In relation to spatiotemporal variables and stiffness variables (Table 4), results did not show significant differences ($p>0.05$) pre vs. post fatigue in speed, stride length, and cadence. However, leg stiffness (Kleg) and vertical stiffness (Kvert) were significantly lower after the prolonged running test ($p<0.01$, ES=0.399, mean diff.= 0.094 kN/m, 95%CI=0.055 to 0.132 kN/m; and $p<0.01$, ES=0.363, mean diff.= 1.158 kN/m, 95%CI=0.64 to 1.676 kN/m; respectively).

Additionally, a significant interaction was found between belonging to a FG and the effect of fatigue on leg stiffness (Kleg) in FG2 and FG3. Specifically, leg stiffness (Kleg) was lower after the prolonged running test in FG2 ($p<0.01$, mean diff.= 0.157 kN/m, 95%CI=0.084 to 0.23 kN/m) and in FG3 ($p=0.022$, mean diff.= 0.1 kN/m, 95%CI=0.022 to 0.177 kN/m). Similarly, vertical stiffness (Kvert) was lower after the fatigue test in FG2 ($p<0.01$, mean diff.= 2.1 kN/m, 95%CI=1.1 to 3.1 kN/m).

No effect related to belonging to different FG ($p>0.05$) was found in the spatiotemporal variables.

Lower-leg angular kinematics response

Lower leg kinematic variables (Table 5) shown significant differences ($p<0.05$) after fatigue protocol at initial contact (TD) with more hip flexion ($p<0.01$, ES=0.427, mean diff.= -0.973°, 95%CI=-1.354 to -0.592°) and greater ankle inversion ($p=0.019$, ES=0.145, mean diff.= -0.918°, 95%CI=-1.673 to -0.163°).

Table 4 Descriptive values of Spatiotemporal and stiffness variables

		FG1	FG2	FG3
Speed (km/h)	PRE	13±1.5	13.5±1.4	13.6±1.2
	POST	13±1.5	13.5±1.4	13.6±1.2
Stride length (m)	PRE	2.59±0.33	2.59±0.3	2.68±0.3
	POST	2.6±0.32	2.58±0.27	2.69±0.28
Step frequency (steps/min)	PRE	168.5±7.6	173.6±5.7	169.8±9.6
	POST	167.7±6.3	174±4.5	169.2±7.8
Kleg (kN/m) *	PRE	1.1±0.3	1.1±0.3†	1.2±0.3†
	POST	1.1±0.3	0.9±0.3	1.1±0.2
Kvert (kN/m) *	PRE	20.1±3.2	20.6±4.1	21.3±3.6
	POST	19.7±3.1	18.5±4.2	20.2±3.2

Results showed as mean±standard deviation. FG: functional group. PRE: Pre fatigue condition (min 0–5'); POST: post-fatigue condition (min 29–30'). *: differences ($p<0.05$) between pre and post (↓Post). †: interaction differences ($p<0.05$) between pre and post (↓Post). (↓Post) refers to a decrease in the mean of the variable after the fatigue test.

Moreover, in the maximum knee flexion (MKF), a greater hip flexion (post) ($p=0.001$, ES=0.25, mean diff.= -0.832°, 95%CI=-1.318 to -0.345°) and greater knee flexion ($p=0.004$, ES=0.213, mean diff.= -0.888°, 95%CI=-1.465 to -0.311°) was observed in post-fatigue condition. During toe-off (TO), only greater hip extension was observed after the fatigue protocol ($p=0.01$, ES=0.444, mean diff.= 1.055°, 95%CI=0.656 to 1.453°).

Finally, concerning swing phase (SW), greater hip flexion ($p=0.006$, ES=0.19, mean diff.= -0.87°, 95%CI = -1.477 to -0.264°) and ankle flexion ($p=0.001$, ES=0.25, mean diff.= -2.82°, 95%CI = -4.47 to -1.17°) were observed after the fatigue protocol.

Regarding to FG differences, only the ankle inversion-eversion variable at toe-off (TO) was different ($p=0.038$, ES=0.166) between FG1 and FG2, with FG2 showing less inversion at toe-off ($p=0.034$, mean diff.= 46.1°, 95%CI=2.72 to 89.4°).

Regarding the differences between FG in the kinematic variables associated to the fatigue (Table 6) results shown differences in leg stiffness (Kleg) ($p=0.010$, ES=0.225) and vertical stiffness (Kvert) ($p=0.015$, ES=0.208), with a higher decreased at FG2 vs. FG1 in Kleg ($p=0.011$, ES=1.259, mean diff.= 0.133 kN/m, 95%CI=0.026 to 0.241 kN/m) and Kvert ($p=0.013$, ES=1.182, mean diff.= 1.747 kN/m, 95%CI=0.311 to 3.182 kN/m).

Furthermore, changes in hip variables at TD ($p=0.011$, ES=0.222) and TO ($p=0.041$, ES=0.162) were observed. Specifically, during TD it was observed a significantly increased hip flexion FG3 vs. FG1 ($p=0.012$, ES=-1.439, mean diff. = -1.354°, 95%CI = -2.455 to -0.252°) and vs. FG2 ($p=0.040$, ES=1.193, mean diff. = -1.346°, 95%CI = -2.642 to -0.049°). And during TO, significantly increased hip flexion at FG2 vs. FG1 was observed ($p=0.038$, ES=0.981, mean diff. = 1.153°, 95%CI=0.048 to 2.258°).

Regarding knee joint, significant differences between FGs were found at TD, MKF, and TO ($p<0.01$, ES=0.809; $p=0.02$, ES=0.299; and $p<0.01$, ES=0.323, respectively). In this sense, during TD after fatigue, knee flexion increased in FG3 vs. FG1 ($p<0.01$, ES=3.013, mean diff. = 2.711°, 95%CI=1.703 to 3.719°) and FG2 ($p<0.01$, ES=4.995, mean diff. = 5.8°, 95%CI=4.618 to 6.990°). However, FG2 reduced knee flexion vs. FG1 ($p<0.01$, ES=-1.014, mean diff. = -3.093°, 95%CI = -4.059 to -2.127°). During MKF, knee flexion increased FG3 vs. FG1 ($p<0.01$, ES=1.622, mean diff. = 2.550°, 95%CI=0.882 to 4.218°), and the same was observed at TO, with greater knee flexion in FG3 vs. FG1 ($p<0.01$, ES=1.584, mean diff. = 2.134°, 95%CI=0.555 to 3.713°) and FG2 ($p<0.01$, ES=1.995, mean diff. = 2.939°, 95%CI=1.081 to 4.797°).

Lastly, an ankle eversion reduction during MKF ($p=0.046$, ES=0.157) was observed in FG3 vs. FG1 and FG2.

Table 5 Descriptive values of lower leg kinematic variables

		FG1	FG2	FG3
Hip TD (°)*	PRE	24.5±3.7	24.7±3.2	25.4±4.5
	POST	25±3.6	25.3±3.2	27.3±4.7
Hip MKF (°) *	PRE	17.8±3	16.1±4	18±3.7
	POST	18±3.3	17.4±4.3	19±3.6
Hip TO (°)*	PRE	-19.6±2.6	-19.6±2.1	-19.3±2.8
	POST	-20.2±3.3	-21.3±2.9	-20.1±2.7
Hip SW (°)*	PRE	16.1±4.1	15.7±4.9	16.8±9.7
	POST	16.7±3.5	16.8±5	17.7±9.7
Knee TD (°)	PRE	13.6±4.5	12.7±5.8	14.6±6.7
	POST	13.9±4.4	10±5.9	17.7±6.5
Knee MKF (°) *	PRE	34.4±5.1	31±7.1	34.9±5.6
	POST	34±5.7	31.8±7.7	37.1±5.8
Knee TO (°)	PRE	12.9±3.6	11.6±3.9	13±4.3
	POST	12±3.7	9.9±4.4	14.2±4.3
Knee SW (°)	PRE	94.2±11.5	92.5±12.8	101.3±8.3
	POST	94.6±10	90.1±11.8	102.8±8.7
Ankle TD (°)	PRE	1.7±4.8	2.6±6.9	5.1±7.6
	POST	2.3±5.3	4.6±8.6	4.4±7.6
Ankle MKF (°)	PRE	-13.7±3.1	-15.8±6.8	-13.6±4.1
	POST	-13.4±3.5	-15.4±7.5	-14.1±4.5
Ankle TO (°)*	PRE	22.5±4.1	17.9±5.8	22.1±4.9
	POST	23.8±4.8	21.6±8.1	23.1±4.4
Ankle SW (°)*	PRE	12.7±5	12.3±9.5	15.1±7.7
	POST	15.9±7	16.5±8.9	16.1±9.2
Inv.Ever TD (°) *	PRE	-1.1±4.4	0±5	-1.5±4.7
	POST	-0.8±4.8	0.7±5	0.3±6
Inv.Ever MKF (°)	PRE	-3.8±4.9	-3.8±5.4	-5±4.4
	POST	-4.2±5.1	-4.4±5.3	-3.4±6
Inv.Ever TO (°)	PRE	123.4±42†	74.8±51.8†	98.4±49.8
	POST	119±42.6†	75.6±49.2†	111.6±47.8
Inv.Ever SW (°)	PRE	132.8±45.8	134.7±47.4	130.6±40.3
	POST	130.5±47	159.1±28.1	137.4±53.9

Results showed as mean±standard deviation. FG: functional group, TD=touch-down, MKF=maximum knee flexion, TO=toe-off, SW=mid-swing phase, PRE: Pre fatigue condition (min 0–5'); POST: post-fatigue condition (min 29–30'), Inv.Ever: inversion-eversion. *Differences ($p<0.05$) between PRE and POST condition. †Differences ($p<0.05$) between FG.

Discussion

The high incidence of RRI in recreational athletes has increased interest in understanding risk factors [9, 10]. Due to limited evidence on traditional factors, new prevention strategies are being sought. This study explores the relationship between BRE, fatigue-induced changes, and the use of unsupervised clustering techniques to identify biomechanical responses that may be associated with an increased risk of injury, particularly in discrete variables recognized as BRE. Our hypothesis suggested that fatigue will induce notable alterations in physiological, perceptual, and angular kinematics, especially in variables associated with knee joint movement.

Our results show that fatigue significantly increases blood lactate levels and heart rate, consistent with previous studies linking marathon split times and heart rate

Table 6 Variations in lower leg kinematic variables after fatigue protocol (Δ pre-post)

Δ Pre-post	FG1	FG2	FG3	P.H. comparisons
Kleg (kN/m) *	-0.024±0.082	-0.157±0.161	-0.1±0.102	↓FG2 vs. FG1
Kvert (kN/m) *	-0.348±1.053	-2.095±2.356	-1.031±1.028	↓FG2 vs. FG1
Hip TD (°) *	0.519±0.951	0.527±1.326	1.873±1.039	↑FG3 vs. FG1 and FG2
Hip MKF (°)	0.198±1.337	1.284±1.503	1.014±1.231	-
Hip TO (°) *	-0.604±1.225	-1.758±1.188	-0.802±0.496	↑FG2 vs. FG1
Hip SW (°)	0.616±1.912	1.087±1.411	0.907±1.212	-
Knee TD (°) *	0.317±0.725	-2.776±1.102	3.028±1.375	↑FG3 vs. FG1 and FG2
				↓FG2 vs. FG1 and FG3
Knee MKF (°) *	-0.346±1.594	0.805±1.542	2.204±1.724	↑FG3 vs. FG1
Knee TO (°) *	-0.932±1.49	-1.736±1.906	1.203±1.058	↑FG3 vs. FG1 and FG2
Knee SW (°)	0.385±3.826	-2.371±4.858	1.428±2.344	-
Ankle TD (°)	0.633±2.715	2.017±2.856	-0.626±1.348	-
Ankle MKF (°)	0.302±1.615	0.413±1.796	-0.531±1.053	-
Ankle TO (°)	1.25±2.993	3.639±4.203	0.996±1.82	-
Ankle SW (°)	3.209±4.351	4.255±5.568	1.005±4.088	-
Inv_Ever TD (°)	0.29±1.96	0.622±1.415	1.843±2.995	-
Inv_Ever MKF (°) *	-0.419±1.695	-0.566±0.994	1.554±3.19	↑FG3 vs. FG1 and FG2
Inv_Ever TO (°)	-4.405±26.47	0.859±26.888	13.213±24.683	-
Inv_Ever SW (°)	-2.309±23.58	24.389±45.506	6.759±25.471	-

Results showed as mean±standard deviation. FG: functional group, TD=touch-down, MKF=maximum knee flexion, TO=toe-off, SW=mid-swing phase, P.H.=post-hoc, Inv_Ever = inversion-eversion, Kleg = leg stiffness, Kvert = vertical stiffness. *Differences ($p<0.05$) between FG.

[28], as well as increases in heart rate and blood lactate observed during moderate-intensity, similarly timed trials [29]. While our findings align with the literature, measuring changes in heart rate variability and complexity would have been insightful, as their decrease is associated with fatigue onset [28].

On the other hand, we also observed an increase in subjective perception of effort at the end of the test compared to the beginning ($p<0.05$). Similarly, consistent

with previous studies [28]. This increase in perceived effort, associated with higher cardiac output [28], is assumed to be linked to greater recruitment of motor units to sustain activity efficiently, ultimately leading to an increase in physiological response [30].

Several studies suggest that fatigue typically alters running kinematics, including decreased speed, step frequency [31], and ankle dorsiflexion [32], and increased trunk flexion and knee internal rotation [33]. However, our results did not show changes in spatiotemporal variables [31], such as step frequency or stride length, despite athletes typically having a preferred step frequency and length that minimize metabolic cost [34]. This might be due to the fatigue protocol not eliciting changes in these variables, or athletes maintaining a steady run to preserve metabolic cost, consistent with a U-shaped relationship between stride length and metabolic cost [34]. Although athletes were fatigued, as indicated by heart rate and blood lactate responses, our results align with previous studies [34], suggesting that using RPE to measure metabolic cost when manipulating spatiotemporal variables is not appropriate.

Our results found a significant decrease in leg and vertical stiffness ($p < 0.05$), consistent with studies showing that fatigue negatively affects leg [6, 35], and vertical stiffness [36]. Normally, leg stiffness is regulated to maintain metabolic cost, which is crucial for running economy and performance [34]. Lower leg stiffness is associated with reduced running economy, increased ground contact time, and greater vertical oscillation of the centre of mass [37]. While some authors have associated changes in leg stiffness with injury occurrence [38], recent work suggests this relationship may not exist [39].

The changes observed in leg stiffness align with the differences noted following the onset of fatigue in angular kinematics, where an increase in hip and knee flexion during initial contact and the maximum knee flexion phase was observed, with these changes being associated with greater displacement of the centre of mass [37]. Our findings align with previous studies that primarily observed changes in hip and knee motion in the sagittal plane [40], although some studies report conflicting or inconclusive results [41].

Our second hypothesis predicts that athletes grouped by their fatigue response will show different levels of changes in angular kinematics, with those exhibiting a stronger negative response likely having more significant alterations. Our study classified athletes into different groups using the K-means algorithm, based on rearfoot biomechanical variables from a recent systematic review [10]. We identified 3 groups with distinct biomechanical responses to fatigue, similar to findings observed in studies involving footwear intervention [12] and analyses of rearfoot kinematics after fatigue [42]. Our study found no

differences in anthropometrics, unlike a previous study [12], foot type, or training volume, though FG3 appeared to represent higher-performing athletes based on training volume and MAS. We also found no differences in physiological response (blood lactate levels and HR) or perceived effort variables related to group membership. Although the use of a maximal effort 5-minute running test on a 400 m track to determine MAS might be seen as a limitation due to potential variability in pacing strategies and external conditions, we selected this method as it is validated in existing literature for field-based assessments of aerobic capacity and offers practicality and ecological validity for recreational athletes [15]. We believe this approach provided a reliable estimation aligned with our study's context, balancing accuracy with the practicalities of real-world application.

Based on the kinematic results, we suggest that FG3 showed greater changes in knee joint angles across all events (TD, MKF, TO) following fatigue. Previous studies indicate that increases in sagittal plane range of motion, associated with fatigue, are often linked to reduced lower-limb stiffness [34]—a factor closely tied to running economy. While we did not directly measure stiffness, these kinematic changes might imply a relative decrease in running economy for FG3 compared to other groups. However, this interpretation should be approached with caution given the study's limitations. Specifically, FG3 showed increased hip and knee flexion at TD, similar to a previous study [36], and at MKF and TO for the knee, consistent with findings by Jewell et al. [44]. The kinematic changes observed in FG3 align with previous studies that associate such changes with a redistribution of joint work after fatigue onset [45], shifting from the ankle to the knee and hip [46]. This redistribution, along with increased knee and hip joint range of motion [46], could impact running economy and may elevate injury risk, particularly as seen in scenarios like low-intensity downhill running [47], or during 10 km run [45].

Furthermore, FG2 showed a significant reduction in leg and vertical stiffness due to fatigue, more so than FG1. Additionally, it was the only group that significantly reduced the knee extension angle at TD compared to FG1 and FG3. Changes in kinematic patterns, such as those observed in FG2, reflect an adaptive response aimed at redistributing load and mitigating injury risk. Previous studies have shown that fatigue can disrupt symmetrical movement, leading to asymmetries that may indicate compromised neuromuscular control or compensatory strategies to maintain performance and reduce overuse stress [48, 49]. The significant reductions in knee extension angle at TD observed in FG2, along with changes in leg and vertical stiffness, suggest a potential asymmetrical response to fatigue, aligning with existing literature where fatigue-induced asymmetries are linked

to increased injury risk and changes in running economy [48, 49].

Our results, similar to those proposed by Langley [42], identified three functional groups using K-means cluster analysis, with each group showing significantly different fatigue-related changes in lower leg kinematics. These findings support the hypothesis that fatigue-induced kinematic changes can group athletes based on their functional response. Identifying different FGs based on fatigue-induced changes in BRF has clear implications for training and injury prevention. Recreational male runners in FG1 and FG2, who show stable or compensatory kinematic responses, could benefit from general endurance and neuromuscular training to maintain efficient running mechanics under fatigue. Conversely, FG3 runners, who exhibit significant kinematic alterations (e.g., increased hip and knee flexion), may require targeted interventions focused on muscle endurance, joint stabilization, and gait retraining to mitigate injury risks. Coaches and sports health practitioners can use these assessments for individualized program design, improving biomechanical resilience and injury prevention through tailored training regimens.

The conclusions from this study should be considered considering its limitations. K-means cluster analysis, used for identifying clusters, is an unsupervised machine learning technique with randomly initialized seed locations, leading to potential variability in cluster centres across different datasets. We determined the number of clusters using the Silhouette method [25]. Our focus was primarily on the angular response of the lower limb, quantifying fatigue through internal load measures (heart rate and blood lactate) and external load measures (RPE). However, this narrow focus limits the scope of our findings. Incorporating more variables and larger sample sizes (including different running level: recreational vs. elite runners or female gender) could improve the generalizability and reliability of our results. Kinematic variables were recorded at the end of a 30-minute period to capture fatigue-related changes, consistent with numerous prior studies [8, 35]. However, recent research suggests that kinematic changes in a 10 km run can appear shortly after the start, which complicates the interpretation of fatigue effects [7]. A possible explanation for this discrepancy could be the intensity of the run; competitive runs are often performed at high intensity, while fatigue protocols are typically sub-maximal. Future studies might consider grouping observations at different points in the trial for more nuanced insights. The intensity of running fatigue protocol (MAS) was estimated by 5-minutes running test [15]. Previous studies have shown that MAS obtained from the 5-minutes running test is closely correlated with MAS measured during treadmill tests, with high correlation coefficients between the 5-minutes

running test and treadmill tests for sub-elite runners (0.86) and for athletes from other disciplines (0.84), indicating strong agreement [15, 50]. However, it is not as accurate for estimating VO₂max [51]. This makes the 5-minutes running test a useful tool for assessing aerobic performance.

Although using participants' own shoes may introduce certain limitations, this approach ensures comfort and familiarity, reducing the risk of discomfort or injury and promoting more natural running biomechanics, as participants are accustomed to the specific characteristics of their own shoes, such as cushioning, support, and fit [52]. Additionally, it enhances the ecological validity of the study, reflecting real-world conditions where recreational male runners typically use their own footwear. While it is true that variability in shoe technologies, such as motion control systems or cushioning, could impact biomechanics and injury risk [53], we deemed this variability acceptable to maintain the study's practical relevance. We acknowledge that differences in shoe wear could introduce confounding factors, as the cushioning and support of worn shoes differ from those of newer ones. However, prioritizing participant familiarity and comfort provided a more realistic and applicable environment, aligning with the study's objectives. Finally, the fact that only male recreational runners were included is also a limitation, which may limit the generalizability of the findings to a broader population. It would be inadvisable to generalize these findings to female athletes, as injury incidence and aetiology often differ between genders. While this may not have a significant impact on the current study's conclusions, future research should consider including both male and female participants to explore potential sex differences in biomechanical responses.

Conclusions

Fatigue-induced alterations in the BRF allow for the functional grouping of recreational athletes. Among the athletes, we have identified three functional groups displaying distinct functional behaviours in lower limb angular kinematics following the onset of fatigue. While two of these groups exhibit similar or compensatory behaviours to maintain their usual running dynamics, one of them, FG3, shows more pronounced changes altering their running pattern, significantly affecting their economy, and therefore presenting different training needs.

Abbreviations

BRF	Biomechanical risk factors
FG	Functional groups
RRI	Running-related injuries
BMI	Body mass index
MAS	Maximal aerobic speed
RPE	Rate of perceived exertion
FPI	Foot postural index

ICC	Intra-Class Correlation Coefficient
HR	Heart rate
TD	Touch-down
MKF	Maximum knee flexion
TO	Toe-off
SW	Mid-swing phase
ANOVA	Analysis of variance
Kleg	Leg stiffness
Kvert	Vertical stiffness

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Authors' Contributions

A.E-M and P.P-S: conceived and designed the study. A.E-M, E.S-R, R.B-L, and R.S-S recruited the participants and prepared the protocol. A.E-M, E.S-R, R.B-L, R.S-S and P.P-S, participate in data collection in laboratory. A.E-M, P.P-S, R.S-S and J.A. performed the statistical analysis. A.E-M, P.P-S, R.S-S and J.A. wrote the draft of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval and Consent to Participate

The study protocol was reviewed and approved by the Ethics Committee of the University of Valencia (No. 2588640). All participants give their informed written consent before participating in the study. The study followed the ethical principles established in the Declaration of Helsinki.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no conflict of interest.

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