# Archivos de medicina del deporte

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#### ORIGINAL ARTICLES

Effect of 3-week progressive overloading and 1-week tapering on performance, internal training load, stress tolerance and heart rate variability in under-19 Brazilian badminton players

Study of sedentary behavior analyzed by self-questionnaire and accelerometry, and its association with cardiovascular risk factors in the adult population from a health center

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Ten years of football (soccer) injuries in the literature. A bibliometric approach

Consumption of energy drinks on cardiovascular and metabolic response and performance. Is there an effect?

Study of heart rate variability following exposure to normobaric hypoxia







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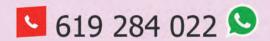
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# Nutritional and health claims made on food products for physical activity and sport

### Declaraciones nutricionales y propiedades saludables en productos alimenticios para la actividad física y el deporte

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Interest in the benefit of nutrients and other substances with a physiological effect has grown in recent years. At the same time, more and more nutrition and health claims have been approved by the European Food Safety Authority (EFSA) and authorised by the European Commission through Regulation 432/2012. It is, therefore, essential that nutrition, physical activity, sports and general health professionals know which nutrients and substances are legally endorsed and authorised under the approval of the EFSA on the basis of scientific evidence. Clearly, a diet which provides the nutrients and other substances with a physiological effect in amounts suitable for the practice of physical activity and sport, based on the criteria of a varied and balanced diet, may prove sufficient. However, the intake of certain nutrients can be increased through a selection of foods to incorporate particular nutrients or the use of food supplements without these being substitutes for a balanced diet. The main difference is the presentation, food matrix or pharmacological form, because, according to Directive 2002/46/EC of the European Parliament, food supplements are foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities'.

It should be noted that when a food product meets the conditions of use established in Regulation (EC) No. 1924/2006 of the European Union, it can voluntarily bear claims on its labelling and/or in its presentation and/or advertising. There are different types of claims: nutrition claims, such as 'low energy', which are explicitly listed in the Regulation, health claims other than those referring to the reduction of disease risk

and children's development and health, as established in Article 13 of Regulation 1924/2006, which are approved through the list authorised in Regulation 432/2012, such as 'Vitamin D contributes to the maintenance of normal muscle function', and claims relating to children's development and health, as highlighted in Article 4 paragraph 1 point b of Regulation 1924/2006, which are approved and authorised on a case-by-case basis through specific Regulations.

These claims are voluntary mentions included on the labelling and/or in the presentation and/or advertising of foodstuffs, known legislatively since 2011 as food information provided to consumers, Regulation 1169/2011. Therefore, if such claims are made through any medium, they must comply with the conditions of use established for each. Although some flexibility is allowed in the wording of the claims, these cannot mislead the consumer and must have the same meaning as those authorised by the European Union. Thus, nutrition claims indicate the presence, absence, increase or decrease of the content of nutrients/substances with a physiological effect/energy, while health claims relate a nutrient, substance, food or food category to health. In the case of health claims, these should not be absolute and must always be preceded by terms such as *helps, contributes, facilitates*, and so on.

Some health claims are associated with children's development, others with risk reduction and others with functional health claims. Some health claims are related to sports activity and focus on improving physical performance.

In order to obtain the beneficial effect mentioned in the claim, the nutrient, substance, food or food category must be present in the minimum quantities established in Regulation (EC) No. 1924/2006. These claims are designed to help consumers make well informed decisions, providing a basis for credibility because, as mentioned earlier, before being approved by the European Commission, the European Food Safety

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Authority (EFSA) assesses the scientific evidence backing them up, and must be 'truthful, clear, reliable and useful'.

Almost 300 claims have now been approved by the EFSA, of which some 30 are nutrition claims and 265, health claims. Of these almost 300 health claims, XX are related to sports activity, of which 48 are of interest to us (0 referring to children's development, 1 to risk reduction and 47 to claims regarding functional health properties).

There are claims of health properties related to physical and sports activities for 26 different nutrients, of which 12 concern minerals (calcium, sodium, copper, chromium, phosphorus, iron, magnesium, manganese, molybdenum, potassium, iodine and zinc), 10 concern vitamins (pantothenic acid (vitamin B5), biotin, folate, niacin (vitamin B3), riboflavin (vitamin B2), thiamine (vitamin B1) vitamin B12, vitamin B6, vitamin C and vitamin D), 2 concern carbohydrates, 2 concern water, 1 concerns protein and 1 concerns creatine. Each of these nutrients or substances with a physiological effect can bear several health claims regarding different functions, for example, 'water contributes to the maintenance of normal physical and cognitive functions' and 'water contributes to the maintenance of normal regulation of the body's temperature'.

Using the search engine made available by the Spanish Food Safety and Nutrition Agency (AESAN), we can point to several claims:

Type: absorption of water; nutrient, substance, food or food category: carbohydrate-electrolyte solutions; claim: carbohydrate-electrolyte solutions enhance the absorption of water during physical exercise; conditions of use of the claim: in order to bear the claim carbohydrate-electrolyte solutions should contain 80-350 kcal/L from carbohydrates, and at least 75% of the energy should be derived from carbohydrates which induce a high glycaemic response, such as glucose, glucose polymers and sucrose. In addition, these beverages should contain between 20 mmol/L (460 mg/L) and 50 mmol/L (1,150 mg/L) of sodium, and have an osmolality between 200-330 mOsm/kg water.

Type: tiredness and fatigue; nutrient, substance, food or food category: (1) pantothenic acid, (2) folate, (3) iron, (4) magnesium; (5) niacin, (6) riboflavin, (7) vitamin B12, (8) vitamin B6, (9) vitamin C; claim: (1)(2) (3)(4)(5)(6)(7)(8)(9) contribute to the reduction of tiredness and fatigue; conditions of use of the claim: the claim may be used only for food which is at least a source of folate as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.

Type: endurance exercise; nutrient, substance, food or food category: (1) carbohydrate-electrolyte solutions, (2) creatine; claim: (1) carbohydrate-electrolyte solutions contribute to the maintenance of endurance performance during prolonged endurance exercise (2) daily creatine consumption can enhance the effect of resistance training on muscle strength in adults over the age of 55; conditions of use of the claim: (1) in order to bear the claim, carbohydrate-electrolyte solutions should contain 80-350 kcal/L from carbohydrates, and at least 75 % of the energy should be derived from carbohydrates which induce a high glycaemic response, such as glucose, glucose polymers and sucrose. In addition, these beverages should contain between 20 mmol/L (460 mg/L) and 50 mmol/L (1,150 mg/L) of sodium, and have an osmolality between 200-330 mOsm/kg water, (2) Information shall be provided to the consumer that: —the claim is targeting adults over the age of 55,

who are engaged in regular resistance training, —the beneficial effect is obtained with a daily intake of 3 g of creatine in conjunction with resistance training, which allows an increase in the workload over time and which should be performed at least three times per week for several weeks, at an intensity of at least 65 %-75 % of one repetition maximum load (\*). (\*) repetition maximum load is the maximum weight or force an individual can exert in a single lift.

Type: muscle function; nutrient, substance, food or food category: (1) (2) protein, (3) carbohydrates; claim: (1) protein contributes to a growth in muscle mass, (2) protein contributes to the maintenance of muscle mass, (3) carbohydrates contribute to the recovery of normal muscle function (contraction) after highly intensive and/or long-lasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle; conditions of use of the claim: (1) (2) the claim may be used only for food which is at least a source of protein as referred to in the claim SOURCE OF PROTEIN as listed in the Annex to Regulation (EC) No 1924/2006. (3) The claim may be used only for food which provides carbohydrates which are metabolised by humans (excluding polyols). Information shall be given to the consumer that the beneficial effect is obtained with the consumption of carbohydrates, from all sources, at a total intake of 4 g per kg body weight, at doses, within the first 4 hours and no later than 6 hours, following highly intensive and/or longlasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle.

*Type:* physical and cognitive functions; nutrient, substance, food or food category: water; claim: water contributes to the maintenance of normal physical and cognitive functions; conditions of use of the claim: in order to bear the claim, information shall be given to the consumer that in order to obtain the claimed effect, at least 2,0 L of water, from all sources, should be consumed per day.

*Type:* energy-yielding metabolism; nutrient, substance, food or food category: (1) pantothenic acid, (2) biotin, (3) calcium, (4) copper, (5) phosphorus, (6) iron, (7) magnesium, (8) manganese, (9) niacin, (10) riboflavin, (11) thiamine, (12) vitamin B12, (13) vitamin B6, (14) vitamin C, (15) iodine; claim: (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14)(15) contribute to normal energy-yielding metabolism; conditions of use of the claim: (1)(2)(3)(4)(5)(6)(7)(8)(9)(10)(11)(12)(13)(14)(15) the claim may be used only for food which is at least a source of iodine as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006

Type: macronutrient metabolism; nutrient, substance, food or food category: (1) biotin, (2) chromium, (3) zinc, (4) Vitamin B6; claim: (1)(2) (3) contribute to normal macronutrient metabolism, (4) contributes to normal protein and glycogen metabolism; conditions of use of the claim: (1)(2)(3)(4) the claim may be used only for food which is at least a source of zinc as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.

*Type:* muscle recovery; nutrient, substance, food or food category: carbohydrates; claim: carbohydrates contribute to the recovery of normal muscle function (contraction) after highly intensive and/or long-lasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle; conditions of use of the claim: the claim may be used only for food which provides carbohydrates which

are metabolised by humans (excluding polyols). Information shall be given to the consumer that the beneficial effect is obtained with the consumption of carbohydrates, from all sources, at a total intake of 4 g per kg body weight, at doses, within the first 4 hours and no later than 6 hours, following highly intensive and/or long-lasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle.

*Type:* physical performance; nutrient, substance, food or food category: creatine; claim: creatine increases physical performance in successive bursts of short-term, high intensity exercise; conditions of use of the claim: the claim may be used only for food which provides a daily intake of 3 g of creatine. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of creatine.

*Type:* immune system; nutrient, substance, food or food category: vitamin C; claim: vitamin C contributes to maintain the normal function of the immune system during and after intense physical exercise; conditions of use of the claim: the claim may be used only for food which provides a daily intake of 200 mg vitamin C. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 200 mg in addition to the recommended daily intake of vitamin C.

*Type*: body temperature; nutrient, substance, food or food category: water; claim: water contributes to the maintenance of normal regulation of the body's temperature; conditions of use of the claim: in order to bear the claim, information shall be given to the consumer that in order to obtain the claimed effect, at least 2,0 L of water, from all sources, should be consumed per day.

*Type*: oxygen transport; nutrient, substance, food or food category: iron; claim: iron contributes to normal oxygen transport in the body; conditions of use of the claim: the claim may be used only for food which is at least a source of iron as referred to in the claim SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S] as listed in the Annex to Regulation (EC) No 1924/2006.

However, some of the health claims come with complementary conditions, restrictions and warnings, such as:

Claim: daily creatine consumption can enhance the effect of resistance training on muscle strength in adults over the age of 55; conditions and/or restrictions of use of the food and/or additional statement or warning: the claim may be used only for foods targeting adults over the age of 55, who are engaged in regular resistance training.

Claim: creatine increases physical performance in successive bursts of short-term, high intensity exercise; conditions and/or restrictions of use of the food and/or additional statement or warning: the claim may be used only for foods targeting adults performing high intensity exercise.

Claim: carbohydrates contribute to the recovery of normal muscle function (contraction) after highly intensive and/or long-lasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle; conditions and/or restrictions of use of the food and/or additional statement or warning: the claim may be used only for foods intended for adults who have performed highly intensive and/or long-lasting physical exercise leading to muscle fatigue and the depletion of glycogen stores in skeletal muscle.

#### Recommended bibliography

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# Effect of 3-week progressive overloading and 1-week tapering on performance, internal training load, stress tolerance and heart rate variability in under-19 Brazilian badminton players

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#### Summary

**Objective:** This study aimed to determine the effect of 3-week progressive overloading and 1-week tapering during a preseason on performance, internal training load, stress tolerance, and heart rate variability in under-19 Brazilian badminton players. **Material and method:** Eight male under-19 badminton players (age  $16.1 \pm 0.6$  years; height  $1.68 \pm 7.7$  m; body mass  $57.2 \pm 5.8$  kg; body mass index  $20.3 \pm 2.5$  kg·m<sup>-2</sup>; body fat  $8.0 \pm 2.7$  %), competing at the state level had physical and physiological monitored over four weeks during pre-season. Players underwent a badminton-specific movement agility test, 5-m multiple shuttle test, Yo-Yo Intermittent Recovery test level 1, and performed vertical jumps before and after the pre-season. During the training, the heart rate variability and internal training load were monitored daily, and weekly were stress tolerance was recorded by psychometric responses.

**Results:** The players showed significant improvements in all performance variables assessed after the training period. The internal training load during overloading was higher (1635  $\pm$  109.9; 2490  $\pm$  124; 2850  $\pm$  210 AU) compared to tapering (1335  $\pm$  100 AU). The stress tolerance decreased during overloading (4.0  $\pm$  0.7; 8.2  $\pm$  1.3; 10.1  $\pm$  1.4) and increased during tapering (5.5  $\pm$  1.5). In addition, higher internal training load during overloading resulted in a greater reduction in root-mean-square difference of successive R-R intervals (InRMSSDmean) (4.2  $\pm$  0.2; 4.1  $\pm$  0.1; 4.0  $\pm$  0.1 ms) and a smaller coefficient of variation (InRMSSDcv) (4.5  $\pm$  2.6; 2.1  $\pm$  1.2; 1.4  $\pm$  0.9 %), and the significant reduction in the internal training load during tapering led to a decrease in InRMSSDmean (1.3  $\pm$  0.5 ms).

#### **Key words:**

Performance. Autonomic nervous system. Periodization. Monitoring.

**Conclusions:** Our results suggest that using badminton training programs during the pre-season, including intermittent high-intensity actions with progressive overloading followed by a tapering is sufficient to result in positive adaptations in performance and led to adaptative changes in internal training load, stress tolerance, and heart rate variability.

Efecto de la sobrecarga progresiva de 3 semanas y la reducción gradual de 1 semana sobre el rendimiento, la carga de entrenamiento interno, la tolerancia al estrés y la variabilidad de la frecuencia cardíaca en jugadores brasileños de Bádminton menores de 19 años

#### Resumen

**Objetivo:** Este estudio tuvo como objetivo determinar el efecto de la sobrecarga progresiva de 3 semanas y la reducción gradual de 1 semana durante una pretemporada sobre el rendimiento, la carga de entrenamiento interno, la tolerancia al estrés y la variabilidad de la frecuencia cardíaca en jugadores de bádminton brasileños menores de 19 años.

**Material y método:** Ocho jugadores masculinos de bádminton sub-19 (edad  $16,1\pm0,6$  años; altura  $1,68\pm7,7$  m; masa corporal  $57,2\pm5,8$  kg; índice de masa corporal  $20,3\pm2,5$  kg·m²; grasa corporal  $8,0\pm2,7$  %), que competían a nivel estatal fueron monitoreados en sus aspectos físicos y fisiológicos durante cuatro semanas en la pretemporada. Los jugadores realizaron un test de agilidad específico de bádminton, el test 5-m multiple shuttle test, el test Yo-Yo y realizaron saltos verticales antes y después de la pretemporada. Durante el entrenamiento, se monitoreó diariamente la variabilidad de la frecuencia cardíaca y la carga interna de entrenamiento. Además, semanalmente se registró la tolerancia al estrés mediante respuestas psicométricas. **Resultados:** Los jugadores mostraron mejoras significativas en todas las variables de rendimiento evaluadas después del período de entrenamiento. La carga de entrenamiento interna durante la sobrecarga fue más grande ( $1.635\pm109,9$ ; 2.490

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 $\pm$  124; 2.850  $\pm$  210 AU) en comparación con la última semana de cargas reducidas (1.335  $\pm$  100 AU). La tolerancia al estrés disminuyó durante la sobrecarga (4,0  $\pm$  0,7; 8,2  $\pm$  1,3; 10,1  $\pm$  1,4) y aumentó durante la última semana (5,5  $\pm$  1,5). Además, las altas cargas del entrenamiento interno durante la sobrecarga resultaron en una gran reducción en la diferencia de la raíz cuadrada media de intervalos R-R sucesivos (lnRMSSDmean) (4,2  $\pm$  0,2; 4,1  $\pm$  0,1; 4,0  $\pm$  0,1 ms) y un coeficiente de variación más pequeño (lnRMSSDcv) (4,5  $\pm$  2,6; 2,1  $\pm$  1,2; 1,4  $\pm$  0,9 %), y la reducción significativa en la carga de entrenamiento interno durante la última semana condujo a una disminución en lnRMSSDmean (1,3  $\pm$  0,5 ms).

Palabras clave:

Rendimiento. Sistema nervioso autónomo. Periodización. Supervisión.

**Conclusión:** Nuestros resultados sugieren que el uso de programas de entrenamiento de bádminton durante la pretemporada, que incluyen acciones intermitentes de alta intensidad con sobrecarga progresiva seguida de una semana de cargas reducidas, es suficiente para dar como resultado adaptaciones positivas en el rendimiento y condujo a cambios adaptativos en la carga de entrenamiento interno, en la tolerancia al estrés y en la variabilidad de la frecuencia cardiaca.

#### Introduction

Badminton has been characterized as a high-intensity sport that combines intermittent actions of very intense anaerobic exercises with changes in direction and longer lower-intensity periods of aerobic exercises<sup>1,2</sup>. Due to these demands, a high level of speed, coordination, agility, strength, and explosive jumps are required from badminton players<sup>1,3</sup>.

The pre-season is considered as a period to develop positive physiological adaptations that maximize physical components (*e.g.*, maximum strength and power, speed, and agility), which are required for the competitive season<sup>4-6</sup>. A strategy that usually occurs during this period involves phases of deliberate overloading (OL), followed by a tapering period (TP) that consist of a gradual reduction in the training load (TL)<sup>4-6</sup>. However, this requires careful individual TL monitoring in an attempt to balance periods of stress and recovery, leading to an increase in performance. Thus, it is important that coaches use physiological, psychological, and performance parameters for measurement and control TL to obtain information on how athletes are responding to different training stressors<sup>5-7</sup>.

Among the measures used to effectively estimate the internal training load (ITL), the session rating of perceived exertion (sRPE) is considered a practical and valid method that demonstrated high correlations with physiological and psychological parameters<sup>7,8</sup>. Furthermore, changes in ITL are associated with changes in stress levels during training programs<sup>7,9</sup>. In this context, previous studies have demonstrated that measures of stress tolerance (ST) evaluated using the daily analysis of life demands for athletes (DALDA) questionnaire<sup>10</sup> are sensitive to increases and decreases in the ITL<sup>4,7,9</sup>, reflecting some positive adaptations<sup>6,7</sup>. In addition to sRPE and DALDA, another important physiological measure used to monitor ITL in different sports is heart rate variability (HRV), which is typically characterized by decreases during periods of higher TL, with increases following periods of lower TL, mirroring recovery, which leads to improvements in performance<sup>4,11,12</sup>.

Some studies involving racquet sports sought to monitor the TL and training effects using tools, such as sRPE, DALDA, heart rate (HR), and physical tests<sup>6,13,14</sup>. Gomes *et al.*<sup>6</sup> when monitoring the ITL and ST of young tennis players during the pre-season, found an increase in stress symptoms in the weeks with higher ITL values, generating adaptive changes in the ST assessed by the DALDA questionnaire. In addition, Moreira *et al.*<sup>14</sup> monitored the training of 12 professional tennis players based on the sRPE and the HR methods and verified an even distribution

for low and moderate intensities zones, but relatively few sessions in high intensity zone. However, specifically with badminton training, previous studies aimed to test the effectiveness of complementary training programs by assessing the performance variables of the players<sup>15,16</sup>, or to monitor a training period using HR and blood markers (*e.g.*, lactate, creatine kinase)<sup>16,17</sup>. Moreover, no study involving badminton players used physical tests, sRPE, DALDA, and HRV to monitor training responses and changes in performance. Thus, we are not aware of studies that clarify adaptive physiology responses during periods of badminton training, especially during the pre-season.

Concerning badminton training, coaches should incorporate periodization models during the pre-season that effectively lead to performance improvements and keep players in an optimal condition to compete <sup>1,15,16</sup>. Therefore, this study aimed to determine the effect of 3-week progressive OL and 1-week TP during the pre-season on performance, ITL, ST and HRV in under-19 Brazilian badminton players. The hypothesis is that the training period improves the performance of the players and that ITL, ST and HRV are different between the training phases.

#### Material and method

#### **Participants**

Eight male under-19 Brazilian badminton players (age  $16.1\pm0.6$  years; height  $168\pm7.7$  cm; body mass  $57.2\pm5.8$  kg; body mass index (BMI)  $20.3\pm2.5$  kg·m<sup>-2</sup>; body fat  $8.0\pm2.7\%$ ), competing at state level volunteered to participate in this study. For a participant's data to be included in the final analysis, the following criteria were adopted: (a) completion of 100% of the training sessions during the pre-season; (b) completion of the sRPE to determine the ITL and DALDA questionnaire to determine the ST; (c) completion HRV daily before each training session; and (d) have no injuries during the training period. Before the study, the participants and their parents were informed about the testing and training procedures, possible risks involved, and provided written informed consent. This study was approved by the Human Research Ethics Committee. All research was conducted ethically according to Helsinki declaration.

#### Design

The experimental protocol consisted of a baseline (BL) period of 4 days during which DALDA and HRV were measured daily. After the

Phasel PhaseII Overload Taper Pre-training tests Post-training tests (1-Week) (3-Weeks) Day 1 Day 2 Day 3 Baseline Day 1 Day 2 Day 3 €0 De Х. **(**) **(**) **(**) 4-Days 24-hour interval between assessments 24-hour interval between assessments Daily Recording ~~ I Daily Recording 1x a week -A-HRV Antropometric VJS DALDA 5-m MST B ITL Badminton Yo-Yo IR1 Agility lest training

Figure 1. Schematic representation of the experimental design.

Note: HRV: heart rate variability; DALDA: daily analysis of life demands for athletes questionnaire; 5-m MST: 5-m multiple shuttle test; Yo-Yo IR1: Yo-Yo intermittent recovery test level I; VJs: vertical jumps; ITL: internal training load.

BL, participants performed the following tests with an interval of 24 h between trials: anthropometric measurements and badminton-specific movement agility test (day 1); 5-m multiple shuttle test (5-m MST) (day 2); vertical jumps tests (VJs) and Yo-Yo Intermittent Recovery test level 1 (Yo-Yo IR1) (day 3). These tests were repeated after the end of the training program following the same procedures. The pre-season training program consisted of two phases. The phase I consisted of a 3-week progressive OL, and the phase II consisted of a 1-week TP. During both phases, HRV and ITL were monitored daily, and DALDA questionnaire evaluated at the end of each training week. The experimental protocol is illustrated in Figure 1.

#### **Procedures**

#### Badminton-specific movement agility test

The test to determine agility was performed with specifics badminton movements using the protocol described previous by Ooi *et al.*<sup>3</sup>. Each participant had 10 min to complete own specific warm-up and two submaximal efforts on the badminton-specific movement agility test. The test was performed on a single badminton court with standardized measures, and required players to perform rapid sideways and diagonal movements with abrupt changes in direction to touch the shuttlecocks with their hands. The test had two phases, in which the players should position themselves in the central base of the court to start and return with at least one foot to the center of the court to validate their execution during and at the end of the test.

The first phase was performed with sideways agility movements, in which the players had to move laterally across the width of the court for a total of 10 repetitions in order to strike each up-turned shuttlecocks placed at each corner. There were five shuttlecocks on each side of the court on the lateral line at a distance of 30 cm between them. In the second phase of the test, there were four shuttlecocks positioned diagonally in the four corners of the court within the service zones

with a distance of 30 cm between them. In this phase, the players moved diagonally in a sequence of four different directions for a total of 16 repetitions. Between the phases the players had five minutes for recovery. The execution time was monitored by a manual stopwatch adjusted by the evaluator; a visual analysis of the movement was performed to verify if the participant was performing displacements with specific Badminton movement. For the data analysis, the duration times of phase 1 and phase 2 were added and it was considered as the total time performance<sup>3</sup>.

#### 5-m multiple shuttle test (5-m MST)

The 5-m MST was performed according to the methods described by Boddington  $et\,al.$  <sup>18</sup> to determine sprint performance. Each participant had 10 min to complete own specific warm-up and two submaximal efforts of the 5-m MST. For this test, six cones were placed 5 m apart from each other in a straight line to cover a total distance of 25 m. The test consisted of six sprints with a change of direction, with run time of 30 s sprint and 35 s recovery time between sprints. To start the test, players positioned themselves in the first cone and upon an auditory signal they sprinted 5 m to a second cone, touched the ground with one hand and returned to the first cone. Then, they sprinted 10 m to a third cone and back to the first cone, etc., until 30 s. The players should accumulate the greatest possible distance within the 30 s execution time. The performance was determined by the total distance (m) (the total distance covered during the 6  $\times$  30-s sprints/shuttles)<sup>18</sup>.

#### Vertical jumps (VJs)

Performances in VJs were measured using an electronic platform (Jump System Pro 1.0 Cefise®, Nova Odessa-SP, Brazil), equipment designed to determine contact time and vertical jump flight time<sup>19</sup>. Participants performed three different vertical jump tests: Squat Jump (SJ), Countermovement Jump (CMJ), and Countermovement Jump with

arms help (CMJA). Before testing, the players performed self-administered submaximal CMJs and SJ as warm-up. In the SJ test, the participants were crouched isometric with his hands on his waist, at the signal of the evaluator performed the jump. In the CMJ tests, the participants stood on the mat, fully erect with his hands positioned at the waist; at the signal of the appraiser, he squatted quickly and then made the jump. Finally, in the CMJA participants followed the same recommendations of the CMJ test but used the aid of the arms to propel themselves. All tests were performed three times, with an interval of 10 s between repetitions and the highest value obtained of height (cm) was used as the performance for analysis<sup>19</sup>.

#### Yo-Yo intermittent recovery test level I (Yo-Yo IR1)

The Yo-Yo Intermittent Recovery Test Level I (Yo-Yo IR1) protocol was conducted according to procedures of the established methods<sup>20</sup>. This test consisted of 20-m shuttle runs performed at increasing velocities, with 10 s of active recovery between runs until voluntary exhaustion. The test was controlled by audio beeps located immediately adjacent to the 20-m long running lanes indicated by markers. The test was considered ended when the participant twice failed to reach the front line in time (objective evaluation) or the participant felt unable to complete another shuttle at the dictated speed. The total distance covered (m) during the Yo-Yo IR1 was considered as the testing score<sup>20</sup>.

#### Internal training load (ITL)

The sRPE method was adopted to measure ITL<sup>21</sup>. Thirty minutes after the end of each training session, players were asked to report RPE for the intensity of the session using the CR-10 scale<sup>22</sup>. To determine ITL, expressed in arbitrary units (AU), the product of session duration (minutes) and sRPE score (CR-10) rated by the player was used. The duration of the training sessions was recorded from the start (warm-up) to the end of the session (cool-down). The weekly-accumulated ITL was calculated intra-individually for the analysis. From the ITL data, strain and monotony were calculated weekly. Monotony was calculated by dividing the average load of the week by the standard deviation, while the strain was calculated by multiplying the monotony by the weekly sum of training loads<sup>21</sup>.

#### Stress tolerance (ST)

To evaluate ST, the Portuguese version<sup>23</sup> of the DALDA question-naire<sup>10</sup> was filled out daily during BL and at the end of each training week. Although it has been suggested that DALDA be used on a daily basis, it was previously used on a weekly basis without having its sensitivity diminished. The DALDA is divided into parts A (9 questions) and B (25 questions), which represents the sources and symptoms of stress respectively. The possible answers for each item are "better than normal", "normal" and "worse than normal". For each training session, the sum of the scores marked as "worse than normal" of the questionnaire was recorded for analysis.

#### Heart rate variability (HRV)

All HRV measures were performed daily during BL and before each training session. The same conditions were maintained during

each HRV measurement. HRV was recorded with a portable heart rate monitor (RS800cx Polar®, Kempele, Finland), previously validated for this purpose<sup>24</sup>. After allowing 1 min for stabilization, HRV were measured for 1 min<sup>25</sup> in a guiet environment, with the participants in a standing position. These data were then downloaded to Polar Pro-trainer 5 software (Polar Electro Ov®, Kempele, Finland). Occasional artefacts and non-sinus beats were replaced with the interpolated adjacent normal cycle. Subsequently, the data were analysed using specialized HRV analysis software (Kubios HRV Analysis®, version 3.0 Biomedical Signals Analysis Group, University of Kuopio, Finland). The vagally mediated HRV parameter used for analysis was the logarithm of the root-mean square difference of successive R-R interval (InRMSSD). Since InRMSSD is considered consistent under paced and spontaneous breathing<sup>26</sup>, all HRV recordings were completed under spontaneous-breathing conditions. The intra-individual weekly mean (InRMSSDmean) and its coefficient of variation (InRMSSDcv) were calculated for the analysis. The InRMSSDcv was calculated as follows:  $CV = ([SD/mean] \times 100)$ .

#### **Training program**

The training program consisted of four weeks of traditional badminton training during the pre-season (*i.e.*, general preparatory) divided into two phases (3-week OL, and 1-week TP). Each training week included three training sessions lasting  $117.8 \pm 7.6$  min. The training focus was on developing resistance, strength, power, mobility, repeat sprints, speed, agility, coordination and pattern movements. Table 1 displays the weekly training microcycles from the training program. Badminton practice sessions (BP) involved the development of technical and tactical skills in conjunction with pattern movement's exercises and anticipation skills during all weeks. Additionally, the agility, coordination, flexibility and lower and upper limb power development, court-based drills were completed during the BP.

Specific resistance training (RT) was performed, involving the multi-shuttle feeding (*i.e.*, shuttlecock control) and continuous displacements in game situations<sup>27</sup>. During RT the players hit eight shuttles which were fed by the trainer. The shuttle feeding was standardized with the trainer serving the shuttles with a badminton racket a frequency of 8 shuttlecocks every 15 s was standardized as described by Wee *et al.*<sup>27</sup>. Sprint-agility training (SAT) was performed daily over all training weeks, requiring participants to complete sprints with specific movement on the court (four points). The number of sprints was gradually increased

Table 1. Microcycle structure during the badminton pre-season training.

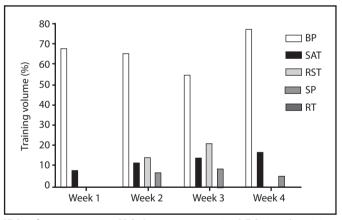
	Week 1	Week 2	Week 3	Week 4
Monday	BP, RT, SAT	BP, SAT, RST	BP, SAT, RST	BP, BP, SAT
Tuesday	_	_	_	_
Wednesday	BP, SAT	BP, SAT, SP	BP, SAT, SP	BP, SAT, SP
Thursday	_	_	_	_
Friday	BP, RT, SAT	BP, SAT, RST	BP, SAT, RST	BP, SAT

Note: RT: Specific resistance training; BP: Badminton practice session; SAT: Sprint agility training in court; RST: Repeat sprint training; SP: Speed training; —: rest.

Table 2. Description of the specific badminton training program during the 4-week pre-season.

	Week 1	Week 2	Week 3	Week 4
Sprint-Agility training (SAT)				
Repetitions	7 to 9 × 20 s	9 to 11 × 20 s	11 to 13 × 20 s	13 to 15 × 20 s
Goal intensity	All-out	All-out	All-out	All-out
Rest period between repetitions	10 s	10 s	10 s	10 s
Repeated sprint training (RST)				
Number of sets	-	2	3	-
Repetitions	-	4 × 30 s	4 × 30 s	-
Goal intensity	-	High	All-out	-
Rest period between repetitions	-	35 s	35 s	-
Rest period between sets	-	3 min	2.30 min	-
Speed training (SP)				
Number of sets	-	3	3	2
Repetitions	-			
	-	5 × 10 m	8 × 10 m	$4 \times 10 \text{ m}$
		$4 \times 20 \text{ m}$	6 × 20 m	$3 \times 20 \text{ m}$
Goal intensity	-	High	All-out	High
Rest period between repetitions	-	20 s	40 s	20 s
Rest period between sets	-	2 min	3 min	2 min
Specific Resistance training (RT)				
Number of sets	3	-	-	-
Repetitions	10 x 15 s			
Goal intensity	High	-	-	-
Rest period between repetitions	30 s	-	-	-
Rest period between sets	1 min	-	-	-

Figure 2. Description of the training volume (%) during the 4-week training period.



RT: Specific resistance training; BP: Badminton practice session; SAT: Sprint agility training in court; RST: Repeat sprint training; SP: Speed training; —: rest.

from seven to 15 during the 4-week training period as described by Waklate *et al.*<sup>15</sup>. A maximal power program was implemented in weeks two and three, which included a repeated sprint training (RST), and speed training (SP), prescribed based on the 5-m MST protocol<sup>18</sup>. During week four, the volume of SP was decreased by decreasing the number of sets while training intensity was maintained. Conditioning program details (i.e. RT, SAT, RST and SP) are displayed in Table 2, the volume of the training program is detailed in Figure 2.

#### Statistical analysis

Statistical procedures were performed using the software Statistical Package for the Social Sciences (SPSS® v 23.0 for Windows, Inc, Chicago, IL, USA). Data normality was verified by the Shapiro-Wilk test and the data are showed as mean  $\pm$  standard deviation (SD). The sphericity was tested with Mauchly's test and Greenhouse-Geisser corrections were made when assumptions of sphericity were violated. One-way analysis of variance for repeated measures (ANOVA) followed by the Bonferroni post-hoc test was used to evaluate differences in ITL, monotony, strain, ST values and HRV parameters across the training weeks. To evaluate differences in performance variables from pre to post training intervention a paired-sample t test was used. Additionally, the percentage change value (%) was calculated for each variable. The level of significance was set at P < 0.05 for all statistical analysis.

#### Results

Table 3 shows the results of SJ, CMJ, CMJA, badminton-specific movement agility test, 5-m MST and Yo-Yo IR1 pre- and post-training. There were significant improvements in all variables pre- to post-training.

Figure 3 demonstrates the ITL, monotony, and strain during the 4-weeks. The ITL increase in week two and three (2490  $\pm$  124 and 2850  $\pm$  210 AU, respectively) compared to week one (1635  $\pm$  109.9 AU). These variables decreased significantly during week four (1335  $\pm$  100 AU) when compared to all training weeks. Regarding monotony, there was no changes during week two and four (1.85  $\pm$  0.1 and 1.63  $\pm$  0.2

Table 3. Mean  $\pm$  standard deviation (SD) and change (%) for performance variables at pre- and post-training (N = 8).

Variables	Pre-training	Post-training	% Change	Р
SJ (cm)	33.2 ± 6.2	34.7 ± 5.4*	5.2 ± 6.3	0.048
CMJ (cm)	$35.0 \pm 6.5$	37.1 ± 5.6*	$6.6 \pm 7.1$	0.028
CMJA (cm)	$41.1 \pm 8.2$	43.5 ± 7.8*	$6.4 \pm 5.7$	0.012
Agility test (s)	57.5 ± 5.1	54.4 ± 3.9*	-5.3 ± 3.2	0.003
5-m MST (m)	556.5 ± 75.8	685.6 ± 72.8*	21.9 ± 12.1	< 0.001
Yo-Yo IR1 (m)	737.5 ± 239.4	1065 ± 337.3*	49.5 ± 47.3	0.011

SJ: Squat Jump; CMJ: Counter Movement Jump; CMJA: Counter Movement Jump with arms help; 5-m MST: 5-m Multiple Shuttle Test; Yo-Yo IR1: Yo-Yo Intermittent Recovery test level 1. \*: Significantly different from pre-training.

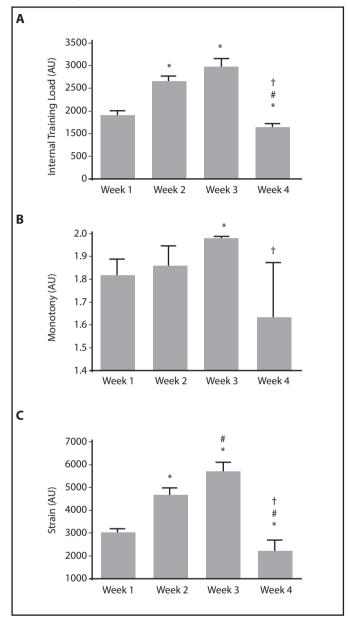
AU, respectively) compared to week one (1.81  $\pm$  0.1 AU), with higher values during week three (2.0  $\pm$  0.0 AU) compared to week one and two. In addition, training monotony was lower during week four (1.63  $\pm$  0.2 AU) when compared to week three (2.0  $\pm$  0.0 AU). Significant increases in training strain were found during week two and three (4615  $\pm$  333 and 5621  $\pm$  419 AU) compared to week one (2962  $\pm$  216 AU), with a significant decrease during week four (2192  $\pm$  447 AU) compared to all training weeks (Figure 3).

Table 4 presents the results of InRMSSDmean, InRMSSDcv and DALDA "worse than normal" scores during BL and throughout the training weeks. When compared to BL, reduction in InRMSSDmean was significant only during week four. In addition, InRMSSDmean was significantly lower during week three compared with week two. During week four, InRMSSDmean was significantly lower compared to all weeks of training. Regarding to InRMSSDcv, there was a significantly reduction during week three compared to week one, while reduction in week four were found when compared to BL and week one. DALDA "worse than normal" scores progressively increased until week three compared to BL, with a decreased during week four compared to week two and three.

#### Discussion

The aim of this study was to determine the effect of 3-week progressive overloading and 1-week tapering during a pre-season on performance, internal training load (ITL), stress tolerance (ST), and heart rate variability (HRV) among under-19 badminton players. The main findings were as follows: an improvement in all performance variables

Figure 3. Overall internal training load (A), monotony (B) and strain (C) in arbitrary units (AU) during the 4-week training period (N = 8).



<sup>\*</sup> P < 0.05 compared to week 1; # P < 0.05 compared to week 2; † P < 0.05 compared to week 3.

Table 4. Mean  $\pm$  standard deviation (SD) for InRMSSDmean, InRMSSDcv and DALDA "worse than normal" score during baseline (BL) and throughout training weeks (N = 8).

Variable	BL	Week 1	Week 2	Week 3	Week 4
InRMSSD <sub>mean</sub> (ms)	4.2 ± 0.2	$4.2 \pm 0.2$	4.1 ± 0.1	4.0 ± 0.1 <sup>†</sup>	3.9 ± 0.1*, #, †, ‡
InRMSSDcv (%)	$2.7 \pm 1.2$	$4.5 \pm 2.6$	2.1 ± 1.2	1.4 ± 0.9 #	1.3 ± 0.5 *, #
DALDA "worse-than-normal"	$2.5 \pm 0.5$	4.0 $\pm$ 0.7 $^{*}$	$8.2 \pm 1.3^{*,#}$	10.1 ± 1.4*, #, †	5.5 ± 1.5 *, †, ‡

Note: InRMSSDmean, weekly mean log-transformed root-mean square difference of successive R-R intervals; InRMSSDcv, coefficient of variation of weekly log-transformed root-mean square difference of successive R-R intervals; DALDA, daily analysis of life demands for athlete's questionnaire.

<sup>\*:</sup> P < 0.05 compared to BL; #: P < 0.05 compared to week 1; †: P < 0.05 compared to week 2; †: P < 0.05 compared to week 3.

after the pre-season, the ITL during OL was higher compared to that during the TP, and the ST decreased during OL and increased during TP. In addition, a higher ITL during OL resulted in a greater reduction in InRMSSDmean and a smaller InRMSSDcv, and the significant decrease in ITL during the tapering period led to a decrease in InRMSSDmean. These results confirm the initial hypothesis of the study.

According to previous research, it is suggested that a badminton training program should address intensities and actions that resemble the competitive reality<sup>2,15</sup>. Thus, it is essential that the physical preparation of badminton players consist of repetitive high-intensity actions of short duration combined with actions of agility, speed, and VJs<sup>2,3,28</sup>. The training program of the present study not only addressed the development of anaerobic power and aerobic endurance, but also sought to promote improvements in the agility, speed, and VJs, which are variables associated to the technical complexity and tactic of badminton players<sup>3,15,28</sup>.

In the present study, the pre-season training led to significant improvements in VJs, badminton-specific movement agility test, Yo-Yo IR1 and 5-m MST performances. Similar to our findings, Gomes *et al.*<sup>6</sup> investigated the effect of four weeks of progressive OL training and a 1-week TP during the pre-season on ITL, ST, immune-endocrine responses, and physical performance in 10 young tennis players was investigated. In addition, similarly to the present study, agility-speed exercises and high-intensity intermittent actions were prescribed. The authors identified significant improvements in the Yo-Yo IR1 and agility T-test as found in our results; however, Gomes *et al.*<sup>6</sup> did not identify a significant increase in VJs (*e.g.*, SJ and CMJ), which was different from our findings.

Regarding the badminton investigations Waklate et al. 15 investigated whether supplementing regular group training with short sessions of badminton-specific agility-sprint training conferred any greater changes in performance than regular training alone during a 4-week pre-season in 12 elite badminton players. The supplementary training group reported improvements in the 300-m shuttle run (2.4  $\pm$  2.7%) and in the badminton sprint protocol (3.6  $\pm$  2.6%); however, the control group did not show significant improvements in any performance variable, which was different our findings. It is important to mention that Waklate et al. 15 did not evaluate the aerobic power and VJs of the players. Wee et al. 16 investigated the effects of four weeks of high-intensity intermittent badminton multi-shuttle complementary training on the performance variables of 18 university badminton players, the authors reported significant improvements in  $\dot{V}$ O<sub>2max</sub>, mean power, leg reactive strength, and agility in the experimental group. However, the control group with regular badminton training showed no improvement in all performance variables.

The training strategy used in the present study sought to integrate specific and general physical exercises  $^{15,16}$ , within a strategy that is characterized by an OL in weeks one to three, followed by a TP during the week four. Concerning the analysis of the training intensity distribution based on the sRPE method, the data showed that ITL at weeks two and three and were higher compared to week one, followed by a significant reduction in week four. Similar to our findings, Gomes  $\it et al.^6$  using a similar training intervention during the pre-season led to a progressive increase in the ITL in 4-weeks ( $\approx 2000$  - 4500 AU), followed by a signifi-

cant reduction in week five ( $\approx$  2000 AU) that promoted improvements in performance of tennis players.

It is known that despite the risk of nonfunctional overreaching or even an overtraining syndrome, high ITL during the pre-season are necessary to generate positive adaptations and increases in performance<sup>13</sup>, which was proven in the present study. Moreover, our results demonstrated that the ITL and strain were aligned with the planned OL and TP training weeks. This finding is in agreement with previous studies that identified a relationship between strain and changes in ITL in racket sports<sup>6,13</sup>. The decrease in ITL and strain identified in the week four is related to the programmed TP strategy.

It is important to mention that the balance between strain and recovery can determine the benefits of the training; thus, monitoring measures related to fatigue (*i.e.*, physiological and psychometric variables) can predict symptoms of overtraining and stress during a training period<sup>7,13</sup>. In our study, we identified a decrease in ST during the OL period compared to the BL, indicated by the greater number of "worse than normal" responses from DALDA questionnaire; changes in the DALDA score of "worse than normal" responses are related to adaptive changes in the ST<sup>4,6,7,13</sup>. Similar ST responses were reported by Gomes *et al.*<sup>6</sup> identified decreases in ST, during OL, indicated by the greater number of "worse than normal" responses from DALDA; followed by an improvement during TP in tennis players during the pre-season. These results confirm that DALDA is a sensitive tool that detects changes in ITL as previously reported<sup>4,7</sup>. However, no previous study sought to monitor ST responses to ITL changes in badminton players.

Concerning the HRV results, we found no changes in InRMSSDmean during the first 2-weeks of OL, but found increases in InRMSSDcv during the first week OL and a decrease during the second week of OL. In addition, during the TP, InRMSSDmean and InRMSSDcv decreased. It is important to emphasize that no study with racquet sports investigated HRV responses during the pre-season, especially during badminton training. These findings during OL are similar to a previous study with female soccer players, which demonstrated that InRMSSDcv increases with no changes in InRMSSDmean during a period of increased ITL<sup>29</sup>. Thus, it seems that the badminton players from the present investigation were not able to cope well with the initial TL. The decrease in InRMSSDcv during the week two of OL was similar to a previous study with rugby sevens athletes during a second exposure to higher ITL, which the authors interpreted as a reflection of an improved ability to maintain cardiac autonomic homeostasis even when ITL is intensified<sup>30</sup>.

Furthermore, during the week three of OL and during TP a decrease in InRMSSDcv and InRMSSDmean was found. These results could be related to the increases in anaerobic workload imposed during weeks two and three. It has been shown that parasympathetic reactivation is highly impaired after repeated sprint training, which is associated with an increase in plasma metabolites and a higher sympathetic activity<sup>31</sup>. The results found during TP are in contrast with previous studies, which demonstrated increases in InRMSSDmean coupled with decreases in InRMSSDcv values after reductions in the ITL<sup>29,25</sup>. However, despite the increase in ST found during TP, we suggest that the reduction in ITL in the last week of training was not sufficient to reestablish the ST and InRMSSD values of the players in relation to the baseline value<sup>4,29,32</sup>, or

was due to some of the persisting effects of fatigue or the inadequate recovery from the higher TLs weeks.

The small sample size in the present study could be considered as a limitation. However, it can be explained by the fact that in Brazil badminton is an incipient modality making it difficult to carry out studies with a large number of players with a higher competitive level. In addition, there are few studies in the literature related to the training and performance of badminton players; therefore, the comparisons in the present study are limited to other modalities. The lack of performance tests at the end of the OL phase could be considered another limitation, which in turn would provide more data related to the effects of increased ITL on performance, ST, and HRV responses.

#### **Conclusions**

Four-week pre-season improved performance in under-19 badminton Brazilian players and the periodized training program evoked a progressive increase in ITL during OL, followed by a significant reduction during TP. Furthermore, modifications in ITL resulted in adaptive changes in ST, indicated by the DALDA questionnaire, and in HRV, demonstrated by the fluctuations in the values of InRMSSDmean e InRMSSDcv. Monitoring individual ITL in conjunction with ST and InRMSSD responses can provide valuable information that leads coaches to strategically manipulate ITLs individually. The results of the present study provide new information on the performance testing and monitoring of ITL, ST, and HRV in under-19 badminton players during a pre-season, which can help coaches to prescribe badminton training programs more successfully.

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#### Conflict of interest

The authors do not declare a conflict of interest.

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### Study of sedentary behaviour analysed by self-report questionnaire and accelerometry, and its association with cardiovascular risk factors in an adult population from a health centre

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#### **Summary**

Introduction: Currently, sedentary lifestyle has been gaining prominence in the daily life of adults, increasing the time they spend seated, with a relationship between sedentary time and increased mortality from any cause, higher incidence of cardiovascular diseases, cancer and type 2 diabetes.

Purpose: The aim of this study are to assess the applicability of subjective and objective recording methods in the field of physical activity and health, and to determine the possible relationships between sedentary behavior and its variables and the level of daily physical activity with the prevalence of one or more cardiovascular risk factors.

Material and method: A group of 64 adults from a Primary Care Center were studied, who were administered the International Physical Activity Self-Questionnaire (IPAQ) and an ActivPal triaxial accelerometer was applied for 72h.

Results: Significant differences were observed between self-reported sitting time and accelerometry values, IPAQ (265.45±129.67 min/day) and ActivPal (387.78±215.06 min/day). Significant differences were also observed between the accelerometry variables related to sedentary behavior and the presence of cardiovascular risk factors (CVRF), sedentary time (H=8.42; df=3; p=.03), number of transitions (H=10.41; df=3; p=.01) and number of total steps (H=13.4; df=3; p=.004).

Conclusions: The results of this study demonstrate the underestimation of sitting time by the population using the IPAQ, the relationship between sedentary behavior variables and the presence of CVRF, and the need to adopt promotional measures for the change towards an active lifestyle using strategies that can generate awareness of the importance in the acquisition of habits that generate transitions from sitting to standing.

#### **Key words:**

Sedentary behavior. Sitting time. Cardiovascular risk factors. Self-questionnaire Accelerometry.

#### Estudio del comportamiento sedentario analizado mediante autocuestionario y acelerometría y su asociación con factores de riesgo cardiovascular en población adulta de un centro de salud

#### Resumen

Introducción: En la actualidad, el sedentarismo ha ido ganando protagonismo en el día a día de las personas adultas aumentando el tiempo que pasan en sedestación, existiendo una relación entre tiempo sedentario y el aumento de la mortalidad por cualquier causa, mayor incidencia en enfermedades cardiovasculares, cáncer y diabetes tipo 2.

Objetivo: Los objetivos de este estudio son valorar la aplicabilidad de métodos de registro subjetivos y objetivos en el ámbito de la actividad física y la salud, y determinar las posibles relaciones entre el comportamiento sedentario y sus variables y el nivel de actividad física diaria con la prevalencia de uno o más factores de riesgo cardiovascular.

Material y método: Se estudió a un grupo de 64 adultos de un Centro de atención primaria a los que se les administró el Autocuestionario Internacional de Actividad Física (IPAQ) y se les aplicó un acelerómetro triaxial ActivPal durante 72h.

Resultados: Se obtuvieron diferencias significativas entre el tiempo sentado reportado mediante auto-cuestionario y los valores de acelerometría, IPAQ (265.45±129.67 min/día) y ActivPal (387.78±215.06 min/día). También se observaron diferencias significativas entre las variables de acelerometría relativas al comportamiento sedentario y la presencia de factores de riesgo cardiovascular (FRCV), tiempo sedentario (H=8.42; df=3; p=.03), número de transiciones (H=10.41; df=3; p=.01) y número de pasos totales (H=13.4; df=3; p=.004).

Conclusiones: Los resultados de este estudio demuestran la subestimación del tiempo sentado por parte de la población mediante el IPAQ, la relación entre las variables del comportamiento sedentario y la presencia de FRCV y la necesidad de adoptar medidas de promoción para el cambio hacia un estilo de vida activo utilizando estrategias que puedan generar consciencia de la importancia en la adquisición de hábitos que generen transiciones de sedestación a bipedestación.

### Palabras clave:

Comportamiento sedentario. Tiempo sentado. Factores de riesgo cardiovasculares. Autocuestionario. Acelerometría.

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#### Introduction

Most epidemiological studies in large cohort populations show an inverse relationship between physical activity and the risk of cardiovascular disease. In turn, regular physical activity is the only behavioural intervention which has demonstrated any usefulness in increasing cardiorespiratory fitness, a strong indicator of good metabolic health, low morbidity and low risk of death<sup>1</sup>.

Sedentary behaviour and its relationship with health is a topic which has been studied for many years<sup>2-4</sup>. Such behaviour has been defined as any waking behaviour characterised by an energy expenditure less than 1.5 times the basal metabolic rate, i.e. 1.5 metabolic equivalents (METs), while sitting, lying or reclining<sup>5</sup>. The concept of sedentary behaviour encompasses the concept of sitting time, defined as the time a person spends in a position in which their weight rests on the buttocks instead of the feet and in which the back is erect<sup>6</sup>. It has been observed that this time represents most of a person's waking time. These studies have proposed that, regardless of the level of physical activity, sedentary time is associated with a higher risk of mortality from any cause and a higher incidence of cardiovascular diseases, cancer and type 2 diabetes<sup>67</sup>. Due to these findings, many health science authorities, such as the American Heart Association, have published guidelines to reduce sitting time and increase active time<sup>8</sup>.

Sitting time is a variable which has mainly been studied using self-report questionnaires and accelerometers<sup>9-11</sup>. The questionnaire which several studies have used is the International Physical Activity Questionnaire (IPAQ), in which the person is asked about the time he or she spends sitting in a day<sup>10,12-14</sup>. This variable has also been analysed in different studies with accelerometry devices like the activPAL accelerometer, which measure the acceleration of movement, at the same time as other variables, such as steps and breaks in sitting time<sup>5,9,10</sup>.

When analysing behaviour, transitions should be understood as interrupted sitting time and we are beginning to understand the biological mechanisms behind the cardiovascular health benefits involved<sup>6</sup>. Transitions can be a strategy to counter the associated risks, such as vascular dysfunctions<sup>15</sup>. Paing *et al.*<sup>16</sup>, in their study, observed effects on glucose control. It has also been observed that more breaks in sedentary time are beneficially associated with metabolic risk variables, in particular with measurements of adiposity, triglycerides and plasma glucose at 2 hours<sup>17</sup>.

Another variable analysed over the years has been the number of daily steps taken and their relationship with cardiovascular risk factors<sup>18–20</sup>. These studies have obtained favourable results in reducing the risk of mortality and developing cardiovascular diseases in those who walk more.

The objectives of this study are to assess the applicability of subjective and objective recording methods in the field of physical activity and health, and to determine possible relationships between sedentary behaviour and level of daily physical activity, and the prevalence of one

or more cardiovascular risk factors (CVRF) in adult patients from a primary health centre on the island of Menorca.

#### Materials and methods

#### **Participants**

Retrospective study with prospective data from a sample belonging to a group of 64 participants (36 men, 28 women) aged 50.1±5.6 (mean  $\pm$  SD) years, weighing 74.4 $\pm$ 1.6 kg., with a height of 167 $\pm$ 1 cm and a BMI of 26.8±4.3, from the quota of patients assigned to a primary health centre in a population on the island of Menorca. The selection was carried out using the common characteristic shared by all the patients: belonging to the guota assigned to the centre and being diagnosed with one or more CVRF. With the help of the health centre database, adult patients between 40 and 80 years of age diagnosed with one or more cardiovascular risk factors were randomly selected from the primary health care quota. This age group was chosen due to the greater presence of CVRFs. The clinical study lasted from 3 January to 5 March 2020. The total number of patients meeting the inclusion criteria came to 580, but due to the state of health emergency, the sample was reduced to those patients who performed the complete protocol prior to its declaration (n=64). The following CVRFs were diagnosed: diabetes mellitus, hypertension, hypercholesterolemia, obesity and overweight (0.62±0.48), and tobacco use.

All the participants were informed of the risks and benefits of the study, and gave written informed consent to take part in the study. Participants could reject the inclusion of their data. The study was carried out following the ethical principles for biomedical research with human beings established in the Declaration of Helsinki of the World Medical Association (updated in 2013) and was approved by the Research Ethics Committee of the Autonomous Government of Catalonia's General Secretariat of Sport (032/CEICGC/2021).

#### Research design

Physical activity and sedentary behaviour were assessed using the IPAQ<sup>21</sup> questionnaire and a triaxial accelerometer (activPAL)<sup>22</sup> fitted on the right lower limb of selected patients for a period of 72 hours. The activPAL accelerometer monitored sitting, standing and activity time

Table 1. Participant data.

Variables	Records	Mean	Standard deviation	Minimum	Maximum
Age (years)	269	50.1	5.6	40	63
Weight (kg)	269	74.45	15.56	47	127
Height (cm)	269	167	8	150	183
BMI	269	26.41	5.7	0	41.8

(time in motion). A triaxial accelerometer is a device which measures the movement of the body in space on different planes (vertical, mediolateral and anteroposterior), using body acceleration to turn it into a quantifiable digital signal. This is a direct, objective measurement<sup>23</sup>.

To select the patients, these were contacted by telephone and asked to come to the health centre or time was used during a regular consultation to explain the objectives of the study and conduct an individual interview with each of them. The data collected in the patients' medical history over the last 6 months were used to determine the degree of control of the condition through: HbA1c in diabetics, blood pressure in hypertensive patients, analytical lipid profile in dyslipidaemic patients, BMI in obese patients and cigarette consumption/day in smoking patients. How many of the patients under study had been recommended physical exercise in the previous year (reflected in their medical histories) and whether there was any difference with those who had not received recommendations was also taken into account.

#### Variables studied

The variables recorded and analysed in this study were those shown in Table 2.

#### Statistical method

The data are presented as the mean  $\pm$  standard deviation (SD). After conducting a descriptive study of central tendency and considering the non-normality of the sample, the Mann-Whitney U test was used to determine possible differences between the variables obtained using IPAQ and activPAL. Subsequently, the Kruskal-Wallis test was used to determine the possible differences between the values of the variables by absolute values and by quartiles in relation to the sedentary behaviour of the CVRF.

Statistical analysis was performed with Jasp software version 0.11.1 (The Jasp Team, Amsterdam, The Netherlands). The significance level for the whole analysis was p < 0.05.

#### Results

The following CVRFs were diagnosed per patient: diabetes mellitus  $(0.1\pm0.31)$ , arterial hypertension (AHT;  $0.1\pm0.31$ ), hypercholesterolemia  $(0.22\pm0.41)$ , obesity and overweight  $(0.62\pm0.48)$  and tobacco use  $(0.44\pm0.49)$  (Table 3). 139 participants had at least one CVRF (50.9%); 96 had two CVRFs (35.1%); 33 had three (12%) and five had all four CVRFs

Table 2. Subjective and objective variables analysed in the study.

Variable	Abbreviation	Type of variable	Description	Unit of measurement
Hypertension	AHT	Objective	Presence of excessively high blood pressure. Systolic blood pressure which is greater than or equal to 140 mmHg and diastolic blood pressure greater than or equal to 90 mmHg.	mmHg
Dyslipidaemia	DLP	Objective	Presence of a high concentration of lipids (cholesterol, triglycerides or both) or a low concentration of cholesterol rich in lipoproteins (HDL).	mg/dL
Diabetes	DM	Objective	A chronic disease which occurs when the pancreas does not secrete enough insulin or when the body does not use the insulin it produces effectively. Greater than 126 mg/dL	mg/dL
Tobacco use	TU	Subjective	Daily tobacco consumption	Yes/no
Obesity/overweight	ОВ	Objective	Relationship between weight and height used to identify overweight and obesity in adults. This is calculated by dividing a person's weight in kilograms by the square of their height in metres (kg/m²). Overweight: BMI equal to or greater than 25. Obesity: BMI equal to or greater than 30.	kg/m²
activPAL sitting time	AST	Objective	Time recorded by the activPAL in the position in which the person remains vertical by resting the pelvis on a support, total or partial	Minutes
IPAQ sitting time	IST	Subjective	Time recorded by the IPAQ in the position in which the person remains vertical by resting the pelvis on a support, total or partial	Minutes
activPAL sedentary time	ASedT	Objective	Time recorded by activPAL of waking behaviour characterised by an energy expenditure <1.5 times the basal metabolic rate, i.e. 1.5 metabolic equivalents of task (METs), while sitting, lying or reclining.	Minutes
activPAL total steps	ATS	Objective	Number of steps counted in a day by the activPAL device	Cumulative count
activPAL transitions	AT	Objective	Number of breaks from sitting time passing to a bipedal position recorded by activPAL	Cumulative count

Table 3. Prevalence of cardiovascular risk factors.

Variable	Records	Sum	Percentage
Hypertension	273	61	22.34%
Dyslipidaemia	273	72	26.37%
Diabetes	273	29	10.62%
Tobacco use	273	120	43.96%
Overweight/obesity	273	168	61.54%

Table 4. Objective (activPAL) and subjective (IPAQ) physical activity and sedentary time variables.

Variables	n	Mean	Standard deviation	Minimum	Maximum
activPAL sitting time (AST)	273	387.78	215.06	0.27	888.06
IPAQ sitting time (min, IST)	273	265.2	129.6	90	600
activPAL sedentary time (min; ASedT)	273	731.17	396.56	0	2,010.63
activPAL total steps (ATS)	273	11,936.01	5,605.01	1,808	31,996
activPAL transitions (AT)	273	47.46	19.87	16	136

(2%). By CVRF analysed, 61 patients (22.34%) were diagnosed with AHT; 72 (26.37%) with dyslipidaemia; 29 (10.62%) with diabetes; 120 (43.96%) with tobacco use; and 168 (61.54%) with obesity or overweight.

As for accelerometry, a total of 273 valid records ( $4.2\pm1$ ) were obtained per patient (Table 4). When it came to sedentary behaviour, the reported sitting time was 265.45 $\pm129.67$  min/day with IST and 387.78  $\pm215.06$  min/day with AST per patient. Statistical analysis determined significant differences in relation to IST and AST (W=115.00; p<.001; SE=-0.99).

Significant differences were observed between the accelerometry variables related to sedentary behaviour and the presence of CVRF, both for the activPAL sedentary time (H=8.42; df=3; p=.03), defined by quartiles <4h, 4-6h, 6-8h, >8h per day<sup>6</sup>, and for the AT (H=10.41; df=3; p=.01). Also in relation to the presence of CVRF diagnosed, significant differences were observed with the total steps variable measured with the activPAL (H=13.4; df=3; p=.004), defined by quartiles <4000, 4000-7999, 8000-11999 and >12000 steps per day<sup>24</sup>.

#### **Discussion**

One of the main objectives of the study was to assess the applicability of subjective and objective recording methods in the field of physical activity and sedentary behaviours. With the data obtained, significant

differences are observed in the amount of time sitting during the day between values obtained by self-report questionnaire (IPAQ) and accelerometer (activPAL). These results support the idea that people tend to underestimate the time they spend sitting during the day, showing that the accelerometer registers an average sitting time 2 hours longer than that recorded in the IPAQ. These results are similar to those obtained by Chastin *et al.*<sup>10</sup>, in which an underestimation of sitting time of 2 hours per day when using the IPAQ compared to the accelerometer, was also observed. Fitzsimons' 12 study obtained daily values with a difference of more than 2 hours.

Another objective of this study was to determine the possible relationships between sedentary behaviour and the level of daily physical activity, and the prevalence of one or more cardiovascular risk factors (CVRF). In our study, we obtained significant differences in the number of CVRFs presented and sedentary time, there being a greater prevalence in the number of risk factors in people with a longer sedentary time. These results are in line with the study by Leiva *et al.*<sup>3</sup>, where a relationship between sedentary time and increased cardiovascular and metabolic risk factors was found. In their study, it was observed that the effect of a sedentary lifestyle on these risk factors was not determined by a higher caloric intake but reduced energy expenditure. Other studies in which the self-report questionnaire and accelerometry have been used have also obtained similar results, revealing an increased presence of risk factors with an increase in sedentary time<sup>25,26</sup>.

One of the most surprising findings of the study by Charles E. M. *et al.*<sup>27</sup> was that those who reported more than 7 hours/week of moderate-to-vigorous physical activity during their leisure time but also watched television  $\geq$ 7 hours/day had a 50% higher risk of any-cause mortality and twice the risk of death from cardiovascular disease compared to those who engaged in the same amount of physical activity but watched TV < 1 h.

One way to reduce sedentary time is to transition from sitting to standing<sup>15</sup>. In our study, a lower presence of cardiovascular risk factors was observed in people with a higher number of transitions. In their study, Paing *et al.*<sup>16</sup> saw improvements in glucose control in people who recurrently interrupted their sedentary time. These results are similar to those obtained by Bergouignan et al. in their study of overweight people who interrupted sedentary time every 20 minutes<sup>28</sup>. Nakayama *et al.*<sup>29</sup> observed that a decrease in sitting time might induce parasympathetic activity during sleep. Therefore, reducing continuous sitting time during the day could contribute, in part, to improving the prognosis of patients with cardiovascular risk factors, not only by preventing muscle loss but also by providing positively influencing parasympathetic tone during sleep.

Peterson C. et al.<sup>15</sup> observed that exposure to acute prolonged, uninterrupted sitting results in significant increases in systolic blood pressure and mean arterial pressure, effects which could be reduced by including transitions to interrupt the prolongation of sitting time. The paper by Dunstan et al.<sup>6</sup> shows similar results. More studies have

observed improvements in variables associated with cardiovascular risk factors<sup>30,31</sup>.

Another variable analysed in this study was the relationship between the number of steps and the prevalence of cardiovascular risk factors, where a lower prevalence of such factors was observed among people who walked more steps in a day. Our results agree with previous studies which have observed that increases in the number of steps are associated with a lower prevalence of risk factors and a lower risk of mortality in adults<sup>25,32,33</sup>. In their study, Katherine S. Hall *et al.*<sup>20</sup> observed that for every increase of 1,000 steps per day at the start of the study, there was a possible reduction in the risk of all-cause mortality (6-36%) over 4-10 years. Pierre F. Saint – Maurice *et al.*<sup>24</sup> obtained results along the same lines as the previous studies and also observed that there are no significant differences regarding walking intensity and the risk of mortality.

Historically, vital signs have been used as key indicators of health status. These include pulse, blood pressure, temperature, breathing rate and body weight (body mass index). As the causes of disease have changed over the last century, new health status measurements have been proposed to address the more contemporary causes of death and disability. Recording physical activity (or exercise) levels is an easy way to meet this need and is recommended as a key health care strategy in the U.S. National Physical Activity Plan. Data on daily physical activity levels should be recorded in the medical history of each patient visiting a primary health facility as a 'new' vital sign<sup>34</sup>.

#### **Conclusions**

From the data obtained, we can conclude that there are differences in the measurement of sedentary behaviour time depending on the method used. The use of subjective methods (IPAQ self-report questionnaire) shows that the recorded minutes of sedentary behaviour, in general, underestimate the duration of such behaviour compared to objective measurement (activPAL accelerometer).

Furthermore, as demonstrated by our results and in line with other authors, greater sedentary behaviour could lead to an increase in the prevalence of associated cardiovascular risk factors.

Regarding the study of the number of steps per day variable, we obtained an inverse relationship between the number of steps and the presence of cardiovascular risk factors, observing a higher prevalence of risk factors in people who take fewer steps in their daily activities.

One of the contributions of our work has been the analysis of transitions from the sitting position to the standing position and their importance in the interruption of sedentary behaviour and the probable incidence of harm to the health caused by prolonged sitting. These transitions from sitting to the bipedal position limit the harmful physiological changes generated by sedentary behaviour.

Therefore, these conclusions reaffirm the need for a decrease in sedentary behaviour time to reduce the prevalence of cardiovascular

risk factors. A useful strategy in public health could involve making transitions with active rest in certain work or academic activities which involve a long time sitting down.

Another area of significant incidence would be the child and adolescent population, ages in which the tendency to use electronic devices and watch television to an excessive degree may lead to long, uninterrupted sedentary behaviour times. Consequently, strategies capable of generating awareness of the importance of acquiring habits which involve transitions from sitting to standing could be useful as part of an active lifestyle in the future.

Finally, the validity and accuracy of the activPAL accelerometer for the measurement of sedentary behaviour and the specific analysis of its components can be reaffirmed. Thanks to its simplicity and manageability, this tool could be included in primary health consultations to obtain physical activity data for use as a vital sign.

#### Conflict of interest

The authors declare that they are not subject to any type of conflict of interest.

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# Creatine improves anaerobic performance and promotes anthropometric changes in Brazilian college soccer players

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#### Summary

**Objective:** This study aimed to evaluate the effects of creatine on the body composition and performance of college soccer players.

**Materials and method:** Sixteen amateur soccer players were supplemented with creatine (n=8) or maltodextrin (placebo, n=8) for four weeks, using a loading protocol (20g/day in the first week, followed by 5g/day for the rest of the study period). Anthropometric measurements and three physical tests were performed before and after the intervention.

**Results:** After the protocol, the Cr group showed increased body mass (pre  $65.1 \pm 8.2$ ; post  $66.4 \pm 8.4$ ; p=0.002), arm (pre  $27.7 \pm 4.3$ ; post  $28.3 \pm 4.3$ ; p=0.01), thigh (pre  $49.9 \pm 4.3$ ; post  $51.3 \pm 4.6$ ; p=0.012), and leg (pre  $34.6 \pm 2.3$ ; post  $34.8 \pm 2.4$ ; p=0.029) circumferences. For bangsbo repeat vertical jump test (BRVJ), there was an increase in maximum power (pre  $2965.4 \pm 691.8$ ; post  $3102.1 \pm 818$ ; p=0.034), maximum relative power (pre  $44.5 \pm 6.5$ ; post  $47.1 \pm 6.5$ ; p=0.045) and average power (pre  $2757.6 \pm 653.2$ ; post  $2937.7 \pm 739.9$ ; p=0.05) only in the Cr group. For running anaerobic sprint test (RAST), there was a significant improvement in average sprint and total times, and in the average power for both groups; however, only the Cr group showed significant improvement in maximum power (pre  $517.93 \pm 118.82$ ; post  $580.15 \pm 119.06$ ; p=0.01) and maximum relative power (pre  $7.9 \pm 1.2$ ; post  $8.5 \pm 1.3$ ; p<0.001). For wingate test (WIN), improvements were observed in maximum power (pre  $456.4 \pm 91.0$ ; post  $508 \pm 79.7$ ; p=0.003), maximum relative power (pre  $450.4 \pm 91.0$ ; post  $410.4 \pm 10.5$ ; p<0.001) and relative average power (pre  $40.4 \pm 10.6$ ; post  $40.4 \pm 10.4$ ; p=0.012) only in the Cr group. **Conclusions:** This study findings demonstrated that Cr supplementation during four weeks promotes positive anthropometric and anaerobic performance changes in college soccer players, especially in lower limbs, but also in upper limbs. However, a possible body mass gain with its use should be considered, so the viability should be individually analysed.

**Key words:** Creatine. Soccer. Performance. Body Composition.

### La creatina mejora el rendimiento anaeróbico y promueve cambios antropométricos en futbolistas universitarios brasileños

#### Resumen

**Objetivo:** Evaluar los efectos de la creatina en la composición corporal y el rendimiento anaeróbico en jugadores de fútbol universitario.

**Material y método:** Dieciséis jugadores fueron suplementados con creatina (Cr) (n=8) o maltodextrina (placebo, n=8) durante cuatro semanas, utilizando un protocolo de carga (20g/día la primera semana, seguidos de 5g/día durante el resto del período de estudio), realizándose mediciones antropométricas y tres pruebas físicas anaeróbicas antes y después de la intervención. **Resultados:** El grupo Cr mostró un aumento en las circunferencias del brazo (antes 27,7  $\pm$  4,3; después 28,3  $\pm$  4,3; p=0,01), muslo (antes 49,9  $\pm$  4,3; después 51,3  $\pm$  4,6; p=0,012) y pierna (antes 34,6  $\pm$  2,3; después 34,8  $\pm$  2,4; p=0,029) y aumento en la masa corporal (antes 65,1  $\pm$  8,2; después 66,4  $\pm$  8,4; p=0,002). No se registraron cambios significativos en el grupo placebo. En la prueba de saltos verticales repetidos (Test de Bangsbo) hubo un aumento en la potencia máxima (antes 2965,4  $\pm$  691,8; después 3102,1  $\pm$  818; p=0,034), relativa máxima (antes 44,5  $\pm$  6,5; después 47,1  $\pm$  6,5; p=0,045) y media (antes 2757,6  $\pm$  653,2; después 2937,7  $\pm$  739,9; p=0.05) solo en el grupo Cr. En la prueba de carrera (RAST), hubo una mejora significativa en los tiempos de sprint total y promedio, y en la potencia promedio para ambos grupos; sin embargo, únicamente el grupo Cr mostró una mejora significativa en la potencia máxima (antes 517,93  $\pm$  118,82; después 580,15  $\pm$  119,06; p=0,01) y máxima relativa (antes 7,9  $\pm$  1,2; después 8,5  $\pm$  1,3; p<0,001). Para la prueba de Wingate en miembros superiores, se observaron mejoras en potencia máxima (antes 456,4  $\pm$  91,0; después 508  $\pm$  79,7; p=0,003), relativa máxima (antes 7,0  $\pm$  0,9; después 7,6  $\pm$  0,6; p=0,012), media (antes 354,8  $\pm$  69,5; después 410  $\pm$  71,5; p<0,001) y potencia relativa media (antes 5,4  $\pm$  0,6; después 6,1  $\pm$  0,6; p=0,012) solo en el grupo Cr.

Palabras clave:

Creatina. Fútbol. Rendimiento. Composición Corporal. **Conclusiones:** Los hallazgos de este estudio mostraron que la suplementación con Cr durante cuatro semanas promueve cambios positivos en el rendimiento anaeróbico y mediciones antropométricas en futbolistas universitarios, especialmente en las extremidades inferiores, pero también en las superiores. Sin embargo, debe considerarse una posible ganancia de masa corporal con su uso, por lo que la viabilidad debe analizarse individualmente.

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#### Introduction

Sports scientists, coaches, and nutritionists look for different ways to improve soccer players' performances¹. Considering the level of competitiveness and early exposure to competitive training², several nutritional strategies, including supplementation, have been used³. Creatine (Cr), caffeine, isotonic drinks, and whey protein are examples of products offered mainly to professional athletes⁴-6.

Cr is a natural amino acid mainly found in skeletal muscle<sup>7</sup>, stored as phosphocreatine (PCr), a fast-consuming energy reserve used as a substrate for the resynthesis of adenosine triphosphate (ATP) through the phosphagen pathway. Thus, athletes and physical activity practitioners can benefit from Cr consumption due to the increase of PCr stocks, especially in modalities that use the anaerobic alactic system as a primary source of energy<sup>7,8</sup>, which is precisely the energy profile of high demand in soccer<sup>2,3,9</sup>.

A 70 Kg adult requires approximately 2-3 g/day of Cr, part of which is synthesized by the body through the combination of the amino acids glycine, arginine, and methionine<sup>10</sup>. The remaining requirement is obtained from dietary sources, especially meat and fish<sup>8</sup>. Cr supplementation can be beneficial in a Cr deficiency. Such supplementation is frequently performed by the "loading" protocol, which consists of two phases. The first one (loading) lasts a week using 20 g/day or 0.3 g/Kg/day of Cr. The second one (maintenance) usually lasts three weeks using a dose of 5g/day or 0.03g/kg/day<sup>11,12</sup>. Furthermore, it is well established that continuous Cr supplementation is safe<sup>8</sup>.

Increased cellular hydration status is one of the ergogenic effects caused by Cr supplementation, which can be beneficial for soccer players since matches and training sessions induce dehydration<sup>13,14</sup>. Other known effects are enhanced muscle and energy recovery, strength and power gain, muscle hypertrophy, and reduction of muscle acidosis<sup>2,3,15</sup>. On the other hand, deleterious effects on the upper airways, cramps, and gastrointestinal discomforts have already been reported<sup>2,16</sup>.

Several studies have shown the potential of Cr supplementation to increase performance in soccer players<sup>5,17</sup>, while other studies have found no positive evidence<sup>18,19</sup>. A current systematic review and meta-analysis<sup>3</sup> showed that the changes in physical test performance in soccer players are dependent on the metabolic pathway involved. Therefore, investigating the impact of Cr supplementation on performance in different soccer-related physical tests can help us better understanding the effectiveness of Cr supplementation in this modality. Thus, the objective of this study was to evaluate the effects of Cr monohydrate supplementation on body composition and performance in a physical test battery in college soccer players, including a muscle region less frequently used in this modality. We hypothesize that the Cr supplementation will improve body composition and anaerobic performance in soccer-related physical tests in university soccer players, including the performance in an upper-limb physical test.

#### Material and method

#### **Participants**

The volunteers were male, between 18-33 years old, who had practiced soccer regularly in the last 6 months without having consumed Cr

in the last 3 months. The recruitment was carried out through academic community social media and direct contact with the coaches of the athletic associations of the local University. This study was approved by the Local Human Research Ethics Committee, under protocol number 2.706.172. The volunteers were properly informed about the study procedures and provided written informed consent to participate in the study, according to the recommendations of the Brazilian Human Research Legislation – Ordinance 466/12.

Initially, 27 players were enrolled in the study. During the experiment, a volunteer manifested an adverse reaction to supplementation, claiming facial and foot swelling; a second volunteer injured a knee ligament during training, and nine participants did not proceed for personal reasons. Thus, complete data were collected from a sample of sixteen players (19.7  $\pm$  2.3 years).

#### **Experimental design**

The experiment consisted of two moments, M1 and M2, defined as an initial assessment before the supplementation (baseline), and the final assessment 28 days after the start of supplementation (post), respectively. Each moment consisted of one meeting to perform physical tests and analyzes of body composition and hydration status. At M1, the volunteers performed anamnesis and answered the PAR-Q questionnaire (Physical Activity Readiness Questionnaire) proposed by Chisholm *et al.*<sup>17</sup>, when only those who had no positive answer were selected. Next, the volunteers were randomly assigned to the Creatine (Cr) (n=8, experimental treatment) or Placebo (PI) (n=8, control treatment with maltodextrin) groups.

#### Anthropometric and hydration assessment

At M1 and M2, body mass (kg) (BM), height (cm), and right-side body circumferences (cm) were measured (arm, forearm, abdomen, thigh, and calf). Circumferences were measured on the right side since all volunteers were right-handed. The BM, height, and circumference measures were obtained through a digital electronic scale (Toledo®, Model 2090), a stadiometer with a 0.5 cm scale (SECA®), and an inelastic measuring tape was used (Cescorf®), respectively. To estimate the fat percentage (%G) and total body hydration (%H2O), a digital bioimpedance scale (Tanita®, IRONMAN BC-558) was used. Urinary density was obtained by collecting urine in a suitable container before the start of physical tests, using an analog refractometer (ATC®, RTP-20ATC).

#### Nutritional assessment

To analyze the participants' nutritional profile, and to verify whether protein consumption could influence the response to supplementation, the volunteers were instructed to maintain their regular eating habits and complete a 3-day food record, two non-consecutive weekdays and one day on the weekend, according to each volunteer's preference 18,19. To provide more accurate assistance for completing the records, illustrative pictures with standardized homemade measurements of some foods were delivered along with the record sheet. The data obtained were entered individually in the AVAnutri® software (Rio de Janeiro, Brazil; Version 4.5.1), and analyzed for energy intake, carbohydrates,

proteins, and fats. After that, the groups' average intake was calculated. To assess whether the protein consumption would impact the results of Cr supplementation, physical performance was compared between the two individuals from each group who ingested the highest (+PTN) and the lowest (-PTN) quantity of protein.

#### **Physical tests**

At M1 and M2, after BM measurement, the participants underwent three physical tests:

#### A) Bosco repeat vertical jump (BRVJ)

To measure the explosive strength of the lower limbs, the BRVJ test was applied on a Jump System NewFit jumping platform (Cefise®). The test consisted of 2 sets of 15 seconds, in which the participants performed the highest possible number of countermovement jumps, with their hands fixed on the waist. A 3-minute passive interval was respected between sets. The volunteers were positioned between the platform sensors in an upright position. After a regressive 10-second count, a beep was emitted, authorizing the start of jumps. During the 15 seconds, the volunteers started from the upright position, flexing their knees up to 90° and making as many jumps as they could, as high as possible without flexing their knees during the jump, then returning to the upright position with both feet at the same time²0. The Jump System® 1.0.4.2 software was used to obtain the results of Maximum Power (MaxP), Maximum Height, Maximum Relative Power (MaxRP), and Fatigue Index (FI).

#### B) Running-based anaerobic sprint test (RAST)

To assess the power of lower limbs, the RAST protocol was used<sup>21</sup>. This test consisted of a traditional soccer warm-up of 5 to 10 minutes, then proceeding to the performance of 6 maximum sprints of 35 meters in a straight line, in a flat area of lawn. Considering that RAST was developed for professional soccer players, and our participants were college players, the physical load of the test was adapted by increasing the original interval between sprints from 10 to 30 seconds. To perform the test, a photocell system (Hidrofit®, MultiSprint Full) and its specific software were used. Data was obtained for MaxP, MaxRP, Average Power (AP), Relative Average Power (RAP), and the FI.

#### C) Wingate of upper limbs (WIN).

To evaluate the influence of Cr consumption over secondary muscles, the Wingate test was performed using an arm crank ergometer (Technogym® Excite TOP 700), focusing on the anaerobic power of the upper limbs. The players were positioned in front of the equipment, with their feet shoulder-width apart and making a pronounced grip on the ergometer crank. The height of the ergometer crank module was positioned at the height of the xiphoid process. The test started with a 30-second warm-up at 50 rpm. At the end of the warm-up, the players applied as much force as possible to maintain the maximum speed on the ergometer for 30 seconds, ending with a 30-second active recovery. At the end of the test, MaxP, AP, RAP, and tiredness level values were obtained.

#### Supplementation protocol

The loading protocol was used to deliver the desired dosages of Cr. It consisted of a loading phase (1 week) and a maintenance phase (3 weeks)<sup>16,18,22</sup>. During the loading phase, all volunteers consumed 20 g of their respective supplementation, distributed in the main meals of the day<sup>7</sup>. The doses were weighed on an electronic scale with an accuracy of 0.01 g (Shimadzu®, Series BL-3200H) and separated into individual doses of 5 g, packed in sterile plastic packages. During the maintenance period, the volunteers were instructed to consume just one dose after lunch.

All the Cr needed for the experiment was provided by a local food supplement company. For PI, 1 Kg sealed packages with unflavored maltodextrin were purchased, aiming to approximate as much as possible the texture and flavor of the experimental treatment.

#### Statistical analysis

The Shapiro-Wilk test was used to analyze the data distribution. There was a non-normal distribution (p <0.05) in the following variables: pre-intervention body fat (group PI), post-intervention MaxP (group PI), and basal FI (group PI) in the RAST protocol, and pre-intervention MaxP and RAP (both groups), and post-intervention RAP (group Cr) in the WIN test. In these cases, the Wilcoxon and Mann-Whitney tests were used to perform intragroup (pre vs. post-intervention) and intergroup comparisons, respectively.

In the other cases of normal distribution, the paired T-test was used in intragroup comparisons, and the independent T-test was used to make comparisons between groups. Cohen effect sizes (ES) were calculated for pair comparisons and classified as small (0.2–0.5), moderate (0.5–0.8), or large (>0.8)<sup>23</sup>. The statistical software SPSS 20.0 was used to perform the statistical analysis. Data were presented as mean  $\pm$  standard deviation (SD), minimum and maximum values. A level of significance was set at p  $\leq$  0.05.

#### Results

#### Energy and macronutrient intake

There was no significant difference in the amount of ingested kilocalories (Kcal) (p=0.067), protein (PTN) (p=0.059), and lipids (LIP) (p=0.594) between groups. However, the PI group consumed a greater amount of carbohydrates (CHO) compared to the Cr group (p=0.049) (Table 1).

The average percentage distribution of macronutrients consumed by the Cr group was 56.2% CHO, 17.1% PTN, and 26.7% LIP, whereas for the Pl group the values corresponded to 57.3% CHO, 18.7% PTN, and 24% LIP. No significant difference was observed in the relative distribution of energy and macronutrients between groups (Table 1).

#### Anthropometry and body composition

There was no significant difference between groups for anthropometric and body composition variables, as well as for hydration status (urine density) before and after the intervention (p>0.05). In addition, the average age was similar between groups (Cr:  $20.5\pm3.0$  years; Pl:  $18.9\pm1.0$  years; p=0.505).

Table 1. Average of absolute and relative energy and macronutrient intake before intervention.

Variable	Cr	PI	р	ES
Energy (kcal)	2136.8 ± 491.4 (1589.4 – 3159)	2585.7 ± 411.1 (1873.1 – 3191.2)	0.067	0.99
CHO (g)	300.4 ± 74 (222 – 436.5)	370.2 ± 54.1 * (281.8 – 449.9)	0.049	0.94
PTN (g)	91.4 ± 24.5 (66 – 138.3)	120.6 ± 31.8 (85.4 – 167)	0.059	1.03
LIP (g)	63.3 ± 18.9 (37.1 – 95.5)	69.2 ± 23.9 (42.3 – 114.8)	0.594	0.27
Energy (kcal/Kg)	$32.8 \pm 5.8$ (25.0 – 42.3)	37.1 ± 7.6 (29.0 – 50.4)	0.226	0.64
CHO (g/Kg)	$4.6 \pm 0.8$ $(3.1 - 5.8)$	$5.3 \pm 0.9$ (4.1 – 6.8)	0.116	0.82
PTN (g/Kg)	$1.4 \pm 0.3$ $(1.0 - 1.9)$	1.7 ± 0.5 (1.2 – 2.6)	0.145	0.73
LIP (g/Kg)	$1.0 \pm 0.3$ $(0.5 - 1.4)$	$1.0 \pm 0.4$ $(0.6 - 1.8)$	0.943	0.0

Kcal: kilocalories; CHO: carbohydrate; PTN: protein; LIP: lipids; ES: effect size; \* represents significant difference between groups (p<0.05).

The intragroup comparisons showed significant increased BM (p=0.002), BMI (p=0.001), biceps (p=0.010), thigh (p=0.012) and calf (p=0.029) circumferences in the Cr group. Moreover, the PI group had a significant reduction in the calf circumference (p=0.009) (Table 2).

#### BRVJ

There was no significant difference between groups for the maximum height achieved in the jump test, as well as for the power and FI values before and after the intervention (p>0.05) (Table 3). However, Cr supplementation significantly increased MaxP (p=0.034), MaxRP (p=0.045), and AP (p=0.050) post-intervention.

#### **RAST**

There was no significant difference between groups for the performance, power, and FI variables obtained in the RAST before and after the intervention (p>0.05) (Table 4).

The intragroup paired comparisons (Table 4) showed significant improvement after the intervention, for both groups, in the total time of the 6 sprints (Cr, p=0.049; Pl, p=0.038), in the average of the sprints (Cr, p=0.050; Pl, p=0.039), and AP (w) (Cr, p=0.014; Pl, p=0.042). The Cr group significantly improved the time obtained in the best sprint (p<0.001), MaxP (p=0.001), and MaxRP (p<0.001). On the other hand, a significant improvement in RAP (p=0.036) was observed exclusively in the Pl group.

#### WIN

After the intervention, significant improvements were observed, only in the Cr group, in MaxP (p=0.003), MaxRP (p=0.012), AP (p<0.001), and RAP (p=0.012) (Table 5).

Table 6 describes the percentage difference in MaxP of the three post-intervention tests of the volunteers who consumed more and less protein in each group.

Table 2. Anthropometric and body composition variables of the groups before and after intervention.

Variable	Group	Pre -intervention	Post -intervention	р	ES
BM (Kg)	Cr	65.1 ± 8.2 (54.8 – 74.9)	66.4 ± 8.4 * (56.2 – 76.3)	0.002	0.16
	PI	$70.3 \pm 6.4$ (63.3 – 83)	70.3 ± 6.7 (61 – 81.9)	0.953	0.0
BMI (Kg/m²)	Cr	20.7 ± 2.9 (17.1 – 25.2)	21.1 ± 2.9 * (17.7 – 25.8)	0.001	0.14
	PI	22.2 ± 1.4 (20.2 – 24.6)	22.1 ± 1.5 (19.5 – 24.5)	0.879	0.07
Body fat (%)	Cr	$7.6 \pm 4.0$ $(5.0 - 16.4)$	7.8 ± 3.3 (5.0 – 14.8)	0.739	0.05
	PI	$9.3 \pm 2.8$ (6.3 – 15.5)	$8.7 \pm 3.0$ $(5.0 - 14.8)$	0.489	0.21
H <sub>2</sub> 0 (%)	Cr	69.2 ± 6.1 (59.3 – 80.3)	69.3 ± 6.1 (61 – 82)	0.930	0.02
	PI	67.7 ± 6.2 (59.4 – 77.9)	66.3 ± 3.5 (60.2 – 71.1)	0.467	0.28
Urine density (g/ml)	y Cr	1.022 ± 0.009 (1.004 – 1.032)	1.021 ± 0.011 (1.006 – 1.036)	0.814	0.1
	Pl	1.024 ± 0.005 (1.020 – 1.033)	1.026 ± 0.005 (1.020 – 1.034)	0.377	0.4
Right biceps circ. (cm)	Cr	27.7 ± 4.3 (22.9 – 34.6)	28.3 ± 4.3 * (23.3 – 35.1)	0.010	0.14
	Pl	29.1 ± 2.2 (26.5 – 32.2)	28.8 ± 1.8 (26 – 31.6)	0.208	0.15
Right forearr circ. (cm)	n Cr	$25.4 \pm 2.4$ (21.8 – 28.4)	$25.8 \pm 2.9$ (22 – 30.5)	0.154	0.15
	PI	27.2 ± 1.3 (24.7 – 28.9)	27.4 ± 1.5 (24.9 – 28.9)	0.212	0.14
Abdominal circ. (cm)	Cr	$72.8 \pm 4.7$ (66.7 – 80.1)	$73.4 \pm 4.5$ (67.4 – 78.9)	0.159	0.13
	PI	75.1 ± 2.8 (71.7 – 80.8)	74.8 ± 3.3 (68.6 – 79.9)	0.449	0.1
Right thigh circ. (cm)	Cr	49.9 ± 4.3 (44.7 – 56.4)	51.3 ± 4.6 * (45.9 – 58.6)	0.012	0.31
	PI	52.1 ± 4.1 (47.2 – 59.9)	52.5 ± 3.9 (47.4 – 59.8)	0.557	0.1
Right calf circ. (cm)	Cr	34.6 ± 2.3 (31.6 – 38.5)	34.8 ± 2.4 * (31.8 – 38.7)	0.029	0.09
	PI	36.9 ± 2.3 (34.3 – 40.3)	36.4 ± 2.4 * (33.7 – 39.8)	0.009	0.21

Cr: creatine group; Pl: placebo group; BM: body mass; Circ: circumference; BMI: body mass index; ES: effect size; \* represents significant intra-group difference (p<0.05).

Table 3. Bangsbo Repeat Vertical Jump.

Variable	Group	Pre-intervention	Post-intervention	р	ES
Maximum height (cm)	Cr	33.4 ± 6.9 (20.8 – 40.7)	36.4 ± 7.5 (25.5 – 50.5)	0.056	0.42
	PI	34.5 ± 7.0 (24.1 – 45.5)	37.2 ± 6.8 (27.9 – 46.1)	0.126	0.39
MaxP (w)	Cr	2965.4 ± 691.8 (1980.8 – 3798.4)	3102.1 ± 818 * (2242.5 – 4469.3)	0.034	0.28
	PI	3227.7 ± 609.3 (2332.1 – 4420.2)	3464 ± 712.6 (2510.6 – 4452.1)	0.202	0.36
MaxRP (w/kg)	Cr	44.5 ± 6.5 (32.4 – 50.9)	47.1 ± 6.5 * (36.9 – 58.6)	0.045	0.4
	PI	45.7 ± 5.9 (36.1 – 53.9)	47.8 ± 5.4 (39.6 – 54.4)	0.120	0.37
AP (w)	Cr	2757.6 ± 653.2 (1804.1 – 3475.1)	2937.7 ± 739.9 * (2064.2 – 3904.6)	0.050	0.26
	PI	3039.6 ± 617.9 (2196.5 – 4213.3)	3130.8 ± 613.5 (2305.5 – 4354.8)	0.182	0.15
FI (%)	Cr	1.0 ± 0.1 (0.9 – 1.1)	1.0 ± 0.1 (0.9 – 1.1)	0.421	0.0
	PI	$0.9 \pm 0.1$ (0.8 – 1)	$0.9 \pm 0.1$ (0.9 – 1)	0.309	0.0

Cr: creatine group; Pl: placebo group; w: watts; MaxP: maximum power; MaxRP: maximum relative power; AP: average power; Fl: fatigue index; ES: effect size; \* represents significant intra-group difference (p < 0.05).

Table 4. RAST results.

Variable	Group	Pre-intervention	Post-intervention	р	ES
Total time (s)	Cr	33.18 ± 1.68 (31.5 – 36.6)	32.56 ± 1.7 * (31.5 – 36.6)	0.049	0.52
	PI	33.82 ± 1.15 (31.80 – 35.11)	32.54 ± 1.37 * (30.09 – 34.16)	0.038	1.01
Best sprint (s)	Cr	5.40 ± 0.29 (5.09 – 5.96)	5.26 ± 0.28 * (4.92 – 5.79)	< 0.001	0.49
	PI	5.41 ± 0.18 (5.05 – 5.60)	5.26 ± 0.21 (4.89 – 5.50)	0.089	0.77
Worst sprint (s)	Cr	5.68 ± 0.29 (5.35 – 6.20)	5.64 ± 0.40 (5.12 – 6.35)	0.642	0.11
	PI	5.89 ± 0.26 (5.52 – 6.21)	5.60 ± 0.29 (5.12 – 6.05)	0.058	1.05
Sprint average (s)	Cr	5.53 ± 0.28 (5.25 – 6.10)	5.43 ± 0.28 * (5.08 – 5.98)	0.050	0.36
	PI	5.64 ± 0.19 (5.30 – 5.85)	5.42 ± 0.23 * (5.02 – 5.69)	0.039	1.04
AP (w)	Cr	480.77 ± 99.95 (321.49 – 579.36)	530.21 ± 110.33 * (349.04 – 677.57)	0.014	0.47
	PI	490.82 ± 96.16 (391.35 – 679.03)	550.73 ± 120.33 * (441.46 – 796.75)	0.042	0.09

(continuated)

Table 4. RAST results (continuation).

Variable	Group	Pre-intervention	Post-intervention	р	ES
RAP (w/kg)	Cr	7.36 ± 1.03 (5.40 – 8.47)	7.80 ± 1.14 (5.75 – 9.36)	0.055	0.41
	PI	6.93 ± 0.72 (6.18 – 8.28)	7.78 ± 1.03 * (6.69 – 9.73)	0.036	0.96
MaxP (w)	Cr	517.93 ± 118.82 (344.46 – 697.82)	580.15 ± 119.06 * (383.87 – 775.16)	0.001	0.52
	PI	551.60 ± 107.99 (449.89 – 781.36)	599.26 ± 125.92 (497.13 – 858.01)	0.093	0.41
MaxRP (w/kg)	Cr	7.9 ± 1.2 (5.8 – 9.3)	8.5 ± 1.3 * (6.3 – 10.3)	< 0.001	0.48
	PI	7.8 ± 0.8 (7.0 – 9.5)	8.5 ± 1.1 (7.4 – 10.5)	0.077	0.73
FI (w/s)	Cr	2.3 ± 1.8 (0.9 – 6.6)	3.2 ± 1.7 (1.2 – 5.8)	0.161	0.51
	PI	3.6 ± 1.1 (1.9 – 5.7)	$3.0 \pm 0.8$ (2.1 – 4.3)	0.232	0.62

Cr: creatine group; Pl: placebo group; w: watts; AP: average power; RAP: relative average power; MaxP: maximum power; MaxRP: maximum relative power; Fl: fatigue index; ES: effect size; \* represents significant intra-group difference (p<0.05).

Table 5. WIN results.

Variable	Group	Pre-intervention	Post-intervention	р	ES
MaxP (w)	Cr	456.4 ± 91.0 (263 – 553)	508 ± 79.7 * (373 – 601)	0.003	0.6
	PI	473.1 ± 111.9 (358 – 650)	517.6 ± 82.1 (430 – 654)	0.088	0.45
MaxRP (w/kg)	PI	7.0 ± 0.9 (4.9 – 8.2)	7.6 ± 0.6 * (6.5 – 8.4)	0.012	0.78
	Cr	6.7 ± 1.0 (5.7 – 7.9)	$7.4 \pm 0.8$ (6.4 – 8.5)	0.141	0.77
AP (w)	Cr	354.8 ± 69.5 (225 – 438)	410 ± 71.5 * (306 – 488)	< 0.001	0.78
	Pl	385.5 ± 86.7 (303 – 522)	413.1 ± 71.9 (335 – 527)	0.096	0.35
RAP (w/kg)	Cr	5.4 ± 0.6 (4.2 – 5.9)	6.1 ± 0.4 * (5.4 – 6.4)	0.012	1.37
	PI	5.5 ± 0.8 (4.8 – 6.5)	5.9 ± 0.7 (4.9 – 6.7)	0.233	0.53
Tiredness level (%)	Cr	63.0 ± 8.1 (53 – 79)	62.1 ± 4.2 (55 – 68)	0.780	0.14
	PI	60.8 ± 5.3 (50 – 68)	65 ± 6.0 (59 – 75)	0.174	0.74

Cr: creatine group; Pl: placebo group; w: watts; MaxP: maximum power; MaxRP: maximum relative power; AP: average power; RAP: relative average power; ES: effect size; \* represents significant intra-group difference (p<0.05).

Table 6. Percentage difference in MaxP according to protein consumption.

Test	Group	Subject	MaxP Pre	MaxP Post	%
BRVJ	Cr	+ PTN - PTN	3798.4 2424.7	4469.3 2572.9	17.7 6.1
	PI	+ PTN - PTN	3646.3 2332.1	3598.2 2510.6	-1.3 7.7
RAST	Cr	+ PTN - PTN	539.0 397.4	597.2 525.6	10.8 32.3
	PI	+ PTN - PTN	576.6 449.9	531.6 511.5	-7.8 13.7
WIN	Cr	+ PTN - PTN	553.0 263.0	590.0 373.0	6.7 41.8
	Pl	+ PTN - PTN	545.0 370.0	519.0 430.0	-4.8 16.2

Cr: creatine group; Pl: placebo group; MaxP: maximum power in watts; + PTN: subject of the group with the highest protein consumption; - PTN: subject of the group with the lowest protein consumption.

#### **Discussion**

The percentage distribution of macronutrients in the diet is in accordance with the nutritional recommendations proposed by Ranchordas et al.<sup>5</sup> (Table 1). Except for CHO consumption, there was no significant difference in energy, protein, and lipid consumption between groups, which is an important aspect to consider before the experimental intervention. Since Cr is endogenously produced, individuals with higher dietary protein intake may experience less benefit from its supplementation. Kreider et al.<sup>8</sup> consider that subjects with reduced protein consumption (lower biological value sources) in the diet would obtain more benefits from Cr supplementation, due to the possible amino acid deficiency for the endogenous synthesis of Cr. Since the protein intake was statistically similar in both absolute and proportional values (g/Kg BM), and the percentage distribution was appropriate, we consider that the dietary factor did not influence the results.

Cr consumption has been appointed as an ergogenic agent that may increase BM<sup>8,24</sup> through an increase in body water<sup>25</sup> or muscle hypertrophy<sup>26</sup>. The results obtained in the present study (Table 2) indicate an increase of approximately 1.3 Kg of BM in the Cr group. This reinforces the rapid effect of consuming this ergogenic resource. However, for athletes in events categorized by BM, such as fighters, weight gain may not be beneficial, reinforcing the need for more rigorous control of the applicability of Cr supplementation.

Body fat was not altered by Cr consumption, which is in line with the findings of previous studies<sup>19,24</sup>. This result can be somewhat expected because Cr supplementation does not interfere with the fat metabolism, but it does interfere in the phosphagens pathway for rapid energy production. To lose body fat, the volunteers must be in an energy deficit, which did not happen. In addition, fatty acid metabolism for energy production starts in activities of submaximal intensity with prolonged duration<sup>27</sup>.

One of the reasons for BM gain may be accumulation of body water following Cr supplementation. However, the results show that

water percentage and urine density indicators were not altered by the Cr consumption, refuting this theory. This result contradicts a study published by Deminice  $et\ al.^{25}$ , where a significant increase in total body water ( $\approx$  5%) after Cr supplementation was found. Therefore, a possible explanation for BM gains would be muscle hypertrophy since circumference increases in the brachial biceps, thigh, and calf for the Cr group was detected, contrarily to the Pl group, in which the calf circumference significantly reduced after the intervention. The increased circumferences may be related to an increased muscle mass <sup>16</sup>. However, in this study, it is not possible categorically to state whether there was myofibrillar hypertrophy, which could only have been confirmed by biopsy or imaging techniques such as magnetic resonance.

An important aspect to be considered is the participants' initial hydration level. According to the urinary density, both groups showed signs of dehydration, with density values above 1.020<sup>28</sup>. However, after the intervention, individuals of the Cr group improved their hydration status, while the Pl distanced itself from it, which may be due to ad libitum water consumption. The practice of physical exercise in a state of dehydration is extremely harmful to athletes' health<sup>29,30</sup>, and soccer competitions have a high risk of dehydrating players<sup>31</sup>. In agreement with our findings, Castro-Sepúlveda *et al.*<sup>14</sup>, and Arnaoutis *et al.*<sup>13</sup> showed how dehydrated soccer players are before and after training sessions and matches, and that hydration ad libitum is not enough to replace the water lost<sup>13</sup>. This reinforces the need of raising awareness and educating the athlete about the importance of performing constant hydration to avoid severe dehydration, which, in addition to impairing the quality of training, will also be harmful to health.

To our knowledge, no study has analyzed the impacts of Cr supplementation on the BRVJ test. The performance data obtained in this test (Table 3) are very interesting since none of the five variables considered significantly improved in the PI group. On the other hand, the Cr group showed noteworthy improvement in MaxP, MaxRP, and AP, as well as an increment in the strength and explosive power of the lower limbs without interfering with the IF. This indicates an important ergogenic

effect since the test protocol is primarily dependent on the ATP-PC system, in which PCr stocks have a decisive influence. These results are contrary to previous studies that evaluated the impacts of Cr supplementation in jump protocols and found no significant improvement in power and maximum jump height 18,32,33. Thus, Cr supplementation can be recommended especially for soccer players who have more active jumping actions, such as attackers, defenders, and goalkeepers. Likewise, athletes from other sports in which the jumping component is also present, like volleyball and basketball, could also benefit.

In amateur female soccer players, Ramírez-Campillo et al.<sup>32</sup> observed a significant improvement in performance in the RAST in the Cr group. At the end of our research, the Cr and Pl groups showed a significant difference in the temporal effect in three analyzed parameters (total time, average of sprints, and AP) (Table 4). Thus, the influencing factor of this behavior cannot be justified by Cr consumption. A possible explanation is that the learning effect can have influenced these parameters since the protocol was applied to the players for the first time. However, the Cr group achieved improvements in the performance of the best sprint and MaxRP. This result, which is directly influenced by PCr stocks, is especially important in the dynamics of a soccer match. Thus, it can be inferred that the proposed supplementation protocol contributed to this result. On the other hand, the Pl group increased the RAP, which represents an unusual result with no reasonable justification, considering that the dietary pattern was similar and that the training activities were maintained.

The groups had no statistically significant changes in the FI results. These results are contrary to Ateş *et al.*<sup>34</sup>, who observed improvements in the FI, an important indicator for the athletic performance of a soccer player, who can be submitted to up to 250 short distance and high-intensity stimuli during the match<sup>35</sup>. Ideally, soccer players should have good repeated sprint ability maintained throughout the match. Considering that the players evaluated were recreational, this may have been a determining factor. Perhaps recreational players could benefit from a longer period of supplementation, which should be investigated in further studies.

In this study, we applied the WIN test to verify how Cr supplementation could impact the performance of a muscle that was not directly trained. Cr supplementation promoted a positive adaptation in four (MaxP, MaxRP, AP, and RAP) of the five monitored parameters (Table 5), while in the level of tiredness there was a similarity between groups, contrarily to the work of Yáñez-Silva *et al.*<sup>2</sup>. These preliminary results of improved performance observed in an untrained musculature are somewhat surprising since the physical stimulus of the exercise has an anabolic local adaptation action over the worked musculature.

To analyze whether protein consumption could interfere with supplementation results, a more detailed analysis was performed in the groups, selecting the individuals who consumed more and less PTN (Table 6). Specifically, in the MaxP variable (common in the 3 tests), it was possible to notice that, in the BRVJ, the individual who had consumed more protein in the Cr group showed better performance than the individual with the lowest consumption (17.7% vs. 6.1%). However, these data are inverted in RAST and WIN, showing an improvement of 10.8% against 32.3% of the individual with lower protein consumption in RAST,

and 6.7% against 41.8% in WIN. Despite what was expected, the results in PI group indicated an improvement of greater magnitude for the individual who consumed less PTN, probably due to the learning effect. These results, analyzed in a particular way, somehow reinforce the theory that the magnitude of the ergogenic effect of Cr consumption may be influenced by the daily protein consumption pattern. An individual who consumes less PTN may benefit more from Cr supplementation, supplying the deficiency of endogenous synthesis resulting from the consequent amino acid deficit.

The side effects reported for Cr consumption include cramps, weight gain, and gastric discomfort<sup>2,16</sup>. During the experimental period, one of the volunteers manifested an adverse reaction to Cr supplementation, showing facial and foot swelling after the loading phase (first week), who was forwarded to the doctor and excluded from the study. Reports of allergic reactions of this nature associated with the consumption of Cr are not common. Cr has been used as an aid in several medical treatments and is considered safe for consumption, even in higher doses<sup>8</sup>.

The final sample size of sixteen individuals can be considered one of the limitations of this study. However, several similar works also had a similar sample size, like Gann *et al.*<sup>24</sup>, with 14 individuals, and Ramírez-Campillo *et al.*<sup>32</sup>, with 20 participants. Other limitations of this study are the training and the food records accuracies. The training record was not performed; however, 13 volunteers from the final sample trained together, and separate training could generate different results. Although there was prior guidance on how to fill out the food record, errors can still occur when registering information, even though this is a usual methodology in studies like this <sup>18,19</sup>.

For future studies, we suggest applying this model to investigate the effects of Cr supplementation on women, athletes from other modalities such as futsal, or even with different age groups. If available, using DXA for body composition analysis can improve the quality of information.

#### Conclusion

From this study, it was found that Cr supplementation for four weeks was able to promote positive anthropometric and anaerobic performance changes in college soccer players, especially in lower limbs, but also in upper limbs. Because it is a low-cost nutritional ergogenic resource, with a low rate of reported side effects, and which has presented positive results on performance, Cr can be recommended for recreational soccer players seeking performance improvements. However, a possible body mass gain due to Cr supplementation should be considered, and its viability should be individually analyzed for each athlete.

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#### Conflict of interest

The authors do not declare a conflict of interest.

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# Ten years of football (soccer) injuries in the literature. A bibliometric approach

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#### Summary

The aim of this research is to analyse the scientific production of publications produced during a decade (2010-2019) about injuries in football (soccer). A bibliographic search was done for publications featuring key terms such as football, soccer, and injuries. We searched for studies in journals that had a five-year impact factor in the Journal Citation Report in the "Sport Sciences" category. The PRISMA methodology was used. The following bibliometric indicators were analysed: number of publications by journal; country of origin; country of publication; publications per year; number of authors; authors' native language; evidence level; type of study; sex; anatomical location and topographic location of injuries. To assess the level of evidence, the Oxford Centre for Evidence-Based Medicine (CEBM) level of evidence classification was used, dichotomised as follows: articles with level 1 and level 2 evidence were 'high evidence', and articles with levels 3, 4, and 5 were 'low evidence'. The statistical tests were performed using SPSS V. 28. A total of 222 articles published in four journals met the inclusion criteria. Production rose as the decade progressed. The highest frequency was in 2013. The British Journal of Sports Medicine (BJSM) had the highest number of publications. Sports Medicine (SM) had the most authors per article and the highest level of evidence. The United States was the main producer. There were more publications regarding injuries in males, and in males and females, compared to publications that only took females into account. More muscle, thigh, and hip injuries were described. The level of evidence was generally low. The increase in scientific production related to football (soccer) injuries during the decade reflect an elevated interest for the subject matter. Publications that prevail around this theme consider injuries in the thigh and lower extremities and in men.

### **Key words:** Football. Soccer. Injuries. Bibliometrics.

Football. Soccer. Injuries. Bibliometrics. Evidence-based medicine.

# Diez años de lesiones de fútbol en la literatura. Una aproximación bibliométrica

#### Resumen

Con el objetivo de analizar la producción científica de publicaciones realizadas durante una década (2010-2019) sobre lesiones en el fútbol, se realizó una búsqueda bibliográfica de publicaciones con términos clave como fútbol, soccer y lesiones, en revistas con mayor Factor de Impacto acumulado en 5 años en el Journal Citation Report, en la categoría "Sport Sciences". Se utilizó la metodología PRISMA. Se analizaron los indicadores bibliométricos: número de publicaciones por revista; país de origen; país de publicación; publicaciones por año; número de autores; lengua materna de los autores; nivel de evidencia; tipo de estudio; sexo; localización anatómica, y localización topográfica de las lesiones. Para evaluar el nivel de evidencia se utilizó la clasificación del Oxford Centre for Evidence-Based Medicine (CEBM) y se dicotomizó en alto (artículos con niveles de evidencia 1 y 2) y bajo (artículos con nivel de evidencia 3, 4 y 5). El análisis estadístico se realizó con SPSS V.28. Un total de 222 artículos publicados en cuatro revistas cumplieron con los criterios de inclusión. La producción aumentó a medida que avanzaba la década, siendo mayor en 2013. El *British Journal of Sports Medicine* (BJSM) realizó el mayor número de publicaciones. La revista *Sports Medicine* (SM) tuvo la mayor cantidad de autores por artículo y el mayor nível de evidencia. Estados Unidos fue el principal productor. Hubo más publicaciones que investigaron las lesiones en hombres, y en hombres y mujeres, en comparación con las publicaciones que solo tomaron en cuenta a las mujeres. Se describieron más lesiones musculares, en muslo y en cadera. El nivel de evidencia en general fue bajo. El incremento de la producción científica durante la década refleja un elevado interés en el tema. Predominaron las publicaciones acerca de lesiones en la cadera y extremidades inferiores y en hombres.

#### Palabras clave:

Fútbol. Soccer. Lesiones. Bibliometría. Medicina basada en la evidencia

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#### Introduction

Bibliometrics can be understood as a research technique that analyses the size, growth, and distribution of publications in a scientific field<sup>1</sup>. This type of studies helps researchers to analyse existing knowledge, through the study of publication patterns using quantitative analysis and statistics<sup>2</sup>. To fulfil their mission, researchers need to identify relevant studies on a topic of interest and to critically assess the level of the evidence presented<sup>3</sup>.

Evidence-Based Medicine (EBM) is defined as the judicious utilisation of the best scientific evidence available to make decisions about the care and treatment of patients<sup>4</sup>. "Evidence-based medicine is defined as a conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients"5. The practice of EBM integrates the individual clinical expertise with the best available external clinical evidence<sup>6</sup>. The need for EBM in the treatment of football (soccer) injuries is justified given the ever-increasing demands of this sport, with more games per calendar year with insufficient recovery time between games, all of this to win games, titles and trophies<sup>7,8</sup>. When injured, athletes need to obtain the best treatment available to return to the field as soon as possible. While physicians need to keep abreast of current knowledge to inform their medical practice<sup>4,9</sup>, the increasing number of academic publications on the subject of football makes it difficult for them to assimilate the never-ending stream of new information. In addition, scientific inquiry into football is saturated with empirical contributions from different sources and networks that have produced misleading knowledge and evidence<sup>10</sup>.

Treating sports injuries is often challenging, costly, and time consuming<sup>11</sup>. Given the increasing participation in football recently, topics related to football injuries are of great interest<sup>10,12,13</sup>. Furthermore, the economic, social, and health burdens resulting from the high incidence of football-related injuries are also the main factors behind the recent interest in football injuries<sup>14</sup>. Given this context, the necessity of a review to quantify the quality of football-related studies and to identify the areas that have attracted the most research interest becomes evident.

The aim of this study is to outline the evolution of publications regarding football-soccer related injuries over a time span of ten years, to show what areas were most studied in order to know which ones carry greater interest and potential for further exploration, to identify which journals have published the most regarding football injuries, and to explore the levels of evidence. Thus, the main objective of this investigation is to analyse the scientific production of football injuries by using the following bibliometric indicators: country of affiliation, country of publication, publications per year, journal production, number of authors per publication, authors' native language, evidence level, type of study, sex, anatomical location of the injuries, and topographic location of the injuries.

#### Material and method

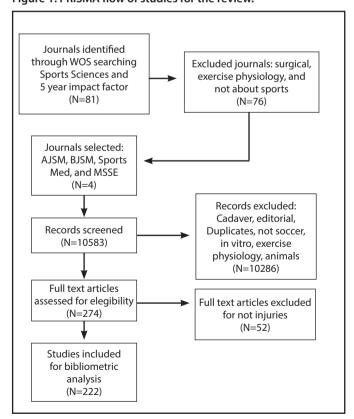
This bibliometric analysis was conducted in accordance with the PRISMA-DTA Statement<sup>15</sup>.

A wide variety of journals with 5- year JIF were selected through searching the Journal Citation Reports™ database (JCR) in the category 'Sport Sciences'. After excluding journals that worked with subjects such as surgery and exercise physiology and those that were not about sports per se, four journals remained: British Journal of Sports Medicine (BJSM), American Journal of Sports Medicine (AJSM), Sports Medicine (SM), and Medicine and Science in Sports and Exercise (MSSE). The journal impact factor (JIF) was obtained from the JCR 2020 edition. In 2019, BJSM had a JIF of 12.68, with a 5-year JIF of 10.67; AJSM had a JIF with a 5-year JIF of 6.8; SM had a JIF of 8.5, with a 5-year JIF of 9.7; and MSSE had a JIF of 4.02, with a 5-Year JIF of 5.09.

Of those four source journals selected, a bibliographic search was developed in the Web of Science database (WoS) collecting records from 2010 to 2019. The following were excluded: articles that studied sports through cadavers; editorials; articles relating to sports other than soccer (such as American football, Australian football, and Gaelic football); studies involving in vitro research; exercise physiology studies; studies with animals; studies that were not related to injuries, and of course duplicates. This search strategy returned 222 articles which were the ones that were bibliometrically analysed. See Figure 1 for a flow diagram.

Data extracted from the articles were as follows: number of publications by journal; country of origin; country of publication; publications per year; number of authors; authors' native language (refers to the language of the institution where the main author is affiliated),

Figure 1. PRISMA flow of studies for the review.



evidence level; type of study (therapeutic, prognostic, diagnostic, and economic); sex (male and female); anatomical location (ligament, joint, tendon, bone, and muscle) and topographic location (head, back, pelvis, hip, thigh, knee, leg, ankle, and foot) of injuries. To assess the level of evidence, the Oxford Centre for Evidence-Based Medicine (CEBM) level of evidence classification was used, dichotomised as follows: articles with level 1 and level 2 evidence were 'high evidence', and articles with levels 3, 4, and 5 were 'low evidence'.

A chi-square test for association was used to analyse most of the categorical and nominal variables; for continuous variables, a Kruskal-Wallis H test was performed. These tests were chosen because none of the variables had a normal distribution. The statistical tests were performed using SPSS V. 28. All statistical comparisons were bilateral; p < .05 was considered statistically significant.

#### Results

#### Country of affiliation

Country of affiliation corresponding to the country of filiation of the first author. Most studies regarding injuries in football were undertaken in the United States (23.9%), followed by the United Kingdom (13.1%) and Sweden (11.7%). These countries produced almost half (48.7%) of the total number of articles. See Table 1.

Table 1. Frequency of publications by country of affiliation.

		Country of affiliati	on
Country	Frequency (N=222)	Percentage	Cumulative percentage
United States	53	23.9	23.9
United Kingdom	29	13.1	36.9
Sweden	26	11.7	48.6
Denmark	16	7.2	55.9
Switzerland	14	6.3	62.2
Norway	12	5.4	67.6
New Zealand	8	3.6	71.2
Qatar	8	3.6	74.8
Australia	7	3.2	77.9
Italy	6	2.7	80.6
Germany	5	2.3	82.9
Canada	5	2.3	85.1
Ireland	4	1.8	86.9
Japan	4	1.8	88.7
Belgium	3	1.4	90.1
Spain	3	1.4	91.4
Finland	3	1.4	92.8
France	3	1.4	94.1
Greece	3	1.4	95.5
Netherlands	3	1.4	96.8
Other*	7	3.5	100.0

<sup>\*</sup>Saudi Arabia. Austria, Brazil, China, Iran, Mexico and Portugal (1 publication; 0.5% each).

#### Country of publication

In the ten-year period under review, British journals (BJSM and SM) published 145 articles (65.3%) and American journals (MSSE and AJSM) published 77 articles (34.7%).

#### Publications per year

The ten-year span was divided quinquennially. British journals published 49 articles in the first quinquennium (33.8%) and 96 articles in the second quinquennium (66.2%). American journals published 57 articles in the first quinquennium (74%) and 20 articles in the second quinquennium (26%). In the first quinquennium, most articles regarding injuries in football were written by American journals (53.7%) whereas in the second quinquennium British journals published more football injury-related articles (82.8%) than American journals. According to this analysis, the differences in publications highlighted above between British and American journals was statistically significant (p < .001), with a moderate association between the variables (p < .001).

#### Journal production

The data shows that half of the articles considered for this analysis come from the BJSM (51,28%), followed by the AJSM (26,6%), the SM journal (13,5%), and the MSSE journal (8,1%). This means that almost 65% of the articles published regarding injuries in football come from British journals. This also shows a trend to rise in article production in all journals, except in AJSM, see Figure 2. Regarding years of publication, the production of journals reached a peak in 2013, with 33 articles published in total (14.9%). Additionally, in this same year, British journals published 21 articles (63.3% of the annual total; 9.5% of the overall total), while American journals published only 12 articles (36.4% of the annual total: 5.4% of the overall total). The graph shows that in the first 5-year period the BJSM and the AJSM published the most articles, compared to the number of articles published by the SM Journal and the MSSE journal. Nonetheless, in the second 5-year period there is a change in the number of publications, with the BJSM and Sports Medicine starting to publish the most, and the AJSM decreasing its rate of publications significantly.

#### Number of authors

The journal with the highest mean number of authors was SM (M = 8.73), followed by MSSE (M = 6.33), BJSM (M = 5.3), and AJSM (M = 5.00). The number of authors was statistically and significantly different between the different journals (p = .025). Post-hoc analyses indicated that the differences were more marked between AJSM and SM (p = .013).

#### Authors' native language

To determine whether the native language of the majority of authors was English, the sample was divided between those with anglophone and non-anglophone authors. It was found that 105 authors (47.3%) did not have English as their native language and 117 (52.7%) had English as their native language.

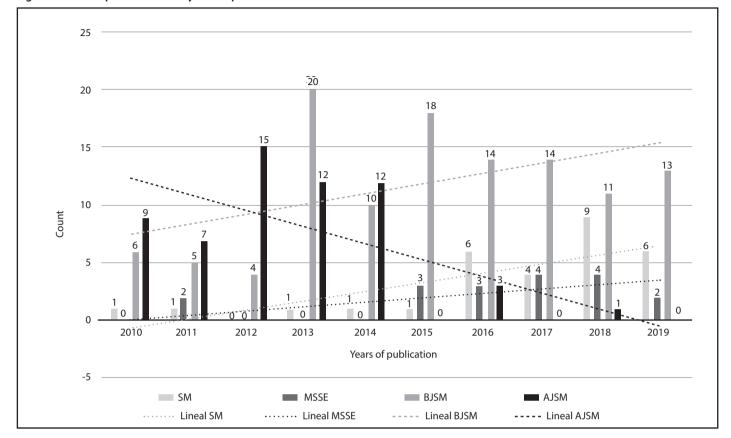


Figure 2. Journal production and years of publication.

The number of non-anglophone authors in BJSM was significantly higher than in the other journals. A greater number of anglophone authors was found in SM, AJSM, and MSSE (p = .003).

#### Evidence level

An analysis of the publications' evidence levels found that 36.4% of the publications presented a level of evidence 2. The level of evidence 1 was least represented with 6.7%. There were 24.3% articles with level of evidence 3 and 22.9% with level of evidence 4. There were 9,4% articles with level of evidence 5. See Table 2.

Dichotomizing the evidence levels in terms of the total number of articles published, 96 (43.2%) presented a high level of evidence and 126 (56.7%) presented a low level of evidence.

SM was the only journal in which most articles presented a high level of evidence (60%). Fifty percent of the articles published in SM were systematic reviews (SRs), cohort studies or ecological studies. Three articles (10%) in SM presented level of evidence 1 and 3 presented level of evidence 3 (p = .013). The BJSM has the most articles in all the levels of evidence. Nonetheless, many of its articles (59,11%) have low levels of evidence. Despite this, the BJSM has 38 articles that are systematic reviews, cohort studies, or ecological studies, which represent a high level of evidence. The AJSM doesn't have articles classified in the first level of evidence, and most of its articles (59,18%) have low levels of

evidence. The MSSE overall has very few articles published regarding injuries in football (soccer). Most of its articles have low levels of evidence (60,9%). see Figure 3.

In order to find out the trend of the evidence levels of the different publications, we have analysed this variable as a continuous variable, this shows us where the evidence levels tend to cluster. Regarding the mean level of evidence per journal, SM presented the highest level of evidence (M = 2.8), followed by AJSM and MSSE, both with a mean of 2.9. BJSM's mean was lowest, M = 3.0, see Figure 4.

Comparing the levels of evidence with the years of publication, no significant associations between the levels of evidence published in the articles were found across the 10-year span (p=.75).

Table 2. Overall evidence level.

Evidence Level	Frequency	
1	15	
2	81	
3	54	
4	51	
5	21	

Figure 3. Evidence level per journal.

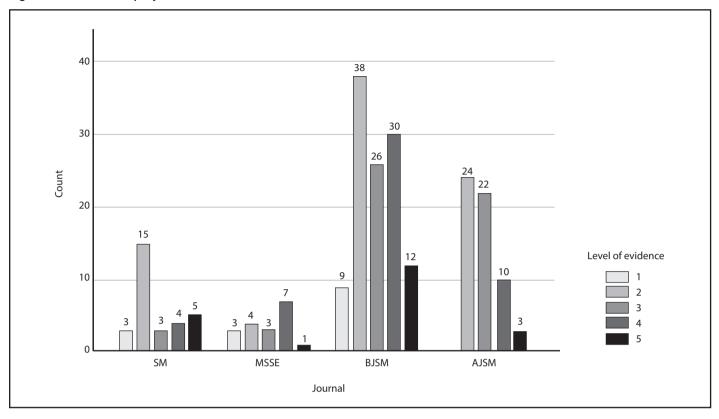


Figure 4. Mean evidence level per journal.

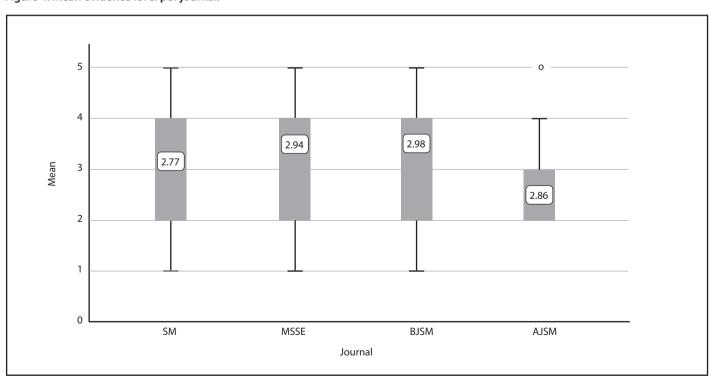


Table 3. Type of study per journal.

	Journal					
Type of study	SM	MSSE	BJSM	AJSM	Total	
Therapeutic	11	1	15	2	29	
Prognosis	10	14	72	44	140	
Diagnosis	2	1	12	6	21	
Economic	7	2	16	7	32	
Total	30	18	115	59	222	

SM: Sport Medicine; MSSE: Medicine and Science in Sport and Exercise; BJSM: British Journal of Sport Medicine; AJSM: American Journal of Sport Medicine.

With respect to native language, anglophone authors published more articles with high levels of evidence, and non-anglophone authors published more articles with low levels of evidence (p = .027).

#### Type of study

Many publications (63%) developed a prognosis and history, followed by economic and decision analysis studies (14.4%), therapeutic, prevention, aetiology and damage studies (13%), and diagnosis studies (9.5%). In terms of the relationship between type of study and journal, BJSM and SM published most of the therapeutic, prevention, and aetiology and damage studies. Most of the economic and decision analysis studies were published in BJSM, followed by AJSM and SM. In addition, it is noteworthy that most MSSE studies concerned prognosis and the history of injuries (p = .002), see Table 3.

No statistically significant associations between the variables were found when type of study was compared with evidence level (p = .872).

#### Sex

A total of 125 (56.3%) studies were conducted with males, 79 studies (35.5%) included both male and female, and 18 (8.1%) included only females.

Of the publications selected for this study, SM published football-related studies involving only males or both sexes but published no studies involving only females. Most of the studies published in MSSE involved only males, with only a few investigating both sexes or only females. Regarding the studies in BJSM, most involved only males, followed by studies involving both sexes, with the least number involving only females; however, it is noteworthy that BJSM published the most studies pertaining to only females. Most of the studies published in AJSM included both sexes, followed by male-only studies and a few that only involved females (p= .005).

After examining the association between sex and years of publication, when considering for the analysis the division into two quinquennium, it was found that studies involving only males were more numerous in the second quinquennium than in the first. In contrast, studies involving both sexes or only females were less numerous in the second quinquennium than in the first (p = .002).

Table 4. Anatomical location of injuries.

Anatomical Location	Frequency	Percentage
Muscle	29	13.06%
Bone	6	2.70%
Tendon	13	5.86%
Joint	17	7.66%
Ligament	21	9.46%
Multiple, others	136	61.26%

#### **Anatomical location**

More than half of the articles (61.3%) described injuries in multiple locations. Reports about specific injury locations ordered by frequency were as follows: muscle injuries 13.1%, ligament 9.5%, joint 7.7%, tendon 5.9%, and bone 2.7%; (p = .037), see Table 4.

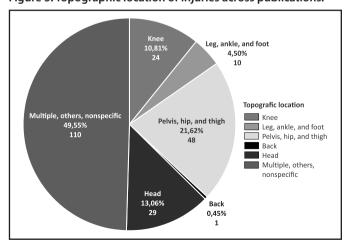
There was a statistically significant association between the variables, implying that studies on injuries to tendons and bones presented higher levels of evidence than studies regarding injuries to muscles, joints, ligaments, and multiple locations (p = .037). There was no significant statistical association between the level of evidence and the anatomical location of the injury (p = .057).

#### Topographic location

Almost half of the articles (49.5%) described injuries in multiple locations; injuries in the hip and thigh comprised 21.6% publications; injuries to the head make up 13.1% of the publications; injuries to the knee were studied in 10.8% publications; and injuries to the leg, ankle, and foot were studied in 4.5% publications, see Figure 5.

Regarding associations between topographic location and country of publication, British journals published more studies involving multiple injuries and injuries to the pelvis, hip, and thigh, while American journals

Figure 5. Topographic location of injuries across publications.



published more studies about injuries to the knee and head. Studies pertaining to leg, ankle and foot injuries were equally represented in British and American journals (p = .009).

An analysis of the relationship between sex and topographic location indicated that most of the studies that included only females investigated multiple injuries, followed by injuries to the knee, and then the head. Most of the studies that included only males in their sample reported multiple injuries, followed by injuries to the pelvis, hip, and thigh; knee; head; and leg, ankle and foot, (p = .002).

#### Discussion

The data shows that there is an overall trend to rise in article production over the past 10 years. This shows that there is a growing interest in developing knowledge around the understanding of football injuries (their prognosis, location, and treatment). The United States had the highest number of studies (23.9%). Nonetheless, most of the articles in this study were published in high-impact British journals (65.31%). This finding coincides with other bibliometric studies in traumatology<sup>16,17</sup>, in which the United States was found to be the country with the highest levels of authorship.

Europe developed 59.46% of the total academic production considered in this analysis, followed by America (27.03%), Asia (6.76%), and Oceania (6.76%). It is noteworthy that countries with an important football tradition, such as Spain and Italy, only developed 4.1% of the total production of studies relating to football injuries.

During the decade considered for this analysis, more than half of the articles were published by BJSM (p = .013) with a peak in production in 2013 (20 publications). This shows a global tendency to increase publications across the four journals. This coincides with the 2012 London Olympics, European Football Championship 2012 (Eurocup)  $^{18}$ , and increased scientific production in football commented on in other bibliometric studies  $^{10,19}$ .

With respect to the number of authors, the journal with the highest number of authors per article was SM, M = 8.73; AJSM had the lowest number, M = 5.00 (p = .025). In other bibliometric studies, it has been observed that having many authors can be interpreted in different ways: it can reflect a high degree of collaboration or an aggrandizement of the number of authors as a consequence of having the presence of honorific or phantom authors, and other studies have reported a 21% prevalence of these types of authors<sup>20</sup>.

Regarding levels of evidence, 56.7% of the studies reviewed had a low level of evidence. This asymmetry in evidence levels may be explained by differences in categories and scenarios regarding the sport of football which affects research infrastructure. Consequently, large studies are limited to the footballing elite. However, this study included meta-analysis and randomized controlled trials (RCTs) in the areas of training endurance and performance, with special focus on the prevention of injuries<sup>21–23</sup>; it also comprised studies that evaluated the efficacy of football programmes, such FIFA's 11+ Kids<sup>24,25</sup>, along with SRs corresponding to FIFA studies in young football players<sup>26</sup>. This constitutes valuable input for medical teams and technicians responsible for managing the training loads and attending to the mental health of football players.

As stated elsewhere, 'where RCTs are often not available, a systematic literature review of other published studies is the next 'best' form of evidence'<sup>27</sup>. Given the low number of RCTs, this could be applied to football. In relation to the SRs analyzed during this study, some of them with meta-analyses, it was found that they refer to topics related to risk factors for injuries and injury prevention and the treatment of sports injuries<sup>28–33</sup>. However, there were 56.7% articles with low levels of evidence; this represents an opportunity for improvement in the area for researchers who are making an incursion into football.

With respect to study type, the majority were prognosis and natural history studies (63%), while the minority were diagnostic studies (9.5%). Most of the studies described injury behaviour and its influence on football players' fulfilment<sup>34–40</sup>, and few investigations were dedicated to the utility of diagnostic tests in pathologies such as tendinopathies or groin pain<sup>41–44</sup>. The high prevalence of economic studies is striking<sup>45–47</sup> (14.4%). As observed in other publications, there is an increase in studies of innovation in football<sup>19,26</sup>, in aspects such as the use of tools for injury prediction<sup>48</sup>, decision-making about the continuity of prevention programmes<sup>25</sup>, analysis of beneficial effects generated by preventive programs<sup>49,50</sup>, adaptations derived from specific skills training<sup>51</sup>, and talent identification<sup>46</sup>.

In relation to sex, there were a greater number of studies involving only males (56,3%) than only females (8.1%). Further, studies reported a lower number of football studies involving females compared to males and an even greater scarcity of publications involving elite female football players, despite an increase in the popularity of females football players, despite an increase in the popularity of females football players, in this study, we observed a lower number of publications about injuries in females; nevertheless, these publications have a high level of evidence. It was found that there was a higher rate of studies with female participants in the first five years 29,36,54, with a special focus on knee injuries in adolescent football players 36; however, there was an unfortunate downward trend in the second five-year period. This suggests a commitment to scientific quality in this field. As a consequence, it is necessary to expand the number of studies with female participants in order to improve their availability to carry out comparative studies with males, which in the end strengthens the possibility of decision-making.

Regarding the anatomical location of injuries, a greater presence of muscle injuries was found, followed by injuries to the ligaments, joints, tendons, and bones. It is striking that tendon and bone studies have a higher level of evidence than those involving other anatomical structures: evaluating their content, it has been observed that they are also epidemiological studies<sup>38,55–58</sup>, or that they evaluate the effectiveness of injury prevention programmes involving structures that are not exclusively tendons, such as the hamstrings<sup>56</sup>. In addition, many of these studies were performed with support from large organizations such as Union of European Football Associations, providing for large population samples<sup>56,58</sup>.

With respect to topographic locations, the distribution found in this study coincides with the epidemiologic distribution described in other reports that noted a general prevalence of muscle and thigh injuries, followed by head injuries<sup>8,20,58</sup>. Furthermore, this study's finding of a high frequency of research reporting hip, joint, and thigh injuries in males coincides with injury patterns reported by UEFA studies, with a higher incidence of such injuries in elite football players<sup>7,59,60</sup>. Regarding

female football, there has been a higher presence of articles reporting head and knee injuries; this finding is coherent with the report of the Football Association (FA) highlighting the importance of knee injuries and the injury profile for females described by Spanish authors in a SR<sup>52,53</sup>, although in the present study there was no special emphasis on head injuries. In contrast with the previous studies, a study conducted with college female football players by the National Collegiate Athletic Association (NCAA) exposes a concern about the rate of this type of injury in females, mentioning that 'the concussion rate in NCAA female soccer is almost twice as high as the rate in male soccer'59. In football, head injuries have been the object of analysis in the field of biomechanics research in order to propose evidence-based rules and policies, as well as quide developments based on agreed criteria regarding return to play after suffering a concussion<sup>61–63</sup>. In this field, American journals stand out in the number of reports about head injuries, with a particular focus on neurocognitive symptoms after concussion<sup>63,64</sup>.

#### Study limitations

This study was limited to 222 papers selected from the WOS database, and thus there may be valuable information in other publications outside the scope of this study. The exclusion of surgery and exercise physiology journals can be considered a limitation as well, since we could have rejected articles related to football injuries. Nevertheless, this report performed a detailed evaluation to exclude articles about Gaelic, Australian, and American football to increase the quality of the study; at the same time, an entirely quantitative approach was avoided and a qualitative assessment of evidence levels in terms of CEBM criteria was included. This approach is intended to contribute to current and future research in this field.

#### **Conclusions**

In conclusion, this review found that during the decade from 2010 to 2019, the frequency of football and sports studies grew, reflecting an increasing interest in this area. We found that BJSM was the journal with the highest number of publications, followed by AJSM, and the United States was the country with the highest level of authorship. A noteworthy finding was that there were more studies involving males than females. Finally, we found that football injury studies in general present a low level of evidence while female football studies exhibit a higher level of evidence.

#### Registration

This review was not registered.

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#### Conflict of interest

The authors do not declare a conflict of interest.

#### Availability of data

Data is available at Repositorio Institucional de la Consejería de Sanidad de la Comunidad de Madrid (https://hdl.handle.net/20.500.12530/54436).

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# Consumption of energy drinks on cardiovascular and metabolic response and performance. Is there an effect?

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#### Summary

Over the years, the search for nutritional strategies that promote improved sports performance has increased. Among the available options, energy drinks appear as potential nutritional resources for this purpose, because they offer, in addition to caffeine, substances that act synergistically to improve performance, such as taurine, carbohydrates, amino acids, vitamins and minerals, promoting improved performance for both amateur and professional athletes. The aim of the study was to verify the effects of ingesting energy drinks with (ED1) and without carbohydrates (ED0) containing 2 mg·kg<sup>-1</sup> of caffeine, and a decaffeinated placebo (PL) on cardiovascular, metabolic and performance parameters during cycling. Twelve male cyclists (age =  $24.4 \pm 6.6$  years old) volunteered to participate in this study. The protocol consisted of three experimental sessions of 60 min of continuous cycling (65-75% of VO<sub>2max</sub>) followed by time-trial 6 km. The subjects ingested ED1, ED0 or a placebo drink (PL) 40 min before beginning the exercise. The heart rate (HR), blood pressure (BP), plasma glucose and lactate concentrations, and the time taken to complete the 6 km time-trial were evaluated. The time taken to complete the time-trial was significantly higher (p < 0.05) in the PL group than in the groups ED1 and ED0. This time significantly decreased after the ED1 consumption relative to that for the ED0 consumption. Heart rate, systolic and diastolic arterial pressure and in the plasma glucose and lactate concentrations were similar in all the considered groups. These results demonstrate that ED1 consumption appears to be more effective at maximizing performance during the last 6 km.

### **Key words:** Caffeine. Taurine. Sport drinks.

Caffeine. Taurine. Sport drinks. Sports performance. Cycling

# Consumo de bebidas energéticas sobre la respuesta cardiovascular, metabólica y rendimiento. ¿Hay efecto?

#### Resumen

Con el paso de los años, se ha incrementado la búsqueda de estrategias nutricionales que promuevan un mejor rendimiento deportivo. Entre las opciones disponibles, las bebidas energéticas aparecen como potenciales recursos nutricionales para este fin, pues ofrecen, además de la cafeína, sustancias que actúan sinérgicamente para mejorar el rendimiento, como taurina, carbohidratos, aminoácidos, vitaminas y minerales, promoviendo un mejor rendimiento para atletas tanto aficionados como profesionales. El objetivo del estudio fue verificar los efectos de la ingestión de bebidas energéticas con (ED1) y sin carbohidratos (ED0) que contienen 2 mg  $\cdot$  kg  $^{-1}$  de cafeína y un placebo descafeinado (PL) sobre los parámetros cardiovasculares, metabólicos y de rendimiento durante el ciclismo. Doce ciclistas varones (edad = 24,4  $\pm$  6,6 años) participaron voluntariamente en este estudio. El protocolo consistió en tres sesiones experimentales de 60 min de ciclismo continuo (65-75% del VO<sub>2max</sub>) seguidas de una prueba contrarreloj de 6 km. Los sujetos ingirieron ED1, ED0 o una bebida placebo (PL) 40 minutos antes de comenzar el ejercicio. Se registró la frecuencia cardíaca (FC), la presión arterial (PA), las concentraciones plasmáticas de glucosa y lactato y el tiempo necesario para completar la prueba contrarreloj de 6 km. El tiempo necesario para completar la contrarreloj en el grupo PL fue significativamente mayor (p <0,05) que en los grupos ED1 y ED0. Este tiempo disminuyó significativamente después del consumo de ED1 en relación con el consumo de ED0. La frecuencia cardíaca, la presión arterial sistólica y diastólica y las concentraciones plasmáticas de glucosa y lactato fueron similares en todos los grupos. Estos resultados demuestran que el consumo de ED1 parece ser más eficaz para maximizar el rendimiento durante los últimos 6 km.

#### Palabras clave:

Cafeína. Taurina. Bebidas deportivas. Rendimiento deportivo. Ciclismo

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#### Introduction

In the last decade, the consumption of energy drinks (EDs) has become popular especially among athletes<sup>1</sup> due to the possibility of an ergogenic effect<sup>2</sup> during competitions.

Most EDs contain caffeine, taurine, carbohydrates and vitamins<sup>3</sup>. Caffeine has often been used as an acute ergogenic substance before performing physical exercise to postpone fatigue and consequently to improve performance<sup>4</sup>. In addition, some studies have also shown the positive effect of an acute ingestion of taurine on physical performance in humans<sup>5,6</sup>.

The presence of carbohydrates in EDs appears to be essential for aerobic events, particularly those that are longer than one hour. Carbohydrates decrease the need to consume muscle glycogen and consequently delay the onset of muscle fatigue<sup>7</sup>. Some studies have shown positive results of a possible enhancement in the ergogenic effect during physical exercise after addition of carbohydrates in caffeinated drinks<sup>8,9</sup>, in contrast, other studies did not observe any effect<sup>10</sup>.

It is becoming quite popular to consume EDs without carbohydrates (sugar free) before physical activity<sup>11</sup>. However, the benefits of these beverages on physical performance are not clear. Some studies with divergent results <sup>12,13</sup> have evaluated performance after the ingestion of this type of EDs. One study reported an increase in the number of sprints during a soccer game<sup>13</sup> and others did not find improvement in the exercise time to exhaustion<sup>12</sup>.

Only the studies by Reis et al., <sup>14</sup> and Reis et al.<sup>9</sup> verified and compared the impacts of energy drinks ingestion with and without carbohydrates, confirming that the consumption of these drinks does not affect the hydro-electrolyte balance and promotes an improvement in the performance of runners.

Considering both the scarcity of studies that compare the effects of EDs with and without carbohydrates on physical performance and the increased consumption of both types of drink before sport practice, identifying which drink has greater ergogenic benefits is important. Therefore, the aim of this study was to investigate the effects of pre-exercise ingestion of EDs with and without carbohydrates on cardiovascular and metabolic parameters and on performance on a cycle ergometer during an aerobic effort.

#### Material and method

#### **Participants**

Twelve male cyclists (age =  $24.4 \pm 6.6$  years, body mass =  $72.7 \pm 7.2$  kg, estimated VO<sub>2max</sub>(VO<sub>2max</sub>e) =  $54.5 \pm 4.8$  ml.kg<sup>-1</sup>.min<sup>-1</sup>), volunteered to participate in this study. Participants were amateurs who were participating to regional competitions, and trained at least three times per week for a minimal duration of 2 hours, for at least two years.

All subjects were considered healthy, as assessed by the PAR-Q questionnaire<sup>15</sup>. The participants were non-smokers, did not consume any alcohol or drugs that could affect food intake and energy metabolism, and did not have a previous history of hypertension, heart disease or diabetes mellitus. The daily caffeine intake of the volunteers was

also low (<200 mg/day), as determined from their response to a food frequency questionnaire that was adapted for caffeine intake<sup>16</sup>.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by local Institutional Review Board for Human Subject Protection (no. 154/2011).

The sample size was calculated on the basis of the formulas proposed by Mera *et al.*<sup>17</sup>. The time taken to complete time-trial (in seconds) was considered the main variable, with a statistic strength of 95%. Standard Deviations values were applied in these calculations, adopting a difference of 15% for the average value of time-trial based on the study realized by Rankin *et al.*<sup>18</sup>. The women were not included in the sample due to hormonal alterations resulting from the menstrual cycle, because the luteal phase can promote alterations in the metabolism of caffeine<sup>18</sup> which could affect the results.

#### Preliminary test

The exercise protocol to determine the maximal consumption of oxygen (VO $_{2max}$ ) was composed of 3 minutes of warm-up with a charge proportional to the body weight (BW) of each participant in the cycle ergometer (SCIFIT ISSO 1000, Oklahoma, USA). Once finalized, 30 W were added each minute until 85% of heart rate (HR) was reached, which had been calculated through the THR equation (training heart rate = % (HR $_{max}$  – HR $_{rest}$ ) + HR $_{rest}$ ) $^{19}$ , where HRmax is calculated with the equation validated for exercise on cycle ergometer\*: HR $_{max}$  = 202 - 0,72 x (age) $^{19}$ .

The  $VO_{2max}$  was estimated on the basis of recommendations of Marsh<sup>20</sup> using the um submaximal cycle ergometer test, aiming to preserve the safety of the subjects. According to this method, individual equations to estimate the  $VO_{2max}$  were formulated by linear regression using the HR (bpm) and the consumption of oxygen ( $VO_2$ ) (ml.kg.min<sup>-1</sup>) values obtained during exercise through the analysis of respiratory gas exchange.

The metabolic gas analyzer (Medical Graphics Corporation®, VO2000) was used to evaluate  $VO_2$ , the cardiac monitor (Polar® RS800cx) was utilized to evaluate the HR, and a software (SigmaPlot®, 11.0) was used to determine the linear regressions. The charge corresponding to the range of 65 a 75% of the  $VO_{2max}$  was also determined by the software in order to use it in the main part of the experimental protocol.

After the preliminary test, the volunteers were instructed to refrain from consuming caffeine or alcohol or performing physical activity for 48 hours before their visit to the laboratory to perform the test.

#### **Experimental protocol**

For the double-blind, randomized crossover design, each subject performed three experimental tests that were separated by at least 2 days. The participants arrived at the laboratory between 6:00 and 9:00 AM after fasting for 10 to 13 hours, and the volunteers then consumed a breakfast (including grape juice, sliced white bread, mozzarella cheese and an apple). The breakfast was based on the recommendations of the Institute of Medicine<sup>21</sup> and provided 15% of the estimated energy requirements (EER), which were calculated for the nutritional needs of each participant. The variables used in this calculation were: age, weight, height and level of physical activity.

The volunteers were instructed to standardize their meals on the previous day and for the remaining days between the three tests. The actual consumption was monitored by a 24-hour dietary recall, which was collected before each experimental protocol.

One hour after breakfast, the subjects consumed their drinks. The EDs differed only in the presence (ED1) or absence (ED0) of carbohydrates, and the placebo solution (PL) was prepared with lemon juice (absence of sugar) dissolved in 500 ml of carbonated water. Table 1 shows the nutritional composition of each drink.

Considering that the flavor placebo was not identical to other energy drinks, the subjects were informed that all drinks had ergogenic substances mixed in their composition.

The beverages were ingested 40 minutes before starting the experimental tests; thus, the caffeine peaks in the bloodstream occur within 30 to 60 minutes of ingestion<sup>22</sup>. The drinks were supplied in opaque plastic bottles to prevent identification.

The amount of liquid consumed (454.7  $\pm$  44.2 ml) was calculated for each individual to provide a dose of 2 mg caffeine/kg of BW. The dosage was selected based on previous studies<sup>23,24</sup> that showed improved physical performance in populations that were similar to the volunteers of the present study.

The exercise consisted of an initial 5-minute warm-up at 45 - 55% of  $VO_{2maxE}$  and the main exercise for 60 min at continuous speed and 65 - 75% of  $VO_{2maxE}$ . All subjects maintained the spinning/cycling rate between 65 to 75 rpm during the 60 minutes of continued exercise. Immediately after the 60 minutes of exercise, subjects realized 6 km time-trial with the same charge carried in the entire test. The verbal encouragement was given to all participants during time-trial, where speed was selected self- selected.

The hydratation procedure adopted during experimental sessions were exclusively realized with water, which corresponds to an individual calculation of 3ml/kg of PC, before exercise and every 15 minutes during exercise, as well as after the time-trial. At those times, the blood pressure

Table 1. Nutritional composition (per 250 ml) of the beverages used in experimental trials.

Ingredients	Energy Drink (ED1)	Energy Drink sugar free (ED0)	Placebo Drink (PL)
Calories (kcal)	110	10	6.37
Carbohydrates	(g) 28	3	0
Protein (g)	0	0	0
Fat (g)	0	0	0
Caffeine (mg)	80	80	0
Taurine (mg)	1000	1000	0
Other ingredients	Glucuronolactone, inositol, sodium, water, and vitamins of group B	Glucuronolactone, inositol, sodium, water, and vitamins of group B	Sodium

ED1: Energy Drink in the presence of carbohydrates. ED0: Energy Drink in the absence of carbohydrates. PL: Placebo solution, prepared with lemon juice (absence of sugar) dissolved in 500 ml of carbonated water.

(BP) was measured using a Tycos® sphygmomanometer. Additionally, the HR was monitored using a Polar® RS800cx (Polar Electro Ltd., Kempelen, Finland) with recordings every 15 seconds, and the value was averaged every 5 minutes.

Every 15 minutes during continued exercised and after the time-trial, participants were asked to provide the index of perceived exertion (IPE) which variants from 6 to  $20^{25}$ .

For each experimental test, blood samples were collected before breakfast (fasting), before the drink ingestion, immediately before the beginning of the exercise, every 20 minutes during the continuous exercise and at the end of the time-trial. After each blood collection, the catheter was filled with 0.9% saline solution to prevent blood coagulation and preserve access for subsequent collections. From each blood sample, 1 ml was removed and immediately transferred to an eppendorf tubes. An automatic pipette was used to select 100  $\mu L$  of this blood, which was injected into a single-use disposable cartridge and analyzed "in situ" by a portable blood analysis system for glucose measurements (i-STAT, Abbott°, Illinois, United States). A drop of blood was collected from the eppendorfs and placed into a portable lactate analyzer (Accutrend°, Roche, Mannheim, Germany) to determine the value of this metabolic variable.

All experimental tests were performed under similar ambient conditions of temperature and relative humidity (23.2  $\pm$  0.91°C and 69.5  $\pm$  5.56%; p> 0.05), as measured by a Hygro Thermometer®.

#### Statistical analysis

All results are reported as the mean and standard deviation. The Shapiro-Wilk test was used to verify the normality. To compare the time-trial duration between the different treatments, a one-way ANOVA test for repeated measures with a post-hoc Tukey HSD test was used. To verify the interaction between the treatments over time, a two-way ANOVA test for repeated measures (split-plot ANOVA test) was used while applying a Mauchly test and a Greenhouse-Geisser correction when the assumption of sphericity was violated. Significant p-values were identified for interaction effects (time x treatment); simple analyses were performed when an influence from the energy drinks was found. Significant values for certain moments were investigated using paired comparisons with Bonferroni-adjusted confidence intervals. The statistical analysis was performed using SPSS (v17.0, USA) and assuming p<0.05 as the significance level.

#### Results

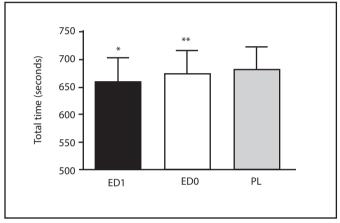
#### Evaluation of physical performance

The time required to complete the 6 km time-trial was significantly higher for the PL group than for the groups that the consumed energy drinks, ED1 (p<0.001) and ED0 (p<0.01) (see Figure 1). The time-trial time after consuming ED1 was significantly lower (p<0.001) than the time after consuming ED0. The mean performance time for the last 6 km was 2.01% and 2.78% faster after ingesting ED1 than after ingesting ED0 and PL, respectively.

#### Cardiovascular parameters

The cardiovascular responses are shown in Table 2. For all treatment groups, there was a significant increase (p<0.001) in the HR and systolic arterial pressure (SAP) during the time-trial relative to the values during the continuous exercise. The diastolic arterial pressure (DAP) remained stable throughout the exercise at 65-75% of VO $_{\rm 2maxE}$  (p<0.05) and increased significantly (p<0.001) during the time-trial for all treatments. No differences were observed in HR, SAP and DAP between the treatments either at rest, during the 60 minutes of continuous exercise, or during the time-trial.

Figure 1. Sprint duration for the three treatments.



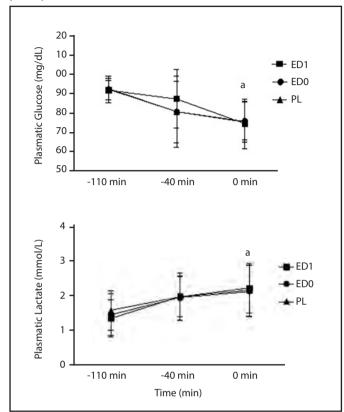
<sup>\*</sup> Significant difference compared with ED0 (p<0.001) and PL (p<0.001). \*\* Significant difference compared with ED1 (p<0.001) and PL (p<0.01).

Table 2. Average  $\pm$  Standard deviation values of the cardiovascular responses at rest, during exercise 65-75% of the VO<sub>2max</sub> and sprint.

		Type of Treatment						
Condition	ED1	ED0	PL					
SAP								
Resting	114.50 ± 9.65	113.00 ± 9.04	115.30 ± 6.51					
Exercise	147.50 ± 14.04*	148.00 ± 16.06*	150.54 ± 14.51*					
Sprint	197.33 ± 19.22§	198.50 ± 19.13§	200.00 ± 17.06§					
DAP								
Resting	70.83 ± 7.93	74.83 ± 9.96	75.16 ± 6.68					
Exercise	$73.95 \pm 5.58$	71.62 ± 9.11	$77.29 \pm 9.38$					
Sprint	90.83 ± 13.11§	90.83 ± 16.21§	93.45 ± 19.23§					
HR (beats/min)								
Resting	55.75 ± 7.16	55.08 ± 7.77	56.75 ± 9.38					
Exercise	145.82 ± 8.93*	148.06 ± 7.77*	146.44 ± 12.17*					
Sprint	173.71 ± 7.37§	171.57 ± 7.05§	173.27 ± 9.86§					

<sup>\*</sup> Significant differences compared to resting and sprint (p<0.001). § Significant differences compared to resting and exercise (p<0.001).

Figure 2. Average  $\pm$  standard deviation of the plasma glucose and lactate levels during fasting (-110 min), before the drink intake (-40 min) and immediately before the beginning of the exercise (0 min).



#### Plasma metabolites

The plasma concentrations of glucose and lactate before breakfast (fasting), before the drink intake and immediately before the beginning of the exercise are shown in Figure 2. No significant differences (p>0.05) were observed between the treatments, indicating that the subjects started the exercise under similar metabolic conditions.

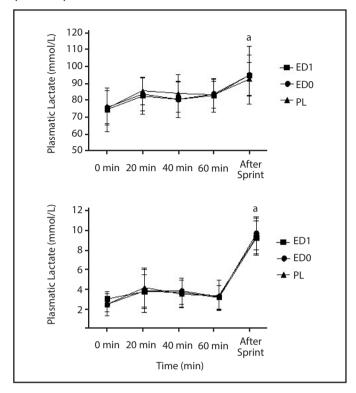
The plasma concentrations of glucose and lactate at rest (0 min), during exercise at 65-75% of  $VO_{2maxE}$  and after the time-trial are shown in Figure 3. For all treatments, the plasma concentrations of glucose and lactate were significantly higher (p<0.001) after the time-trial than those at rest and during the continuous exercise. No significant differences were observed in the plasma levels of glucose and lactate between the treatments at rest, during the continuous exercise and after the time-trial.

#### Index of perceived exertion (IPE)

The IPE remained stable throughout continued exercise (13.41  $\pm$  0.59 for ED1, 13.41  $\pm$  0.44 for ED0 and 13.48  $\pm$  0.65 for PL) and increased significantly (p<0.001) after time-trial in all treatments (18.25  $\pm$  1.35 for ED1, 18.5  $\pm$  1.62 for ED0 and 19.15  $\pm$  1.07 for PL). The IPE value at the end of the time-trial was higher in the treatment with PL compared to ED1 (p=0.012) and to ED0 (p=0.022).

SAP = Systolic arterial pressure; DAP = Diastolic arterial pressure; HR = Heart rate.

Figure 3. Average  $\pm$  standard deviation of the plasma glucose and lactate concentrations during the experimental protocol. (a) Significant difference (p<0.001) compared with the other conditions (0-60 min) for all treatments.



#### **Discussion**

The aim of this study was to verify the effects of pre-exercise ingestion of an ED with and without carbohydrates on cardiovascular, metabolic and performance parameters during an aerobic effort on a cycle ergometer.

The main finding of this study was that the use of EDs with and without carbohydrates decreased the time to complete a 6 km time-trial after 60 minutes of aerobic training relative to the PL time-trial time (Figure 1). This improvement in performance after the ED ingestion is consistent with other previous studies<sup>8</sup>.

The mechanisms by which EDs improve performance are not fully understood. Some authors believe that caffeine is the main active ingredient of EDs and is responsible for the ergogenic effects on physical performance<sup>12</sup>. Several mechanisms may be involved in these effects. These mechanisms include the following: action on the sarcoplasmic reticulum by increasing the availability of calcium to potentiate muscle contraction<sup>26</sup> an antagonist effect on adenosine receptors, leading to increased activation of the central nervous system and plasma epinephrine<sup>26</sup>; and changes in potassium concentrations that assist in the maintenance of the membrane excitability of contractile muscle during exercise<sup>27</sup>.

Although caffeine is considered the main active ingredient in energy drinks, taurine may also contribute to the ergogenic effect on exercise<sup>28</sup>. Some studies reported that taurine supplementation

can increase the ability to prolong exercise<sup>5,6</sup>, possibly by stabilizing the phospholipids of the membrane and increasing the availability of calcium in the muscle<sup>29</sup>.

The combination of the ingestion of caffeine and taurine in the same formulation showed significant improvements in the ventricular function, increasing the stroke volume<sup>6</sup> compared to drinks only containing caffeine. This way, these components can act synergistically and improve the performance in training as well as in competitions.

ED1 seems appears to increase performance more effectively because it reduced the 6 km time-trial time by 2.01% relative to the time for ED0. The results corroborate those of other studies indicating that the combination of caffeine and carbohydrates can improve the ergogenic effect during exercise<sup>30</sup>. Caffeine has also been linked with increased intestinal glucose absorption and utilization of exogenous carbohydrates<sup>31</sup> thus minimizing the depletion of muscle glycogen and delaying the onset of fatigue during aerobic exercise.

The HR values gradually increased during the 60 minutes of continued exercise and during time-trial, although no significant difference was observed between the treatments (Table 2). Caffeine inducts the liberation of adrenaline during exercise, higher HR values were expected in treatment with energy drinks. However, some studies reported a decrease in HR during submaximal exercise after the ingestion of an ED containing taurine<sup>32</sup> suggesting that taurine could alter cardiovascular physiology.

Corroborating this hypothesis, Baum<sup>6</sup> observed that taurine in combination with caffeine significantly increases the volume of ejection in athletes after endurance exercise. This is due to the greater end diastolic volume and to the decrease of the end systolic volume of the left ventricle, as the fractional shortening increases significantly, and contributes to lower values of HR. If confirmed, these responses in the cardiovascular system can have an important effect after a training session in athletes who train for extended durations, since it acts like a factor of cardioprotection.

As found in a previous study<sup>33</sup>, our mean values of SAP were lower, but not significantly, after the consumption of ED1 and ED0 compared with those in the PL group. Besides, no significant differences between treatments were observed in the DAP values.

Ragsdale<sup>34</sup> analyzed the effects of two energy drinks on cardiovascular function during two hours of rest. Those drinks were similar to the drinks used in this study (ED1 and ED0), and those researchers reported that ingesting EDs did not cause a significant difference in BP relative to the BP after ingesting PL.

The lack of studies that has analyzed BP during exercise after the consumption of energy drink brings difficulties in the comparison and the interpretation of the results. Consequently, additional studies are necessary to verify if the consumption of ED effects the pressure values both during rest and exercise, principally when consumed chronically and not as acute in this study.

The IPE significantly increased after the time-trial compared with the values obtained during the continuous exercise. The IPE for the time-trial was significantly higher after PL consumption than after ED1 and ED0 consumption (Table 2). Similar results were observed in the study realized by Hahn *et al.*, 35 after the ingestion of drinks comparable to placebo.

It is believed that caffeine reduces the feeling of pain through its antagonist effect on adenosine<sup>36</sup>. Due to the similarity between the molecular structures of adenosine and caffeine, caffeine occupies some adenosine receptors and minimizes the effect of this neurotransmitter on the body<sup>36</sup>. Thus, dopamine becomes less inhibited, increasing the dopamine concentrations during exercise. Another ergogenic mechanism of caffeine is to decrease the expression of tryptophan hydroxylase (TPH); the degradation of tryptophan modulates the amount of serotonin that crosses the blood-brain barrier<sup>37</sup>. A lower serotonin/dopamine ratio reduces central fatigue and improves performance<sup>38</sup>.

Our results show no differences among the treatments in the plasma glucose levels during the continuous exercise and the time-trial (Figure 3). Thus, the energy drinks were equally effective at maintaining plasma glucose levels during the continuous exercise and the time-trial. The maintenance of blood glucose is crucial because hypoglycemia causes fatigue, leading to exhaustion and forcing an individual to stop the exercise<sup>39</sup>. Our results indicate that the presence or absence of carbohydrates in the ED did not affect the plasma glucose kinetics, which were most likely influenced by the breakfast that was consumed before the exercise.

No differences were observed in the plasma lactate concentrations between the treatments (Figure 3). Lactate has an important role in the onset of fatigue during high-intensity exercise<sup>40</sup>, as shown for the 6 km time-trial in the present study. The results indicated better performance after ED consumption than after PL consumption but did not show significant differences in lactate concentration. Therefore, it is possible that this improved performance could be linked to specific cellular adaptations.

Consuming an ED can also cause ergolytic effects. The most common problem in these beverages appears to be associated with the high levels of caffeine, rather than the other substances in these drinks. Doses above 6 mg/kg of BW can be toxic to the body, decreasing the stability of the upper limbs and causing insomnia, irritability, anxiety, nausea and gastrointestinal discomfort<sup>41</sup>. Campbell<sup>22</sup> indicated that ingesting a dose of caffeine of 2 mg/kg of BW between 10 and 40 minutes prior to exercise increased performance without ergolytic effects or any risk for the athlete, as was further confirmed in this study.

The limitations of the present study include the absence of either a muscle biopsy to analyze the levels of muscle glycogen or an electromyographic examination that could provide relevant information about the different muscle responses to the work load.

#### Conclusion

Considering the assessed population group (regular cyclists with a low daily intake of caffeine) and the conditions of the exercises performed in this study, it can be concluded that consuming ED1 (2 mg of caffeine/kg of BW) 40 minutes before 60 minutes of moderate aerobic exercise improved the performance in a 6 km time-trial. Further research that uses other doses and other population groups (such as women and consumers of caffeine) and that measures the effects in other sports should be undertaken to expand upon the scientific evidence presented in this study.

#### Conflict of interest

The authors do not declare a conflict of interest.

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# Study of heart rate variability following exposure to normobaric hypoxia

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#### Summary

Heart rate variability (HRV) is a tool capable of analysing and assessing the vegetative activity of the heart in various activities and situations. It consists of measuring the time that elapses between every two heartbeats over a period of time and expressing it in terms of mathematical and statistical equations. Other authors have analysed the influence of different stressors on HRV. In this work we are looking for the action of normobaric hypoxia (NH) on HRV. NH consists of breathing oxygen-depleted air simulating altitude training. The aim of the study is to determine the influence of HN on the time and frequency domains of HRV. We subjected 13 healthy subjects (recreational athletes) to two HN sessions. We used the iAltitude Trainer v2.7° simulator. The first was a hypoxia tolerance test (HTT) (10 minutes,  $11\% O_{2^2}$  equivalent to 5050m) and the second was an intermittent exposure (HNI) ( $14\% O_{2^2}$ , 3250m) in which periods of 4 minutes of hypoxia alternated with 4 minutes of normoxia for 64 minutes. For HRV analysis, a Polar H10° heart rate monitor, the HRV-elite° application and the Kubios-Standard° software were used. Data were taken 5 minutes before and after each session, and these values were compared using the Student's t- test for paired data. None of the variables in the time (RRmean, SDNN, rMSSD, pNN50) or frequency (VLF, LF, HF, LF/HF) domains of HRV showed significant changes in either situation. HN did not cause changes in the stress levels of these subjects and was well tolerated, clinically and electrocardiographically. A tolerance test and a session of exposure to intermittent normobaric hypoxia are not sufficient stimuli to cause acute changes in HRV.

#### **Key words:** Heart rate variability. Intermittent normobaric hypoxia. Hypoxia tolerance.

## Estudio de la variabilidad de la frecuencia cardiaca tras la exposición a la hipoxia normobárica

#### Resumen

La variabilidad de la frecuencia cardiaca (VFC) es una herramienta capaz de analizar y valorar la actividad vegetativa sobre el corazón ante diversas actividades y situaciones. Consiste en medir el tiempo que trascurre entre cada dos latidos cardiacos durante un periodo de tiempo y expresarlo en función de ecuaciones matemáticas y estadísticas. Otros autores han analizado la influencia de diferentes estresores sobre la VFC. En este trabajo buscamos la acción de la hipoxia normobárica (HN) sobre la misma. La HN consiste en respirar aire empobrecido de oxígeno simulando el entrenamiento en altitud. El objetivo del estudio es determinar la influencia de la HN sobre los dominios de tiempo y frecuencia de la VFC. Sometimos a 13 sujetos sanos (deportistas recreacionales) a dos sesiones de HN. Usamos el simulador iAltitude Trainer v2.7°. La primera mediante un test de tolerancia a la hipoxia (TTH) (10 minutos, 11%  $O_2$ , equivalente a 5.050 m) y, la segunda, con una exposición intermitente (HNI) (14%  $O_2$ , 3.250 m) en la que se alternaron periodos de 4 minutos de hipoxia con 4 de normoxia durante 64 minutos. Para el análisis de VFC se utilizó un pulsómetro Polar H10°, la aplicación HRV-elite° y el software Kubios-Standard°. Se tomaron los datos de los 5 minutos previos y posteriores a cada sesión, comparándose estos valores mediante el test de T-student para datos pareados. Ninguna de las variables de los dominios de tiempo (RRmedio, SDNN, rMSSD, pNN50) ni de frecuencia (VLF, LF, HF, LF/HF) de la VFC mostró cambios significativos ante ninguna de las dos situaciones. La HN no provocó modificaciones en los niveles de estrés de estos sujetos, siendo bien tolerada, clínica y electrocardiográficamente. Un test de tolerancia y una sesión de exposición a hipoxia normobárica intermitente no son estímulos suficientes para provocar cambios aqudos en la VFC.

#### Palabras clave:

Variabilidad de la frecuencia cardiaca. Hipoxia normobárica intermitente. Tolerancia a la hipoxia.

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#### Introduction

Heart rate variability (HRV) has been defined as the variation in the time interval between consecutive heartbeats, termed R-R intervals¹. HRV can be assessed through linear methods, analysed through time and frequency domains, and non-linear methods².³. In the time domain, the following statistical indices are highlighted: Mean R-R (mean of the R-R intervals); SDNN (standard deviation of the normal-tonormal intervals; RMSSD (root mean square of successive RR interval differences); pNN50 (total percentage of successive RR intervals that differ by more than 50 ms); and, in the frequency domain: VLF (very low frequency); HF (high frequency); LF (low frequency); LF/HF (ratio of low frequency to high frequency)⁴-6.

Traditionally, HRV has been used in the area of sports for the purpose, among others, of improving the adaptation or performance of athletes<sup>7</sup>. Apart from sport, it has other uses in the field of medicine, given that it has been used in different pathologies such as cardiac<sup>8</sup>, anxious depression<sup>9</sup> and currently in the COVID-19 disease as a potential acute inflammation marker <sup>10</sup> or as a method to detect the effects triggered by lockdown on this variable<sup>11</sup>.

Many and varied factors can influence heart rate and, therefore, they can also have an influence on the analysis of heart rate variability. These factors can be divided into intrinsic or extrinsic to the body. With regard to the former, the following are established: age (HR decreases with age)<sup>12</sup>, sex (in general, HR is greater in women)<sup>13</sup>, body position (HR is lower in the supine position)<sup>14</sup> and work-related stress<sup>15</sup>. With regard to extrinsic factors, of particular note are: humidity<sup>16</sup>, the time of day at which the measurement is taken (HR is higher in the early morning)<sup>14</sup>, ambient temperature<sup>17</sup> or the intake of substances such as caffeine<sup>18,19</sup>. One stimulus that has been little investigated is the influence of normobaric hypoxia (breathing air with a reduced oxygen fraction at constant atmospheric pressure) on HRV.

With increased altitude, the barometric pressure drops exponentially, giving rise to a progressive reduction in the partial pressure of oxygen ( $pO_2$ ) in the atmosphere, this type is referred to as hypobaric hypoxia<sup>20</sup>. However, with technological progress, altitude simulators have been developed that make it possible to reduce the amount of oxygen in the air without altering the atmospheric pressure, giving rise to what is known as normobaric hypoxia (HN)<sup>21</sup>.

Training in places with a reduced oxygen fraction has been a particularly important resource in the area of sport. It can represent a stimulus for the body, improving the hypoxia adaptation systems and, consequently, acting as a supercompensation mechanism<sup>22</sup>.

Depending on the Hypoxia exposure time, it is possible to differentiate between chronic exposure, made over long periods of time, and acute exposure and, within the latter, intermittent normobaric hypoxia (INH) which consists in applying periods of hypoxia followed by periods of normoxia (normal oxygen fraction).

The aim of this study is to determine the variations arising in the

domains of heart rate variability following exposure to normobaric hypoxia.

#### Material and method

This investigation is a prospective intervention study. A non-probability convenience selection method was used to select the participants. Each participant signed an informed consent prior to data collection. This consent stated the study objectives, the conditions in which the measurements would be taken, the confidentiality and security of the information obtained. Prior to the investigation, approval was obtained from the Ethics Committee of Investigation of the University of Murcia. In all cases, the study met the requirements of the Code of Ethics of the World Medical Association (Declaration of Helsinki) for trials with human subjects.

#### **Participants**

Our study comprised thirteen subjects (53.3% women). The age of the participants ranged between 20 and 29 years. They did not suffer from any prior cardiac or respiratory disease.

The inclusion criteria were as follows: 1) Express a willingness to participate in the study, having understood the scope of the same, the risk and benefits of the intervention, confirming this willingness to participate by signing the informed consent. 2) Be aged between 20 to 30 years. Excluded from the study were those with pathologies that contraindicated the testing; the presence of a body temperature of more than 37°C and/or a positive antibody test. 3) Have had the Covid-19 disease and, at the time of the study, have no negative PCR tests or not completing either of the two hypoxia tests for reasons not related to the tests.

#### Instruments

For the anthropometric study, the height (SECA 213°) and weight (In Body 120°) were recorded. The waist and thigh circumferences were obtained with a Holtain° flexible metal tape measure and a Lux° device was used to measure the blood cell count. The blood pressure and auscultation were taken with a conventional stethoscope and sphygmomanometer (Littmann Clasic°).

The tolerance test and the training session were conducted with a Hypoxia simulator, namely the iAltitude Trainer v2.7° connected to a specific mask. During both tests, all participants were monitored for muscle oxygenation through the Humon Hex° device in the anterior rectus muscle of the right thigh, for oxygen saturation with a pulse oximeter (Nonin°) in the left ear lobe, and heart rate variability with a Polar H10° heart rate monitor.

Furthermore, due to the special circumstances resulting from CO-VID-19, temperature was taken using the Yuwell® digital thermometer and the 2019-nCoV lgG/lgM®" antibody test was used to rule out the presence of the SARS-CoV-2 virus.

#### Prior procedure

After voluntarily signing the informed consent, each participant underwent the measurement of the different anthropometric parameters followed by a medical examination to rule out any alteration that could contraindicate the performance of the tests. The prior procedures and both hypoxia tests were conducted at the Biomedical Research Laboratory (LAIB) of the University of Murcia. The room temperature was always constant at 25 °C.

The body temperature was taken first, this was followed by the antibody test, the determination of the red blood cell count and the anthropometric study. The participant then completed a questionnaire on diseases and/or family history. This was checked by a physician through a direct interview.

Secondly, the blood pressure was taken in a supine position, the participant was auscultated and an electrocardiogram at rest was performed (Figure 1).

#### Performance of the hypoxia tests

Having checked for the absence of pathologies that could contraindicate the test, the participant was then subjected to breathing in thin air while continuously monitoring heart rate variability. A tolerance test was first performed to check the adaptation of the individual to hypoxia before conducting the hypoxia exposure session. In both tests, if the hypoxia simulator detected arterial oxygen saturation values of less than 83%, then it indicated the withdrawal of the mask through visual and audio signals, thereby breathing in normoxic conditions and recovering the normal concentration of oxygen.

For both tests, the participants were in a seated position in an armchair, allowing them to rest their heads and flex their hips and knees 90°. A foam rubber wedge was positioned to give correct back support, giving a comfortable and relaxed position (Figure 2). Participants wore a Polar H10° chest strap linked to a tablet by Bluetooth connection and data were recorded through the HRV elite° application.

Figure 1. Prior examination of a participant.



Figure 2. A participant performing a hypoxia test.



#### Hypoxia tolerance test

In the position described above, the subject remained seated and relaxed for 5 minutes before the start of the tolerance test to record their HRV at rest and in normoxia.

The tolerance test was then conducted. It consisted in continuously breathing in under hypoxic conditions (11% oxygen, equivalent to an altitude of 5,050 m) for a maximum time of 10 minutes. Once the test had ended, the subject remained seated in order to record the 5 minutes subsequent to the completion of the tolerance test.

Therefore, two measurements of the HRV were taken, both under normoxic conditions The first reading shows the five minutes prior to subjecting the subject to the hypoxia tolerance test while the second one records the five minutes subsequent to the completion of this test.

#### **Exposure session**

Once the tolerance test had been completed, the volunteer remained seated in the position described above in order to start the second hypoxia test. The heart rate variability was recorded during the 5 minutes prior to the test. When this time was up, the intermittent hypoxia exposure session commenced. The duration of the session was 64 minutes, with 14% oxygen (equivalent to an altitude of 3,250 m). The intermittent mode was applied. In other words, a period of hypoxia (4 minutes) was followed by a normoxia period (4 minutes) alternating until the completion of the 64-minute session. At the end of the session, the sunbsequent 5 minutes were recorded in order to analyse the HRV.

Therefore, two measurements of the HRV were taken, both under normoxic conditions The first record corresponds to the 5 minutes prior to the start of the hypoxia exposure session and the second record to the period immediately after completion of the session. Both records had a 5-minute duration.

#### Statistical analysis

Based on the data from the different computer applications and software, a spreadsheet was prepared using the Excel® program and, in turn, the data were analysed with the SPSS 24.0® statistical software.

The quantitative variables were described through the mean and standard deviation. The Coefficient of Variation was used to check the data dispersion. The homogeneity of variance was determined with the Levene test. Intra-individual comparisons were found through the paired T-test and the inter-group comparisons with the T-student test. Significant differences or relationships were considered to occur when p<0.05.

#### Results

The study population comprised 6 males and 7 females. Table 1 describes the characteristics of the population in general and separated by sex. Significant differences were observed (p<0.05) between sexes in the variables for height, weight and waist circumference, obtaining higher values in the male study population.

When comparing the HRV values prior and subsequent to the performance of the tolerance test, no significant differences were found in any of the domains: time (Table 2) and frequency (Table 3).

Table 1. Characteristics of the overall population and separated by sex.

Variable	Population	Mean	SD	CV (%)	t	Sig. (bilateral)
Age	Overall (n = 13)	23	2.58	11.22		
(years)	Males $(n = 6)$	22.0	1.26	5.73	-1.41	0.194
	Females (n = 7)	23.86	3.19	13.37		
Height	Overall (n = 13)	173.75	9.24	5.32		
(cm)	Males $(n = 6)$	182.18	4.97	2.72	6.129	0.000
	Females (n = 7)	166.51	4.25	2.55		
Weight	Overall (n = 13)	72.72	12.80	17.60		
(kg)	Males $(n = 6)$	83.21	5.27	6.33	4.270	0.001
	Females (n = 7)	63.72	10.01	15.71		
Waist	Overall (n = 13)	77.02	8.33	10.82		
C.(cm)	Males $(n = 6)$	83.21	4.99	6.00	3.411	0.006
	Females (n = 7)	71.71	6.82	9.51		
Hip	Overall (n = 13)	98.13	6.40	6.52		
C. (cm)	Males $(n = 6)$	101.11	4.69	4.63	1.667	0.124
	Females (n = 7)	95.57	6.87	7.19		
BMI	Overall (n = 13)	23.94	2.74	11.45		
(kg/m²)	Males $(n = 6)$	25.08	1.44	5.74	1.45	0.176
	Females (n = 7)	22.97	3.30	14.37		

Waist C.: waist circumference; Hip C.: hip circumference; CV: coefficient of variation; SD: standard deviation; Sig. (bilateral): bilateral signification.

Table 2. Differences in the time domain component of heart rate variability before and after the tolerance test (n=13).

Time domain variables	Mean	SD	Mean of the differences	SD of the mean	t	Sig. (bila- teral)
Start RRmean	869.35	143.16	4.20692	60.03661	0.253	0.805
End RRmean	865.14	109.06	4.20092	00.03001	0.255	0.803
Start SDNN	73.21	21.19	-6.59908	24.80853	-0.959	0.356
End SDNN	79.81	35.09	-6.59908	24.00033	0.555	0.550
Start RMSSD	50.40	23.73	1.11462	11.52709	0.349	0.733
End RMSSD	49.29	24.03	1.11402	11.32709	0.549	0.733
Start pNN50	26.09	19.76	1.56176	9.44434	0.500	0.562
End pNN50	24.53	19.70	1.301/0	9.44434	0.596	0.562

RRmean: mean of RR intervals; SDNN: Standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive RR interval differences; pNN50: percentage of successive RR > 50 ms: ST: standard deviation.

Table 3. Differences in the frequency domain component of heart rate variability before and after the tolerance test (n=13).

Frequen- cy domain variables	Mean	SD	Mean of the differen- ces	SD of the mean of the differences	t	Sig. (bila- teral)
Start VLF	2299.84	1664.02	-1866.11	5979.313	-1.125	0.282
End VLF	4165.95	5925.52	1000.11	3777.313	1.123	0.202
Start LF	2341.87	1972.07	-25.82077	1580.123	-0.059	0.954
End LF	2367.69	2039.74	-23.02077	1360.123	-0.039	0.934
Start HF	980.11	668.23	29.19838	249.249	0.422	0.68
End LF	950.91	716.12	29.19030	249.249	0.422	0.08
Start LF/HF	3.05	2.45	-0.3728	1.487	-0.904	0.384
End LF/HF	3.42	2.39	-0.3726	1,40/	-0.904	0.364

VLF: very high frequency; HF: high frequency; LF/HF: low frequency to high frequency ratio; LF: low frequency; SD: standard deviation

During the intermittent hypoxia session, no significant differences were obtained when comparing the values of the time domain

prior to the INH session with those obtained at the end of the same (Table 4). Neither were significant differences (p<0.05) found in the frequency domain (Table 5).

Table 4 Differences in the time domain component of heart rate variability before and after the Hypoxia session (n=13).

Variables	Mean	SD	Mean of the differences	SD of the mean of the differences	t	Sig. (bila- teral)
Start RRmean	854.55	106.70	36.47	58.58	2.157	0.054
End RRmean	891.02	120.05	30.47	36.36	2.137	0.054
Start SDNN	81.74	35.92	-1.73	18.81	-0.319	0.756
End SDNN	80.01	24.45	-1./3	10.01	-0.519	0.730
Start RMSSD	47.09	23.70	0.134	8.83	0.055	0.957
End RMSSD	47.23	17.47	0.134	0.03	0.055	0.957
Start pNN50	21.48	17.06	2.25	5.44	1.436	0.179
End pNN50	23.73	15.,72	2.25	J.44	1.430	0.179

RRmean; mean of the RR intervals; SDNN: Standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive RR interval differences; pNN50: percentage of successive RR > 50 ms; ST: standard deviation.

Table 5 Differences in the frequency domain of heart rate variability before and after the INH exposure session (n=13).

Variable	Mean	SD	Mean of the differen- ces	SD of the mean of the differences	t	Sig. (bila- teral)
Start VLF	3085.77	2073.66	349.61	3059.15	0.396	0.700
End VLF	2736.16	3362.20				
Start LF	2289.60	1708.52	-213.92	1068.16	-0.694	0.502
End LF	2503.52	2068.12				
Start HF	731.98	461.18	-146.22	482.91	-1.049	0.317
End HF	878.19	696.04				
Start LF/HF	3.43	2.62	-0.24	1.67	-0.505	0.623
End LF/HF	3.67	2.31	-0.24	1.07	-0.505	0.023

VLF: very high frequency; HF: high frequency; LF/HF: low frequency to high frequency ratio; LF: low frequency; SD: standard deviation.

#### Discussion

This study analysed the heart rate variability response following a tolerance test and a session with exposure to normobaric hypoxia. It has been demonstrated that hypoxia, at the altitude and temperature described, does not cause significant changes in the HRV domains for time and frequency.

The population of this study comprised 13 subjects, a size that is in concordance with that of other authors<sup>23-25</sup>. The same is true for the mean age of the subjects (23±2.58 years) being very similar to that described in other studies<sup>24,26</sup>. This could be due to the fact that, at present, there are few studies that analyse this phenomenon and, as a prior step, it was conducted on young, healthy individuals so that it will be possible to perform it in the future on other populations subject to greater risks, thereby ensuring that studies are conducted safely.

The altitude simulated and the exposure time used were different depending on whether a tolerance test was being conducted or a session with exposure to hypoxia. In our case, during the tolerance test, an altitude of 5,050 metres (11%  $O_2$ ) was simulated for a maximum period of 10 minutes, while Buchheit *et al*<sup>23</sup> subjected their participants to a slightly lower altitude at 4,800 metres (11.5%  $O_2$ ) during this test. However, the protocol used by these authors differs from ours, given that they alternated periods of hypoxia at rest with hypoxia during physical exercise, making a comparison with our study difficult.

## Heart rate variability in the time and frequency domains

We have verified, through other studies, how the HRV response can vary depending on whether it is: a short exposure<sup>23,25-27</sup> or more prolonged exposure to hypoxia<sup>28,29</sup>; the type of hypoxic administration, in other words, gradually<sup>29-31</sup> or suddenly<sup>23,25</sup>; the altitude intensity: high<sup>23,29,31</sup>, moderate<sup>24</sup> or low<sup>30</sup>; as well as the different types of hypoxia used: normobaric<sup>23,26</sup> or hypobaric<sup>31,25</sup>, given that the body's response to each one is different and makes comparison difficult. For this reason, it is important to determine which duration, intensity and type of hypoxia favourably influence HRV

Some authors agree that exposure to normobaric hypoxia induces a decrease of the RMSSD and an increase in the LF/HF ratio, in other words, a greater activation of the sympathetic nervous system <sup>25,32</sup> while other authors uphold an increase in the parasympathetic nervous system <sup>24,31</sup>. However, the tolerance test conducted in our study showed no significant differences in the HRV time and frequency domains. This finding could corroborate the point that other authors are suggesting, in that, in order to induce significant changes in some domains, such as the LF/HF ratio, a prolonged duration of at least 30 minutes is necessary<sup>33</sup>. However, Botek *et al*<sup>26</sup> specified a 10-minute exposure, but with the difference that their participants were subjected to an altitude of 6,200 m (9.6% O), which was higher than the one described in the literature. These authors<sup>26</sup> obtained a decrease in LF and an increase in HF.

Therefore, it appears that, while in 2001 Bernardi *et al*<sup>33</sup> indicated that a 30-minute exposure to hypoxia was necessary to generate changes in the HRV domains, years later Botek *et al*<sup>26</sup> managed to influence the HRV domains by maintaining acute exposures (10 minutes) while increasing the altitude stimulus. For this reason, our study demonstrates that an altitude of 5,050 metres for 10 minutes is not sufficient to affect the HRV, meaning that it would be necessary to further reduce the amount of oxygen inspired.

During the training session, the exposure time was more prolonged (64 minutes) than for the tolerance test, yet reducing the altitude and applied intermittently (intermittent normobaric hypoxia). The 3,250 metres at which the second hypoxic test was conducted in this study may be insufficient to generate changes in heart rate variability despite the fact that exposure duration was longer. In this way, these findings are in line with Yamatho  $et\ al^{34}$ , who also found no significant changes in their study. These authors a hypothesis which supports the findings of our present study.

Some studies used exposure to hypoxia simultaneously with the practice of physical exercise<sup>34,35</sup>. In this case, there does appear to be more consensus with regard to a greater variation in the absolute time and frequency domains than if hypoxia is administered at rest. However, this may be due to the actual stimulus of physical exercise and not to hypoxia. For this reason, we consider that there is a need to clarify what times and altitudes are necessary in order to generate a beneficial effect on heart rate variability before synchronously including a further stimulus such as physical exercise.

#### Conclusion

Heart rate variability shows no significant changes neither in the time domain nor in the frequency domain following exposure to normobaric hypoxia. We therefore consider that these stimuli were not sufficiently stressful to cause acute changes.

#### Conflict of interest

The authors have no conflict of interest at all.

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- Gestión Integral del Riesgo Cardiovascular 22
- · Ingeniería Biomédica (1)
- Investigación en Ciencias Sociosanitarias ②
- · Investigación en Educación Física y Salud ②
- Neuro-Rehabilitación @
- Nutrición Clínica (1)
- · Nutrición y Seguridad Alimentaria ②
- Nutrición en la Actividad Física y Deporte w
- · Osteopatía y Terapia Manual (2)
- · Patología Molecular Humana ②
- Psicología General Sanitaria (1)

(1) Presencial (2) Semipresencial





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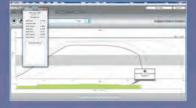




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