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Sumario / Summary

Sports Medicine in times of COVID

La Medicina del Deporte en tiempos del COVID

Pedro Manonelles

Presidente de la Sociedad Española de Medicina del Deporte.

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December 2019, and the Chinese city of Wuhan will not easily be erased from our memory, given that this is the starting point of the COVID-19 pandemic, a type 2 acute respiratory syndrome caused by the SARS-CoV2 virus.

In Spain, the first case was confirmed on 31st January 2020 in the island of La Gomera and the first death occurred on 13th February in Valencia[1].

At this stage, the way of life of all citizens has had to adapt to this unexpected circumstance brought by fate, and Medicine and Sports Medicine are not external to this. Furthermore, at the time of writing this editorial, the pandemic is far from being under control.

The second outbreak of the pandemic, with a heterogeneous distribution, is characterised by its extensive spread, in many cases through certain groups, young people, leisure gatherings, many of a family nature and different types of crowds.

Although mortality has decreased and treatment appears to be more effective, the clinical picture frequently continues to be serious, and frequently lethal, with long stays in the ICU. On the other hand, the after-effects and complications of the disease are numerous and extremely important in many cases and this also affects athletes, even asymptomatic athletes, in which cardiomyopathies and other problems have been described, making it necessary to remain extremely vigilant[2].

Role of Sports Medicine in the context of the pandemic

The role of Sports Medicine, represented by the Spanish Society of Sports Medicine (SEMED) is, at all times, to care for athletes and to help and protect the doctors working in this discipline.

Doctors, out of conviction and due to the very nature of their profession and their legal remit[3], have the inexcusable obligation to "provide appropriate technical and professional healthcare to their patients", both from a therapeutic and preventive point of view. They are required to perform this duty even when this involves decisions that are not to the liking of patients or the population. To give a typical case, that of the amputation of a limb due to gangrene.

The Spanish Society of Sports Medicine, by virtue of its responsibility, has progressively taken decisions that are always in line with the situation of the pandemic. It has given an answer to citizens and professionals alike with regard to those matters that, due to its experience and knowledge, come within its competence. At the time of writing this editorial, the SEMED had published a total of eleven documents (announcements, action guidelines, recommendations and advice) specifically related to infection from the SARS-CoV2 virus, including matters concerning training, health promotion through physical activity, nutrition, sports practice, professional exercise and protection measures[4].

The severe consequences of the pandemic has made it necessary to adopt measures directed at preventing the germ from spreading and the subsequent risk of infection. For this reason, the SEMED, through a group of experts from Society and with the close collaboration of the Consejo General de Colegios Oficiales de Médicos (General Council of the Official Association of Doctors), has been working on the preparation of the aforementioned documents.

There have been controversial recommendations related to the very nature of sports activities. Closeness and contact between athletes, rivals and also team mates, is inevitable in combat sports, team sports and racket sports (such as squash and padel for example), but also in individual sports in which closeness between athletes is a constant factor, as is the case of a pack of cyclists and long-distance runners, in canoeing and rowing (in team specialities), to give but a few examples. This closeness and contact promotes the transmission of the virus.

The other factor inherent in sport is ventilation. It was necessary to conduct an information campaign in order to explain the significance

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of ventilation, based on the location of a person at any given time, a phenomenon that is well known among sports doctors. For a normal adult, ventilation at baseline conditions is around 5-7 litres per minute. However, depending on the effort made, this respiratory flow not only increases with regard to the distance travelled by the exhaled air but also in volume, reaching 150-180 litres per minute for amateur athletes and up to 200-250 litres per minute for top level resistance athletes.

This fact has two extremely significant consequences. The first is that the social distancing recommendation of 1.5 metres is absolutely insufficient for sports activities. The second is the large amount of accumulated air that is breathed in by people in enclosed spaces in which sport or any other physical activity is practised, with little ventilation and the simultaneous presence of many people. If anyone is infected, even asymptomatically, then this will contaminate the air breathed in by this group and the disease will be transmitted.

In Sports Medicine it is well known that there is an intense focus on prevention (of injuries, of sudden death, etc.) and this is becoming the norm in daily professional practice. Evidence of this is the fact that a number of documents have been issued, setting out extensive measures to prevent the disease. For this reason, a recommendation was made, namely the use of face masks, which has created discontent in some sport sectors.

It is clear that the use of surgical face masks is a very important measure in the context of sports activities. In this way it is possible to limit the disease transmission and infection and, therefore, the use of face masks is recommended in non-professional and amateur sports.

Despite the fact that this pandemic has only recently appeared, scientific material is gradually becoming available and which serves to support the decisions that are being taken. With regard to the use of face masks, it can be said that no health risks have been found with regard to their correct, although continuous use. Although there is little evidence available on the matter, a study conducted on healthy adults[5] comparing the cardiorespiratory, metabolic, performance and comfort parameters in maximum effort tests while wearing either a surgical or FFP2 mask compared to that observed when no face mask was used, found that the use of FFP2 face masks considerably reduced practically all the parameters studied, while the use of surgical face masks only affected some comfort parameters (heat, breathing resistance, itching, pressure, discomfort). The SEMED has initiated a similar study on athletes.

Although the SEMED has been criticised for making this recommendation, it is not the duty of doctors and their representative entities to either recommend or fail to recommend anything that is not acceptable to some members of society. Instead, based on their experience, evidence and responsibility, it is their duty to advise on what they consider to be the best way to protect health at all times.

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Original article

Comparison of quadriceps muscle activation in exercises with different duration of concentric and eccentric contractions

Gisele Freire da Silva[1,2], Frank Douglas Tourino[1], Rodrigo César Ribeiro Diniz[1], Lucas Túlio de Lacerda[1], Hugo César Martins Costa[1,3], André Gustavo Pereira de Andrade[1], Mateus Camargos Gomes[1], Mauro Heleno Chagas[1], Fernando Vitor Lima[1]

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Summary

Aim: The objective of the present study was to compare the amplitude of the electromyographic (EMG) signal of the quadriceps muscle portions vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF) and the activation ratio (VM/VL, VM/RF and VL/RF) in protocols with different durations of concentric and eccentric muscular actions.
Material and method: Twelve female volunteers performed the knee extensor exercise with two different protocols [1s for concentric muscle action and 5s for eccentric muscle action (1:5); 5s of concentric muscle action and 1s of eccentric muscle action (5:1)] and 3 sets of 6 repetitions, 180s of pause between each sets and a intensity of 50% of 1RM. The root mean square of the amplitude of the normalized EMG signal was calculated for each repetition in each series.
Results: it was observed an increase in the activation of the VM and VL portions in equivalent repetitions of each series and for the VL portion, the 1: 5 protocol provided greater activation compared to the other protocol. No differences were found for muscles activation ratios VM/RF and VL/RF, being that for the VM/VL ratio there was only change at one repetition.
Conclusion: The results suggest that the portions of the quadriceps muscle may present different EMG responses in similar protocols, but this fact may not interfere in the synergism between them. The reduced degrees of freedom of the knee extension exercise and the characteristics of the protocols adopted may be the elements that contributed to the limited alterations that occurred in the present study.

Key words:
Resistance Training.
Electromyography.
Quadriceps muscle.

Comparación de la activación muscular del cuádriceps en ejercicios con diferente duración de las contracciones concéntricas y excéntricas

Resumen

Objetivo: El objetivo del presente estudio fue comparar la amplitud de la señal electromiográfica (EMG) de las porciones de músculo cuádriceps vasto medial (VM), vasto lateral (VL) y recto femoral (RF) y la relación de activación (VM/VL, VM/RF y VL/RF) en protocolos con diferentes duraciones de acciones musculares concéntricas y excéntricas.
Material y método: Doce mujeres voluntarias realizaron el ejercicio extensor de rodilla con dos protocolos diferentes [1 s para la acción muscular concéntrica y 5 s para la acción muscular excéntrica (1: 5); 5s de acción muscular concéntrica y 1s de acción muscular excéntrica (5: 1)] y 3 series de 6 repeticiones, 180s de pausa entre cada serie y una intensidad del 50% de 1RM. La raíz media cuadrática de la amplitud de la señal electromiográfica normalizada se calculó para cada repetición en cada serie.
Resultados: se observó un aumento en la EMG de las porciones de VM y VL en repeticiones equivalentes de cada serie y para la porción de VL, el protocolo 1: 5 proporcionó una mayor activación en comparación con el otro protocolo. No se encontraron diferencias para las relaciones de activación de los músculos VM/RF y VL/RF, siendo que para la relación VM/VL solo hubo cambios en una repetición.
Conclusión: Los resultados sugieren que las partes del músculo del cuádriceps pueden presentar diferentes respuestas de EMG en protocolos similares, pero este hecho puede no interferir en el sinergismo entre ellos. Los grados reducidos de libertad del ejercicio de extensión de la rodilla y las características de los protocolos adoptados pueden ser los elementos que contribuyeron a las alteraciones limitadas que se produjeron en el presente estudio.

Palabras clave:
Entrenamiento de fuerza.
Electromiografía.
Músculo cuádriceps.

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Introduction

The vastus medialis (VM), vastus lateralis (VL) and rectus femoris (RF) are portions of the quadriceps that act synergistically to produce knee extension torque for many movements[1] and knee extensor equipment has been used both for training and for scientific research on electromyographic activation (EMG) of this muscle group in different muscle strength performances. This equipment limits the movement in its trajectory due to its rigid axis, allowing the body segment to only move in one direction, unlike other equipment such as free bars or Smith machine where knee extension is also performed.

Pincivero *et al.*[2] evaluated the EMG activity in a single set of knee extension at 50% of one repetition maximum 1RM with the maximum number of repetitions and verified increased activation in the concentric action without differences between portions. In the eccentric action, these authors recorded an overall reduction in activation but the activation of the VL remained greater than the other portions. Akima and Saito[1] observed a gradual increase in the EMG with time, similar to the concentric action of the study by Pincivero *et al.*[2], while Rabita *et al.*[3] found an increase only in RF activation compared to the control group after four weeks of isometric training. Matheson *et al.*[4] verified that different velocities produced different EMG of RF, VL and vastus medial oblique (VMO) in multiple series of isokinetic knee extension. The results of Hatzel *et al.*[5] suggest the influence of repetition duration on quadriceps activation, with greater activation at 60º/s compared to 15º/s on eccentric action. Yavuz *et al.*[6] pointed that different magnitudes of external load can result in different activations in the comparision between quadriceps muscle portions and Ema *et al.*[7] recorded different EMG activations for RF at leg press and knee extensor exercíses compared to VM and VL, suggesting alterations in the synergism.

Despite these different responses in studies, the results of Ribeiro *et al.*[8] point out that when demand increases on quadriceps it is shared between the portions in synergistic action and Laine *et al.*[9] showed that the VM and VL share the neural drive when activated synergistically.

The analysis of the activation ratios (VM/VL, VM/RF, VL/RF) can help to understand the relative contribution of each quadriceps portion compared to the others, allowing to verify how the nervous system strategy can alter synergism in the production of a specific knee extension performance. Coqueiro *et al.*[10] indicate that patellar misalignment may be due to dysfunction of VMO compared to VL (VMO/VL) and other studies have suggested that changes in the activation ratio (VM/VL)[11,12,4] may favor the onset of Patellofemoral Pain Syndrome, where individuals presenting the syndrome have a lower VMO/VL ratio than healthy individuals[13,14].

Wong and Ng[11] verifyed an increase in the VMO/VL ratio in both a low intensity and high volume training protocol, as well as in a higher intensity and lower volume training protocol, with no difference between them. Beyond this, VMO activation started before VL after the training period, a fact that did not occur in the pre-training period. Other studies[4,15,16] found higher values for the VM/VL ratio with the increase in angular velocity, suggesting the interference of repetition durations; however, the methodological differences between the studies, such as the type of exercise used, limit the comparisons.

The results available keep open the different possibilities of investigating how the portions cooperate to produce movement in response to different manipulations. In addition, studies have been investigating equipment that apply external resistance in different ways and care should be taken with the transfer of results between them, for example, knee extensor and squat or leg press, due to the differences in degrees of freedom and allowed movement trajectories.

Therefore, analyzing the EMG responses of the quadriceps portions in protocols performed with different durations of muscular actions for the same repetition duration, will allow to increase the understanding of how they act synergistically in order to produce muscular strength in weight training equipments. Therefore, the present study aimed to analyze the amplitude of the electromyographic signal of the VM, VL and RF and the activation ratios VM/VL, VM/RF and VL/RF in protocols with multiple series, equal repetition duration and different durations of muscular actions in knee extension exercise.

Material and method

Sample

A experimental design of repeated measurement was used, with 12 female university students (age 21.4 ± 3.6 years, body weight 55.9 ± 7.3 kg, height 1.62 ± 0.07 meters), participating in recreational physical activities in the last six months and absence of musculoskeletal lesions in lower limbs, spine and pelvis. To participate in the study, volunteers should perform the 1RM test with, at least, 25 kg. This value refers to twice the resistance offered by the movable structure of the equipment (support of washers, mechanical arm, camus). This procedure ensured that they all performed training protocols with the determined intensity of 50% of 1RM.

The volunteers were instructed not to perform physical activities on the days of testing sessions, and on the day before the same. The sample calculation used GPower software (version 3.1.7). It was used the design of repeated measurements (ANOVA Repeated measures, within interaction), an alpha error of 0.05, a power of 0.8, a correlation between the repeated measures of 0.73 and a correction of non-sphericity of 1, considering the 2 experimental groups (1: 5 and 5: 1) and the 3 measures (equivalent repetitions: 1, 7 and 13, etc). For the effect size variable a value of 0,37 was, obtained through the study of Pincivero *et al.*[2], in the data of the rectus femoris muscle that obtained the highest coefficient of variation (CV). Through this information the software determined a sample size of 10 individuals. The study was approved by the local Research Ethics Committee.

Procedures

A knee extensor machine (Master Equipment®) was adjusted to a 110 degree angle between the backrest and the seat. A belt was placed near the iliac crest in order to minimize accessory movements in the hip. Fixed to the axis of rotation of the equipment, a linear potentiometer of 10 kΩ, with linearity error of 2%, voltage range of +10V to -10V, allowed the measurement of the angles in the equipment. To perform the Maximum voluntary isometric contraction (MVIC) at each joint angle, a manual system was used to lift the support with weights up to the height that corresponded to the desired angle in the equipment, determined by the potentiometer.

An experimental design of repeated measurements was used and the volunteers attended 4 days at the laboratory separated by a minimum of 48 hours and a maximum of 96 hours. All testing sessions took place at the same time of the day for each volunteer.

In session 1, the volunteers were positioned with the hip at an angle of 110º, the lateral epicondyle of the femur aligned with the potentiometer and the distal support of the equipment 3 cm above the medial malleolus[17]; this positioning was maintained in all sessions. The 1RM test followed the guidelines of Diniz *et al*.[18], with a maximum number of six attempts, five-minute pause between each attempt and gradual weight progression. The onset of concentric action was at 100º of knee flexion and the weight was progressively increased until it was not possible to reach 30º of knee flexion (0º = extended knee) in the concentric action. Ten minutes after the 1RM test, familiarization with the duration of muscle actions was performed in a series of 6 repetitions without additional weight. In session 2, the 1RM test was performed again and after 10 minutes another familiarization with the durations of muscular actions was performed.

In sessions 3 and 4, the MVIC test was performed and the EMG signal of VM, VL and RF was recorded. The electrodes on the vastus medialis were placed at 80% on the line between the upper anterior iliac spine and the joint space in front of the anterior border of the medial ligament. On the vastus lateralis the locaction was 2/3 on the line from the upper anterior iliac spine to the lateral side of the patella, and on the rectus femoris the electrodes were placed at 50% on the line from the upper anterior iliac spine to the superior part of the patella; these are the recommendations of Surface electromyography for the non-invasive assessment of muscles (SENIAM)[19]. After the placement of the electrodes, a semi-permanent pen was used to draw the edges on the patella (reference electrode) and muscles, so as to allow replication in the subsequent sessions. The electrical activity of the muscles was recorded using a surface electromyography equipment (Biovision, Germany), with the electrodes configured with a gain of 1000 times. The electromyograph information was synchronized and converted into digital signals by an A / D card (Biovision, Germany) with an input range of -5 to +5 Volts and directed to a computer. For the acquisition and treatment of the signals, a specific program (Dasylab 11.0, Ireland, Dasytech Laboratories, 12 bits), calibrated with 2000 Hz sampling frequency was used. Ag / AgCl type surface electrodes (Kobme Bio Protec, Korea), with a capture area of approximately 1 cm^2, were positioned in the direction of the muscle fibers according to the recommendations of SENIAM[19].

Before the electrodes were placed, the area of the skin was trichotomized, hygienized with alcohol and cotton, rubbing heavily the cotton in the place in order to guarantee the cleanliness and a reduction of the impedance of the skin[20]. The electrodes were positioned in pairs with a center-to-center distance of 2 cm.The MVIC test was performed with two 5s trials at angles of 30º, 50º, 70º and 90º of knee flexion (knee extended = 0º) and pause of three minutes between each angle and each attempt. The volunteers performed maximum force against the arm of the equipment that was fixed so that it could not be moved.

Table 1. Reliability of EMG measurements in the MVIC test.

Portion	Protocol	Mean ± SD (mV)	ICC $_{(3.1)}$	SEM (mv)
Vastus medialis	1-5	0.82 ± 0.40		
	5-1	0.75 ± 0.28	0.65	0.25
Rectus femoris	1-5	0.95 ± 0.28		
	5-1	0.95 ± 0.28	0.87	0.21
Vastus lateralis	1-5	0.68 ± 0.28		
	5-1	0.69 ± 0.24	0.90	0.20

Standard deviation (± SD); ICC - intraclass correlation coefficient; EPM – standard error of measurement. N = 12.

The MVIC was used as a reference for normalization (normalization test). Initially, a smoothing of the electromyographic signal of MVIC of each muscle was performed in each attempt at the angles of 30 °, 50 °, 70 ° and 90 ° through movable windows using the root mean square (RMS). EMG normalization considered the position with the highest mean value of the two trials at the respective testing session. The potentiometer data was filtered with a lowpass filter of 10 hz and EMG with a 20-500hz band pass filter, 2nd order Butterworth type. Electromyographic activity was processed in the time domain with DASYLAB 11.0. The RMS of the EMG signal during the protocols was extracted and the values were divided by the reference value previously described, resulting in normalized percentages of EMG. The reliability of the MVIC measures for each portion was determined by intraclass correlation coefficient (ICC) and standard error described in Table 1.

Protocols

After the MVIC, one of the protocols was performed, which consisted of 3 sets of 6 repetitions at 50% of 1RM, with pauses of 180s between sets. These values are in accordance with those recommended for muscle hypertrophy training[21] and muscular actions times used were the same ones investigated by Goto *et al*.[22]:

- 1: 5; concentric muscle action of 1s and eccentric of 5s.
- 5: 1; concentric muscle action of 5s and eccentric of 1s.

The order of the protocols was balanced.

Statistical analysis

Statistical packages SPSS 20.0 and SISVAR were used for data analysis and the significance level adopted was P <0.05. The EMG analysis was performed between the equivalent repetitions of the series, with 1, 7 and 13 being the first repetition of each series; 2, 8 and 14 the second repetition of each series and so on. The first repetition was also compared with the last one in each series. Initially, a descriptive analysis of the data was performed. All variables passed the normality test (Shapiro-Wilk) and homogeneity of variances (Levene). A two-way ANOVA with the protocol and repetiton factors with repeated measures was used to compare the EMG mean values for the equivalent repetitions of each series in RF, VM and VL and the ratios VM/VL, VM/RF and VL/RF between the protocols and between the repetitions. When necessary, the Bonferroni post hoc was used to locate the differences.

To reflect the magnitude of the differences in each treatment the eta square was calculated by dividing the square sum of the effect by the total sums of squares. According to Cohen (1988), one can consider η 2 = 0.140 as large, η 2 = 0.060, as mean and η 2 = 0.010 as small.

Results

The durations of the concentric and eccentric muscular actions in the 1:5 and 5:1 protocols were respectively: 1:5 (1.06 ± 0.20 CONs, 4.95 ± 0.61s EXC) and 5:1 (4.56 ± 0.31 s CON, 1.41 ± 0.33 s EXC).

In this study, 18 comparisons were performed between the equivalent repetitions in each series, with repetitions 1 to 6 located in the first series, 7 to 12 in the second and 13 to 18 in the third series. In all series, muscle EMG in the last repetition was greater than in the first one.

For the VM portion it was observed significant main effect only for the repetition factor (F 17.187 = 64,384, *p* <0.001, power = 1.000; effect size = 0:40), showing an increase in the activation of the equivalent repetitions in some repetitions for both protocols. There were no differences between the protocols (Figures 1 and 2).

For the VL portion, it was verified main effect of the protocol ($F_{2,22}$ = 5,317; *p* < 0.019; power = 0.721; effect size = 0.15) and repetition ($F_{17,187}$ = 57,594; *p* < 0.001; power = 1,000; effect size= 0.37). The 1:5 protocol had higher activation compared to the 5:1 protocol and there was an increase in activation in some equivalent repetitions in both protocols (Figures 3 and 4).

For the RF portion, a significant interaction protocol x repetition ($F_{34,374}$ = 2,200; *p* < 0.05; power = 0.775; effect size = 0.02) was verified, showing that changes in muscle activation were different for repetitions and series between protocols (Figures 5 and 6).

For the VM/VL ratio it was verified significant effect of repetition ($F_{17,187}$ = 1746, *p* < 0.05, power = 0.523, effect size = 0.01). Only the repetition 2 of the second series showed an increase in EMG ratio to its

Figure 1. Normalized EMG (% MVIC) in the vastus medialis portion. @ series 1 different from series 3; $ series 2 different from the series 3; & the first repetition lower than the last repetition of each series.

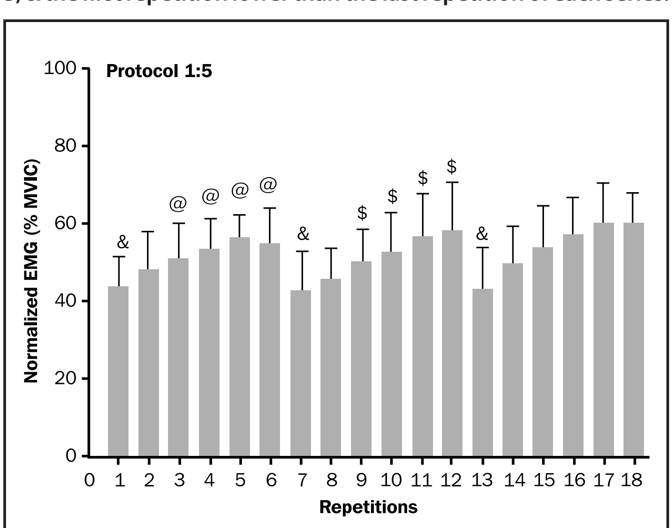


Figure 2. Normalized EMG (% MVIC) in the vastus medialis portion. @ series 1 different from series 3; $ series 2 different from the series 3; & the first repetition lower than the last repetition of each series.

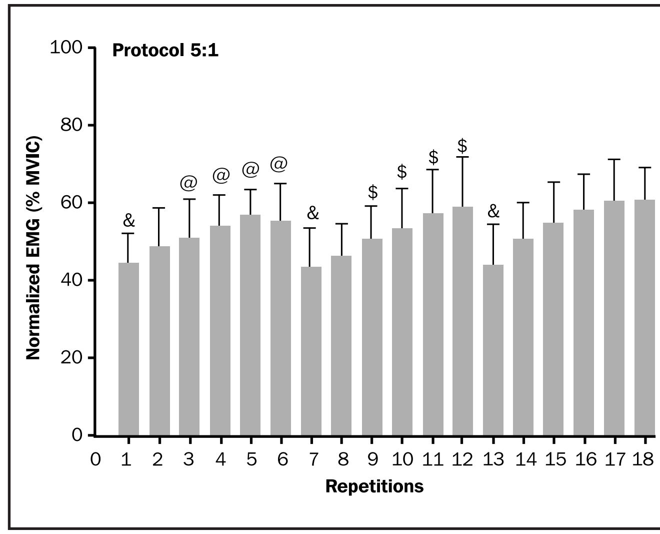
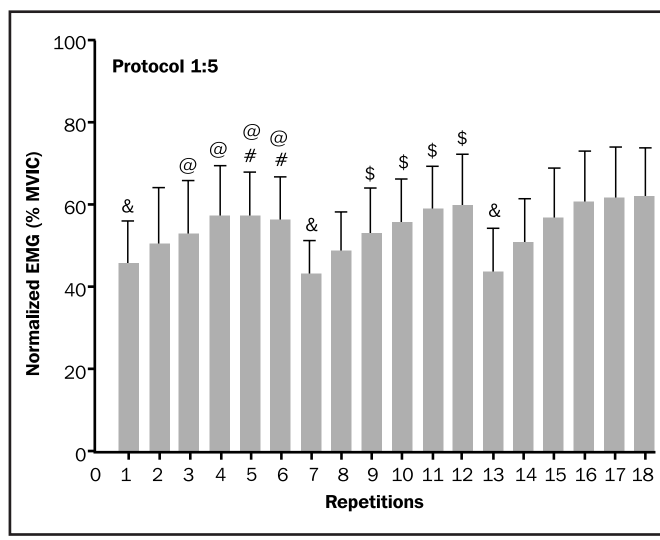


Figure 3. Normalized EMG (% CIVM) in the vastus lateralis. @ series 1 different from series 3; $ series 2 different from the series 3; # series 1 different from series 2; & the first repetition lower than the last repetition of each series. * 1: 5 protocol greater than 5: 1.



equivalent in the third series, with no difference between the protocols. The VM/RF and VL/RF ratios did not present significant differences.

Discussion

This study investigated VM, VL and RF EMG in protocols with differences in muscle actions time. Differences were observed when comparing initial and final repetitions and the equivalent repetitions in second half of each series, with an increase in EMG from the beginning

Figure 4. Normalized EMG (% CIVM) in the vastus lateralis. @ series1 different from series 3; $ series 2 different from series 3; # series 1 different from series 2; & the first repetition lower than the last repetition of each series.

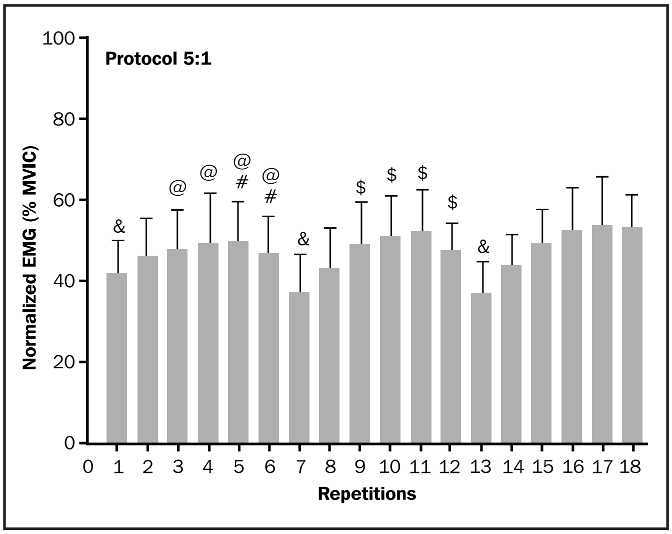
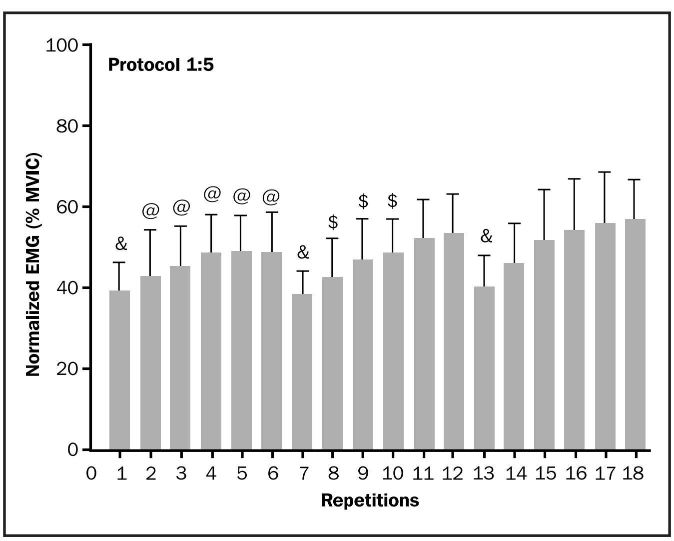
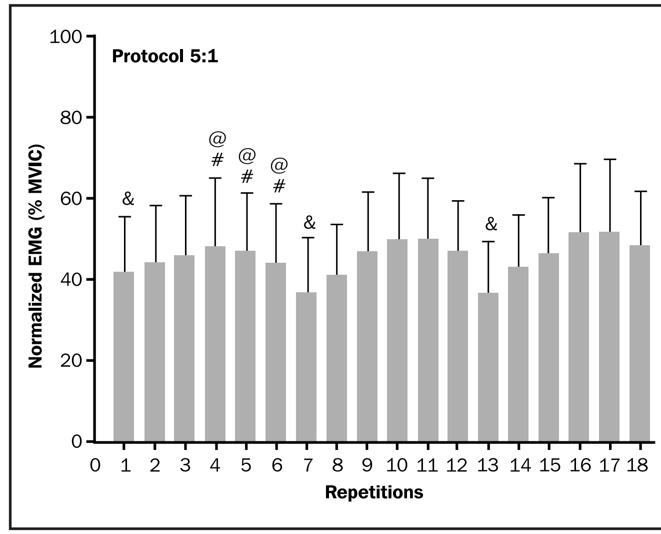


Figure 5. Normalized EMG (% MVIC) in the rectus femoris. @ series1 different from series 3; $ series 2 different from series 3; # series 1 different from series 2; & the first repetition lower than the last repetition of each series.



to the end of series. Walker *et al*.[23] also observed an increase in the EMG amplitude along the repetitions for the VM and VL, with higher EMG for repetition 8 compared to repetition 2 in all series analyzed; Pincivero *et al*.[2] found that the portions of the quadriceps presented an increase in EMG between 10 and 20%, 20 and 30%, 40 and 50% of total time spent at single series and Akima and Saito[1] also verified larger EMG in the final repetition compared to the initial one at the knee extension exercise with intensities of 50 and 70% of 1RM and repetitions until fatigue.

Figure 6. Normalized EMG (% MVIC) in the rectus femoris. @ series1 different from series 3; $ series 2 different from series 3; # series 1 different from series 2; & the first repetition lower than the last repetition of each series.



These results may reflect a characteristic of strength training, where this increase in muscle activation can be explained by the higher recruitment of motor units to produce the force required to complete the task along the protocol. However, other factors may influence the EMG amplitude as the firing frequency and/or increased synchronization of motor unit activation. On the other hand, Burd *et al*.[24] verified a differential increase of the EMG throughout the series between two repetition durations in the knee extensor, being that these responses were common to the VM, VL and RF, differently from our study. However, these studies did not analyze the EMG in the equivalent repetitions between series, and this was a different analysis carried out in the present study that allows adding an additional understanding of the recruitment of synergistic musculature for the production of strength.

In the present study, the results showed different activations for VM, VL and RF. An increase in EMG was observed in some equivalent repetitions between the 3 series for both protocols for the VM. For VL, the EMG for the 1:5 protocol was higher than the 5:1 protocol and the change in EMG occurred differently among protocols for RF The 1:5 protocol had a greater EMG response than the 5:1 protocol for VL and it may be related to the need to produce larger peak forces to accelerate the weight with the concentric duration of 1s. The high effect size (0.15) and the results of the study by Sakamoto and Sinclair[25] reinforce this explanation. These authors verified higher EMG in concentric actions when shorter repetition durations were performed. However, it is not possible to determine why this difference occurred only in VL in our study. No differences were observed between the protocols for the VM, and for the RF it was verified protocol x repetition interaction, showing that the differences did not occur in the same equivalent repetitions in the series for each duration of the muscular actions. However, the effect size for RF was small (0.02), which reinforces the need for caution in highlighting these difference. Besides this, taking into account that the

RF is biarticular, such characteristic has been considered when analyzing muscle activation in the knee exensor. Cramer *et al*.[26] investigated the impact of eight different velocities of movement and observed a differentiated behavior for RF, suggesting that this result may be due to differences in fiber composition and muscle architecture between portions. Using isometric knee extension, Rabbita; Pérot; Lensel-Corbeil[3] showed an increase only in the RF activation after 4 weeks and justified this difference by its biarticular condition. Ebenbichler *et al*.[27], verified a differentiated increase in activation between the portions of quadriceps in protocols until fatigue; the VM and the VL presented increased EMG throughout the task, while the RF presented a slight increase in intensities 30 and 50%, but registered decrease with 70% of the MVIC. According to these authors, the neurophysiological control of synergism between mono and biarticular muscles can be mediated by different mechanisms of neural control in the central nervous system. There is a suggestion that the differences between the mono and bi-articular muscles may be related to the presence of two different groups of neurons in the cerebral cortex, one that encode positions[27] and the other that encodes the direction of the resulting force[28]. Ebenbichler *et al*.[27] suggested that the organized control of different muscles acting synergistically would involve the group of neurons that control the direction of the resulting force mainly for the monoarticular muscles and both types of neurons would act for the biarticular ones; this author also point out that a monoarticular task such as knee extension would be able to stimulate these two groups of neurons, which would justify the differentiated EMG behavior of RF.

The study by Matheson *et al*.[4] found that at 60º/s the RF was more activated than VL and VMO, at 180º/s there was no difference and at 300º/s VL and VMO activated more than RF. The authors justify these differences mainly by the absence of a thigh strap for hip stabilization and also by the fact that RF is the only biarticular portion. In the present study the volunteers were stabilized in the hip region with a belt attached to the seat to minimize accessory movements such as hip flexion and/or extension that could alter both the length and activation of the RF. However, there may have been a force application on the trunk near the back of the seat, resulting in some subtle alteration in hip joint positioning. Ema *et al*.[29] analysed VM, VL and RF activation at knee extension in fully extended and 80º hip flexion and no effect of hip position were observed on muscles activation, but higher activation was present at faster velocity (180º/s vs 80º/s).

The analysis of the activation ratios showed that the durations of the muscular actions adopted altered only the repetition 2 of the second series for VM/VL when compared to its equivalent in the third series, with no difference between the protocols. For the VL/RF and VM/RF, no significant differences were found. Despite the increase of the EMG verified, the synergism was not altered. To the best of our knowledge, no study investigated the VM/RF and VL/RF ratios. The studies by Matheson *et al*.[4], Sczepanski *et al*.[15] and Yoo[16] investigated the impact of different velocities and suggest that higher velocities may produce higher values on the VM/VL ratio. The results of Wu *et al*.[30] showed that the VMO/VML activation ratio was higher than 1 at the standing unilateral knee extension, with no significant differences at nine joint ranges of motion. Other studies reported a lower VMO/VL value in individuals with patellofemoral pain syndrome (PFPS)[12,13] compared to healthy individuals[12,13] and an increase in this ratio would imply a possible increase in patellar medialization[11], which would lead to a better distribution of the compressive forces acting on the patellar femoral joint, improving the pain symptoms

Considering the available literature, it should be emphasized that many of the results have been obtained in isokinetic equipment that does not reproduce the conditions of the resistance applied by traditional weight training equipment.

Taking into account the volume of analyzes performed, it can be pointed out that only a small number of differences were detected in EMG between the portions of the quadriceps analyzed and this can also be attributed to the characteristic of the exercise and equipment used in the experiment. The knee joint can be described as a hinge joint between the femur and the tibia and flat between the femur and the patella, and it acts on a single plane, the sagittal; this configuration does not allow a variation of the trajectory of the movement, due to the limited degrees of freedom. These degrees of freedom of the movement can not be altered by the different durations of the muscular actions adopted, not leading to changes in the characteristics of the movement that resulted in a differentiated drive despite the architectural differences between the portions analyzed, so, the synergism between the portions of the quadriceps is maintained in a stable manner. Each portion will perform its task with no possibility of changes in the trajectory of the movement that could be influenced by the durations.

Conclusions

The present study concluded that multiple series protocols with different durations of muscle action may result in a differential increase in the EMG response between the quadriceps portions. The results also indicate that despite this differentiated increase between the portions, the activation ratios between did not change, suggesting that these changes in EMG were not sufficient to alter the synergism between the portions. However, the number of differences verified was limited by the volume of comparisons. The reduced degrees of freedom of the exercise and the characteristics of the protocols may be the elements that contributed to the limited alterations that occurred in the present study.

Limitations of study

Through these results, it is not possible to suggest precise directions for the prescription of the training that guarantees a greater selective activation of some portion of the quadriceps with the manipulation of the durations of the muscular actions adopted. It should also be considered that different values for the training load volume, intensity and density may lead to another results, mainly in conditions of maximum demands of volume and/or intensity. In addition, performing knee extension on other equipment with greater degrees of freedom in the knee and hip joints may have different results from the present study.

Conflict of interest

The authors declare no conflict of interest.

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Original article

Sweat rate and environmental factors in junior Laser class sailors

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Summary

Introduction: Recent studies have reported differences in sweat rate (SR) in laser class (LC) sailors under extreme environmental conditions (EC). This study aimed to determine a 'standard-like' SR in junior Laser 4.7 sailors under 'not-extreme' weather conditions to achieve an adequate fluid replacement rate for training and racing sessions. Additionally, we analysed the hypothetical relationship between SR and certain environmental factors, not just considering them as independent variables, but also including them as a whole factor, usually known as 'windchill' (WCh).
Material and method: Nine male elite junior Laser 4.7 class athletes were included in this descriptive study. They were monitored during the entire year of training and racing sessions, including national and international championships. Body mass changes as well as their food and fluid intake were measured for each sailor before and after sailing to estimate SR, absolute body mass change, and percentage body mass variation for all the sessions. Athletes were asked to maintain *"ab libitum"* fluid and food intake during the study. Environmental temperature, wind speed, and relative humidity were measured.
Results: Significant differences (p = 0.012) were observed between training and racing sessions with respect to SR, 0.18 (± 0.14) $L.h^{-1}$ *vs*. 0.23 (± 0.12 $L.h^{-1}$). Environmental temperature and WCh during racing showed an inverse relationship with SR.
Discussion: This result suggests that increased fluid and food intake are required under cold weather conditions to maintain the 'target' weight during the competitions and improve performance.

Key words:
Nutrition. Youth.
Exercise. Performance.

Tasa de sudoración y factores ambientales en regatistas juveniles de clase Láser

Resumen

Introducción: Recientemente, algunos estudios han reportado diferencias en la tasa de sudoración (SR) en regatistas de clase láser (LC) bajo condiciones ambientales (EC) extremas. Este estudio pretende determinar una especie de "SR standard" en regatistas juveniles de Láser 4.7 bajo condiciones "no extremas", con el objetivo de alcanzar una adecuada reposición de líquidos tanto para entrenamientos como en competición. Adicionalmente, se ha analizado una hipotética relación entre SR y los factores ambientales, considerándolos no únicamente como variables independientes, sino también como una variable compleja, conocida habitualmente como "sensación térmica" (WCh).
Material y método: Nueve regatistas juveniles de Láser 4.7 han participado en este estudio descriptivo y prospectivo. Todos ellos han sido monitorizados durante todo un año de sesiones de entrenamiento y competición, incluyendo regatas nacionales como internacionales. Los cambios en la masa corporal, así como la ingesta de líquidos y sólidos han sido registrados de forma individual para cada regatista, antes y después de cada sesión para estimar la SR, los cambios totales en la masa corporal, así como las variaciones en el porcentaje de masa corporal. Se instó a los regatistas a ingerir comida y bebida *"ab libidtum"* a lo largo del estudio. La temperatura ambiental, la intensidad de viento y la humedad relativa fueron registradas.
Resultados: Se observaron diferencias significativas (p = 0,012) para la SR entre los entrenos y las competiciones 0,18 (± 0,14) $L.h^{-1}$ *vs*. 0,23 (± 0,12 $L.h^{-1}$). La temperatura ambiental y la WCh durante las competiciones mostraron además una relación inversa con la SR.
Discusión: Estos resultados sugieren que una mayor ingesta de líquidos y sólidos bajo condiciones de "frío ambiental" es necesaria para mantener el peso ideal, mejorando así el rendimiento durante la competición.

Palabras clave:
Bicarbonato sódico. Alta intensidad.
Rendimiento físico. Personal militar.

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Introduction

Dehydration is a term that usually reflects any loss of body water and is associated with poor athletic performance, heat-induced illness, skeletal muscle cramps, and acute renal failure in athletes. The American College of Sports Medicine uses non-invasive measures to define dehydration (a urine specific gravity of ≥1.020 and/or urine osmolality of ≥700 mOsmol/kgH_2O). Additionally, in 2007, the American College of Sports Medicine proposed that 'dehydration (loss of water greater than >2% of body weight) decreases aerobic exercise performance in temperate and warm-hot environments and that greater levels of dehydration will further decrease aerobic exercise performance'[1].

While adequate intake of carbohydrates, fluid, and electrolytes is an important factor for improving endurance sports performance[2,3], progressive levels of exercise-induced dehydration during sport activities have shown to be associated with increased thirst and negative mood ratings; this, in turn, would limit cognitive function, reducing aerobic endurance exercise performance and resulting in increased body temperature, heart rate, and perceived exertion[4].

Although a small decrement in hydration status has been shown to impair the physiologic function and performance of runners while running in the heat[5], some studies suggested that athletes of endurance sports who run regularly ingest less than adequate fluid to maintain hydration, even if provided with ad libitum drinks[6]. Moreover, ab libitum fluid intake is not enough to prevent dehydration in suboptimally hydrated young soccer players[7]. Several studies using body weight and urine indices as outcome measures support the conclusion that athletes must drink beyond the physiological dictates of thirst to maintain adequate hydration levels[1,8]. This insufficient self-voluntary fluid intake indicated that thirst is not an adequate mechanism to appropriately maintain hydration status during prolonged endurance exercise[9,10]. However, there was disagreement between the results of previous studies on the rates of voluntary dehydration between athletes during practise[7].

In one of the first studies on hydration and sport performance[11], explained that 'during exercise, sweat output often exceeds water intake, which results in a body water deficit or hypohydration. Aerobic exercise tasks are likely to be adversely affected by hypohydration; moreover, the warmer the environment, the greater the potential for performance decrements'.

A recent review concluded that dehydration has a negative impact on physical performance for activities lasting more than 30 seconds. Although exercise itself can help achieve a 'hypohydration state', the level of dehydration induced may depend on several, factors, including the type, intensity, and duration of the exercise and the temperature and humidity of the environment[12].

In early 90s, some researchers suggested that SR and water loss seem to be affected by environmental conditions (EC)[13]. Therefore, it seems logical to suspect that the variability of environmental factors and their effects on sport performance could be increased when associated with outdoor sport practice. Although several studies have examined the physiology of players participating in dinghy racing and other sports involving the use of small sail boats in the past 20 years, evidence-based knowledge on sailing and the associated physiological demands is limited compared to that on other endurance sports. However, the current interest in research by groups, such as Olympic and America's Cup teams, has led to beneficial changes[14].

The Laser class (LC) is one of the most popular single- handed dinghies in the world. The Laser became a men's Olympic class boat at the 1996 Summer Olympics in Atlanta. A version with a smaller sail, the Laser Radial was first sailed as a women's Olympic class boat at the 2008 Summer Olympics. Laser 4.7 is the version with the smallest sail and is often sailed by young sailors, usually aged below 18 years, before reaching the greater versions. Fast Laser sailing requires an advanced level of fitness, especially in the quadriceps, abdominal muscles, and upper back muscles, to endure the straight legged hiking and body-torque techniques essential in getting upwind and reaching quickly[15,16]. Physiological requirements in Elite dinghy sailors (including LC) seem to be similar to other endurance sports, such cycling and running[14,17]. Evaluation of the energy demands of LC sailors revealed that there was a direct correlation between wind velocity and the energy demand during sailing[18]. Although some authors initially suggested that dinghy sailing performance is primarily sustained by the anaerobic metabolism, their studies did not reproduce real racing conditions, because the mean duration of exercise on water varied from 10 to 20 minutes[19,20]. In fact, regattas last for 30–45 minutes; thus, most recent studies suggested that aerobic capacity could be a determinant of an elite Laser athlete's performance.

On reproducing real on-water racing conditions for 30 minutes or more, some studies reported that the physiologic response to sailing in moderate winds could be used to determine the energy requirements, which are largely met by the aerobic metabolism especially in higher skilled Laser sailors[18].

Although the evidence suggest that the maintenance of hydration status is a fundamental factor to achieve the best level for athletic performance, only a limited number of studies examined the hydration requirements of Olympic class sailors compared with other endurance sports[16]. Studies showed that most participants experience a negative fluid balance after three consecutive races (95 minutes of racing and 5 hours on water) in a Laser and Laser radial club level regatta. The results showed that most participants presented with negative fluid balance after racing (men: mean, −2.1% [95% confidence limits, −1.7% to −2.5%]; women: mean, −0.9% [0% to −1.8%]), most likely due to low voluntary fluid intake. However, the periods of 'rest' between races could result in the underestimation of these results[17].

Previously[21], reported that the elite New Zealand Olympic class dinghy sailors also demonstrated a lack of knowledge regarding sports nutrition and guidelines for fluid consumption during exercise. Moreover, no significant differences were observed in the nutrition habits between sailors who received nutrition *sport science support* (SSS) and those who did not receive SSS in 1994–1995[22]. In a recent study assessing the hydration status of elite young Laser athletes of a Greek national sailing team during a 4-day World Championship competition, results showed that pre-race body weight was significantly and progressively decreased on days 2, 3, and 4 compared with the 3-day baseline value, indicating progressive dehydration. The highest degree of dehydration was observed on the 4th racing day (pre: −2.9±0.1% and post: −5.8±0.2% of body weight)[23].

In another study including 11 male Olympic sailors, a fixed volume of 11.5 $mL.kg^{-1}.h^{-1}$ was consumed during 'warm environmental condition (WEC)' training, and hydration status was maintained by preventing changes in body mass and urine specific gravity. However, in the same study, it showed that the ab libitum fluid consumption in cold environmental conditions (CEC) was insufficient in preventing a decrease in body mass and blood electrolyte concentration post-training. Additionally, notable differences between laboratory and on-water SR for warm conditions were observed[16].

Purpose of the study

Therefore, it is important to note that laboratory sweat testing results did not directly correspond to the on-water SR. This finding may guide further research on the hydration requirements of sailors in different EC. The present study aimed to evaluate the on-water training and racing sweat rates (SRs) in young Laser 4.7 elite athletes. It also aimed to determine the hypothetical relationship between EC and SR.

Material and method

Participants

Nine male LC 4.7 young sailors volunteered for an investigation to assess body mass changes under diverse weather conditions during training sessions (TS) and racing sessions (RS) for a year (October 2015 to September 2016). The mean age and weight at the beginning of the study were 15,9 ± 1.4 years and 64,1 ± 8.1 kg. Body mass index (BMI) was 23 ± 4,2 (Table 1).

All participants and their legal guardians were informed of the objective of the study, and they provided written informed consent prior to the start of the study. The study was approved by the Research Ethics Committee of Galicia (Spain) and was carried out in accordance with the Helsinki Declaration. Support for the methodological and data analyses was obtained from the Clinical Researching Committee, Unidad de Investigación Clínica y Ensayos Clínicos (UICEC) de A Coruña (Spain). Athletes who were invited to participate in the Spanish National LC 4.7 Championship and without chronic illness were included.

Table 1. Demographic characteristics of participants.

	M	SD
Age (years)	15.9	1.4
BMI (kg/m^2)	23	4.2
Weight (kg)	64.1	8.1
Height (cm)	173.9	6.9
Sailing experience (years)	6.8	1.8

BMI: Body Mass Index.
Body mass index (BMI) is a value derived from the mass (weight) and height of a person (Keys *et al,* 1972). The BMI is defined as the body mass divided by the square of the body height, and is universally expressed in units of kg/m^2.

Design

Body weight after voiding was measured digitally and recorded to the nearest 100 g (±0.1 kg) (Model 803, Seca, Hamburg, Germany) approximately 10 minutes before departure. Athletes were weighed again 10 minutes after each session. They were towel dried and weighed while wearing minimal clothing. Data from sailors confirming bladder/bowel evacuation between pre and post measurements were removed from the analysis.

The participants had ad libitum access to water and food intake while sailing. Data were individually collected and recorded for each session and sailor. Athletes were asked to keep all food and fluid packaging for recording.

Amount of fluid consumption were measured to the nearest 10.0 mL of translucent, graduated bottles before and after each session, and the difference were recorded.

Food was provided as energy bars (Powerbar©, Active Nutrition International GmbH, Munich), 55 g per bar (fat 2,8 g + carbohydrates 35 g + protein 5,3 g). Athletes were instructed to consume all the energy bars to avoid subestimation of food intake.

The athletes dressed accordingly during sailing sessions; light, non-restrictive attire was worn underneath life jackets, with neoprene wetsuit completely covering their bodies, arms, and legs. They were asked to maintain their usual dietary practices, but a special effort was made to athletes for recovering their body mass losses after sailing, with the aim of reaching their baseline body weight before the next session, especially when sessions were conducted on consecutive days.

Measures

Data collection was performed in A Coruña (Spain) during TS.
RS measurements were obtained during the National Laser 4.7 Championship in Cádiz, Spain, May 2016; the European Laser 4.7 Championship in Crozon, France, July 2016; and the Laser Under-21 World Championships in Kiel, Germany, August 2016.

Change in body mass

Changes in body mass (g) over the sessions were defined as follows (equation 1):

(pre-exercise body mass(g) – post-exercise body mass(g)

Sweat rate

Changes in body mass, weight of food and water consumed, and duration of sessions were used to calculate SR (millilitres per hour), with the assumption 1 kg = 1 L, using the following formula[24] (equation 2):

[(pre-exercise body mass (g) – post-exercise body mass(g)) + food(g) + fluid intake]/practice length (h)

Percent of hypohydration (equation 3)

Percentage change (PC) (%) in fluid balance was calculated using the following formula[24] (equation 3):

[(post-exercise body mass(g) – pre-exercise body mass(g))/pre-exercise body mass(g)] x 100

This 'percentage of dehydration' assumes euhydration at the time of pre-launch weighing.

Windchill (equation 4)

Windchill (WCh) is an index used to indicate how cold the weather feels to the average person. It is determined by combining temperature and wind velocity values into one number to reflect the perceived temperature.

$$Twc = 13.12 + (0.6215 \times Ta) - (11.37 \times V^{+0.16}) + (0.3965T \times V^{+0.16})$$

where Twc is the windchill index, based on the Celsius temperature scale, Ta is the air temperature in degrees Celsius, and v is the wind speed at 10 m (33 ft) standard anemometer height in km.h^{-1}.

Duration of sessions

The duration of sailing was also recorded, which is expressed as the time since the group of athletes' departure until the time of their arrival.

Environmental conditions

Environmental temperature, relative humidity, wind speed, and wind speed peaks were measured every 10 minutes using a portable weather station (Kestrel 3000 Pocket Weather Meter, Nielsen-Kelerman company, USA), in a boat as close as possible to the sailing area by the athletes' support crew. Main values were obtained for each session and then recorded for the study.

Data analyses

A descriptive analysis was be performed for all. Continuous variables were expressed as means±standard deviations or interquartile ranges. Dichotomous or categorical variables were expressed as absolute numbers and percentages. Pearson's or Spearman's correlation coefficient was used to determine the correlation between quantitative variables. All tests were two tailed, and a p value of <.05 was considered significant. Data were analysed using IBM SPSS Statistics 21.

Results

About 202 valid measurements were obtained from 45 group sailing sessions (128 training and 74 racing); the number of measurements for each athlete depended upon their availability.

Data of those participants that evacuated their bladders while sailing were excluded from the analysis for that day (18 sessions). Up to eight sessions were not included in the study as the body mass of the athletes exceeded beyond the baseline measurement 15 minutes after arriving. Another five sessions were not included because of the variations in the environmental conditions during on-water period were more than 20%.

Data were presented as the mean (range) for all descriptive statistics and within conditions, with the level of confidence set at p<0.05 to determine its significance unless specified.

Duration of sessions (Table 2)

Average of sailing session duration was 3,579 ± 1,532 (h). The shortest session lasted for 30 minutes, and the longest session lasted for 495 minutes. The mean duration of TS was 3,0488 ± 0,9495 (h) and that of racing sessions was 4,945 ± 1,664 (h).

Table 2. Duration of sailing sessions, environmental factors and mean fluid/food intake.

Characteristics of sessions	Training sessions		Racing sessions	
Number of sessions	128		74	
	M	SD	M	SD
Duration of sessions (h)	3.05	0.95	4.95	1.66
Environmental factors				
Environmental temperature (Cº)	17.5	1.62	15.78	3.66
Relative humidity (%)	70.16	11.43	69.19	17.45
Wind speed (km.h^{1})	9.00	4.65	17.62	6.72
Wind speed peaks (km.h^{1})	16.58	6.11	24.12	9.04
Fluid and food intake				
Mean fluid intake (ml)	87.9	203	524	534
Mean food intake (g)	34.9	105.6	64.6	64.1
Mean fluid intake per hour (mL.h^{-1})	9.3	67.8	104.8	106.2
Mean food intake per hour (g.h^{-1})	11.6	35.4	12.9	12.8

All values except number of sessions are expressed as mean +/- standard deviation. "Fluid Intake" and "Food intake" are referred to mean intake along the sessions by person. "Mean inteake per hour" and "Mean fluid intake per hour" are referred to mean intake per hour by person.

Mean fluid/meal intake (Table 2)

The mean fluid intake during the TS was 88 ± 203 (ml) and that during RS was 524 ± 534 (ml). The mean food intake during the TS was 34,9 ± 105,6 (g) and that during the RS 64,6 ± 64,1 (g).

Environmental conditions (Table 2)

The mean environmental temperature, relative humidity, and wind velocity during TS were 17,50 ± 1,62 (°C), 67 ± 17,55 (%), and 9,0 ± 4,65 (km.h^{-1}), respectively, and those during RS were 15,78 ± 3.66 (°C), 70,28 ± 11,43 (%), and 17,62 ± 6,72 (km.h^{-1}) respectively. The mean wind speed peak for TS was 16,58 ± 6,11 (km.h^{1}) and that for RS 24,12 ± 9,04 (km.h^{1}).

Change in body mass

The mean body mass changes during the TS were negative: −554 ± 432 g and that during RS was also negative: −1121 ± 387 g.

Sweat rate

Significant differences in SR were found between TS and RS (p=0.012). The mean SR during TS was 184,4 ± 140,6 mL.h^{1} and that during RS was 234,8 ± 129,0 mL.h^{1}.

Percentage of dehydration

The mean percentage change in fluid balance during TS was −0.87 ± 0.69% (minimum: 0% and maximum: −3.16%, respectively). The mean percentage change in fluid balance during RS was −1.73 ± 0.99 (minimum: 0% and maximum: 3,93%, respectively (Figure 1).

Changes in body mass and SR were described as the mean value ± p25 and p75 values, respectively (Figure 1).

Figure 1. Fluid balance and sweat rate expressed as mean ± p25 and p75 standard deviation. Fluid balance and sweat rate were increased during racing sessions when compared to training sessions.

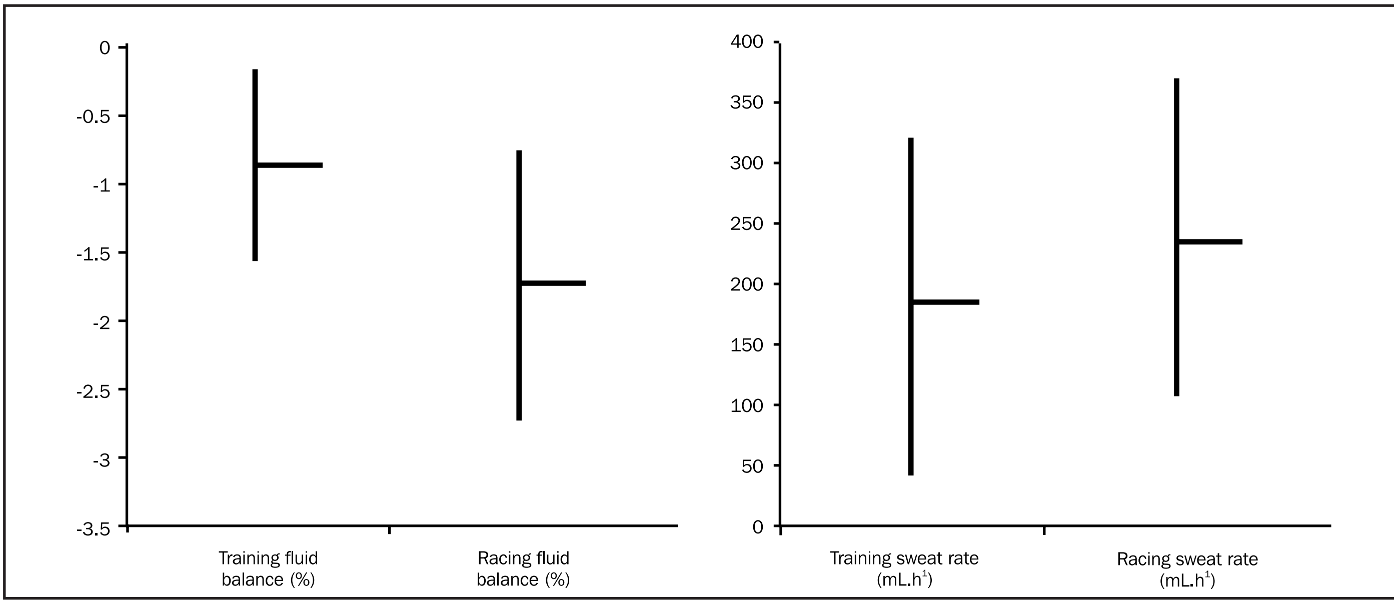
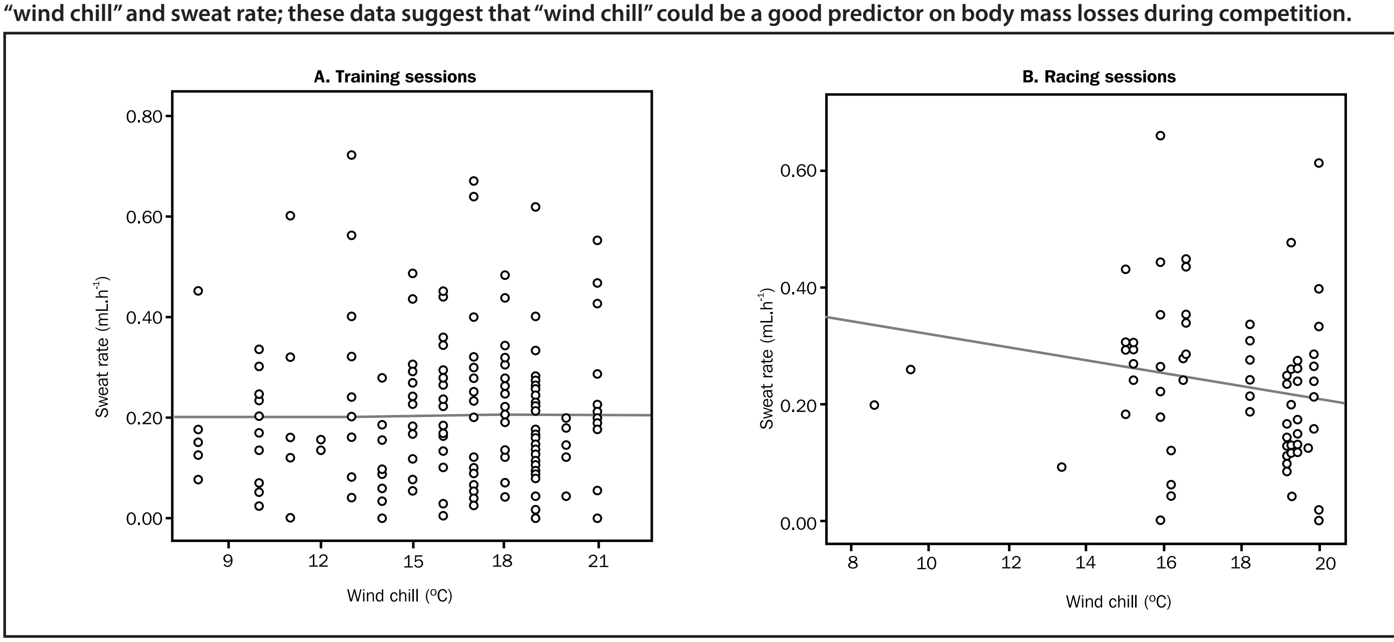


Figure 2. Sweat rate and "wind chill" for training and racing sessions. During racing sessions, an inverse correlation was found between "wind chill" and sweat rate; these data suggest that "wind chill" could be a good predictor on body mass losses during competition.

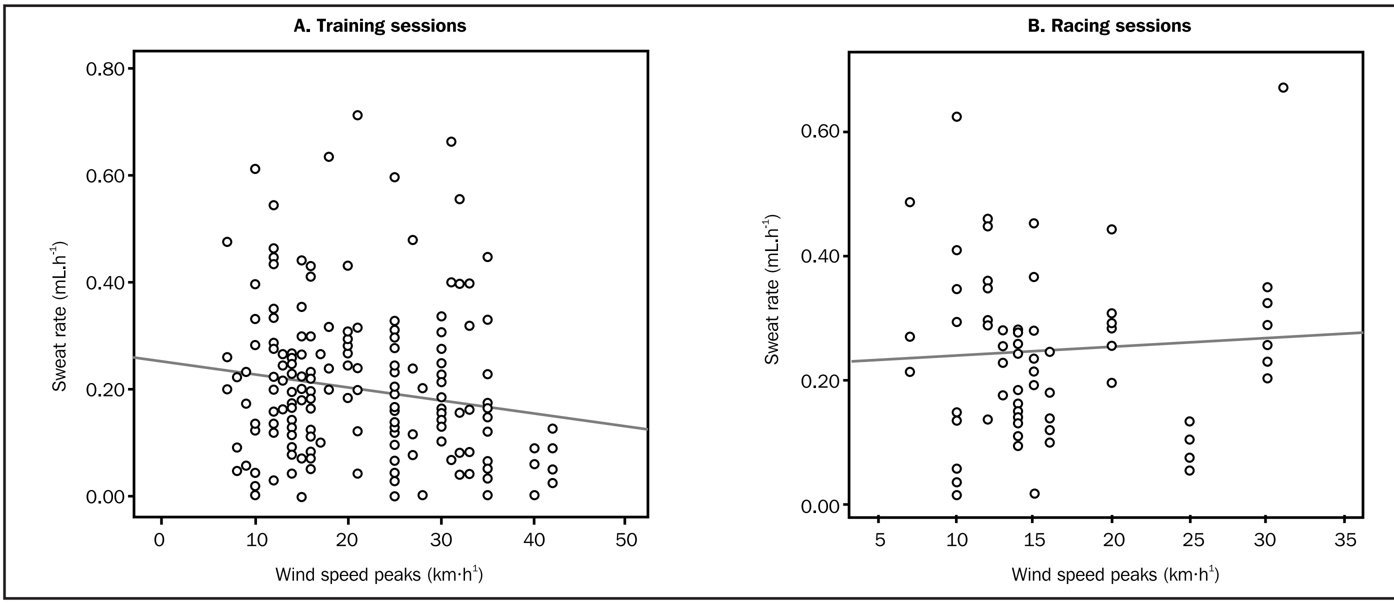


Sweat rate, percentage of dehydration, and environmental conditions

An inverse relationship was found between environmental temperature, WCh, and SR. This finding suggests that athletes would require not only an increased amount of fluid intake, but also a longer intake of carbohydrates when the environmental temperature is below 21°C and/or WCh is below 21°C, to recover those metabolic losses (fluid and calories per hour) (Figure 2).

When analysed as independent variables, not environmental temperature, relative humidity, nor mean wind speed showed a significant statistic relationship with SR or percentage of dehydration after exercise. Our results have shown, however, a significant statistic relationship between peak wind speed and fluid requirements, but only during RS, not during TS. This could be explained by the intense performance during competitions (Figure 3).

Figure 3. Significant statistic relationship between peak wind speed and fluid requirements was founded, but only during RS, not during TS. Not environmental temperature, relative humidity, nor mean wind speed showed a significant statistic relationship with SR or percentage of dehydration after exercise.



Discussion

Maintaining the 'target' weight for each sailor is a major factor for achieving better results during racing. This 'target' value depends on the body composition of each athlete and his tactical and strategical preferences during competitions. Variations in this 'target' weight for each sailor often imply poor athletic performance.

Our study showed that young elite sailors had insufficient fluid intake, even if they were instructed to consume enough fluids, which is consistent which other studies. Lewis *et al.*[16] suggested that fluid requirements could be different for warm and cold conditions during TS: a recent study showed that a consumption of 11.5 mL.kg^{-1}.h^{-1} exceeded the requirements of different Olympic class sailors at warm conditions (environmental temperature: 19°C [17.0–23.3]). The fluid requirements of the participants during WEC were anticipated to reflect the average laboratory SR of 1,470 mL.h^{-1} measured at 21.8°C. The participant's average on-water SR was only 611.8 ± 47.2 mL.h^{-1}. This rate was 41.5% lower than the pre-study laboratory SR of 1,470 mL.h^{-1}. However, at CEC, participants had ab libitum fluid consumption, which indicated that 'they arrived for training in a borderline hypohydrated state' after attending a 2.5-hour RS[25].

In contrast, we found a significant difference (p=0.012) in SR between TS and RS, suggesting that increased liquid intake is needed during racing, regardless of the weather condition.

In our study, the inverse relationship between SR and some isolated environmental factors like temperature (<21°C) as well as the direct relationship between SR and wind speed peaks under racing conditions were considered as important factors for predicting the fluid and food requirements in young sailors before racing or training depending on the weather condition.

This study had some limitations. The study only included 'high-skilled' young sailors. The results were inconsistent as the level of technical and physical performance differed between athletes.

Although our results were in agreement with those of previous studies with similar populations, further investigations are necessary to confirm the influence of EC and determine a better strategy to help reduce the impact of these factors on the study results. The possibility of using WCh as a global environmental indicator should be analysed and discussed to confirm its accuracy as a predictive factor.

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Any opinions, conclusions, or recommendations expressed here are those of the authors.

Conflict of interest

The authors declare no conflict of interest.

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Original article

Psychometric properties of the Chilean version of the Quick Disabilities of the Arm, Shoulder and Hand (Quick DASH) questionnaire for patients with shoulder disorders

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Summary

Introduction: The literature provides psychometric properties Quick Disabilities of the Arm, Shoulder and Hand (DASH) similar to the original DASH. The potential advantages of this instrument include the shorter time needed to answer it and the elimination of some less relevant items. The cross-cultural validity for the Chilean version of the Quick DASH has already been developed, but its psychometric properties have not yet been studied in the Chilean population.
Aim: To determine the internal consistency, test-retest reliability, minimum detectable change, minimum important change, relevant clinical change, and sensitivity of the Quick DASH subjective questionnaire in patients with common shoulder pathologies in the Chilean population.
Material and method: 81 patients with shoulder pathologies were recruited by completing the Quick DASH on 3 occasions. After visiting their attending physician, starting physical therapy, and after completing 10 sessions of physical therapy.
Results: The questionnaire shows an internal consistency of 0.92, test-retest reliability of 0.95 (0.91-0.97), minimum detectable change of 19.6%, minimum important change of 25.5%, relevant clinical change of 37.1%, and effect size (sensitivity) of 1.1.
Conclusions: The psychometric properties described show that the Quick DASH can be used reliably in both clinical and research for Chilean patients with shoulder pathologies.

Key words:
Outcome measures. Reliability of results. Shoulder.

Propiedades psicométricas de la versión chilena del cuestionario *Quick Disabilities of the Arm, Shoulder and Hand* en pacientes con patologías de hombro

Resumen

Introducción: Los cuestionarios basados en la percepción subjetiva del paciente sobre las disfunciones asociadas a su patología son comúnmente utilizado como instrumentos de evaluación, para definir manejo terapéutico y evaluar estados de avance tanto en la clínica como investigación. Es relevante que los cuestionarios seleccionados midan lo que proponen de manera válida y confiable, pero que además sea factible de utilizar considerando su simpleza como el tiempo empleado en su uso. Existen diversos cuestionarios comúnmente utilizados en las patologías de hombro. Entre estos se encuentra el cuestionario *Quick Disabilities of the Arm, Shoulder and Hand (Quick DASH)* que puede ser utilizado en diversas disfunciones de extremidad superior y está clasificado entre los mejores cuestionarios subjetivos autoadministrados considerando sus propiedades psicométricas. Las potenciales ventajas de este instrumento incluyen el menor tiempo necesario para contestarlo y la eliminación de algunos ítems menos relevantes. La validez transcultural para la versión chilena del Quick DASH ya ha sido desarrollada, pero sus propiedades psicométricas aún no han sido estudiadas en la población chilena.
Objetivo: Determinar la consistencia interna, confiabilidad test-retest, cambio mínimo detectable, cambio mínimo importante, cambio clínico relevante y sensibilidad del cuestionario subjetivo Quick DASH en pacientes con patologías de hombro en la población chilena.
Material y método: 81 pacientes con patologías de hombro fueron reclutados completando el Quick DASH en 3 ocasiones. Tras visitar al médico tratante, cuando comiencen su rehabilitación kinésica y tras completar 10 sesiones de kinesioterapia.
Resultados: El cuestionario muestra una consistencia interna de 0.92, confiabilidad test-retest de 0.95 (0.91-0.97), cambio mínimo detectable de 19.6 %, cambio mínimo importante de 25.5%, cambio clínico relevante de 37.1% y tamaño del efecto de 1.1.
Conclusiones: Las propiedades psicométricas demuestran que el Quick DASH puede ser usado de manera confiable tanto en clínica como en investigación para pacientes chilenos con patologías de hombro.

Palabras clave:
Confiabilidad de resultados. Cuestionarios subjetivos autoadministrados. Hombro.

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Introduction

Shoulder-related pathologies constitute the third highest reason for muscular-skeletal injury consultancies in medical practice. Questionnaires based on the patient-reported outcome measure (PROM) linked to the pathology in question, are commonly-used assessment instruments to determine therapy options and to evaluate clinical and research states of progress[1-3]. It is important for clinicians and researchers to choose a PROM that is able to validly and reliably measure the element, whilst also being feasible for use considering its simple nature as well as the time invested in it. There are different commonly-used PROM for shoulder pathologies. One is the Quick Disabilities of the Arm, Shoulder and Hand questionnaire (Quick DASH), which can be used for different upper extremity dysfunctions[4], and is positioned among the best PROM given its psychometric properties[5-7]. The Quick DASH comprises 11 questions addressing the degree of difficulty the patient experiences in performing physical activities due to shoulder, elbow or hand problems (6 questions); the intensity of pain and tingling sensation (2 questions); and the effects these problems have on the patient's social, work and sleep activity (3 questions). The cross-cultural validity of the Quick DASH into Spanish has been published8, but its psychometric properties have not yet been studied for the Chilean population. The aim of this study is to establish the internal consistency, test-retest reliability, minimal detectable change, minimal important change, relevant clinical change and sensitivity of the subjective Quick DASH questionnaire on patients with common shoulder pathologies in Chile.

Material and method

Study type

Internal consistency, test-retest reliability, minimal detectable change (MDC), minimal important change (MIC), relevant clinical change (RCC) and sensitivity of the Quick DASH questionnaire were all assessed on a cohort of Chilean patients with shoulder pathologies, via an observational analytical prospective test-retest study.

Sample

The patients were recruited from medical centres, hospitals and clinics in the Pontifical Catholic University of Chile Health Network between October 2018 and January 2019. The group considered for this study were patients with medically diagnosed shoulder pathologies from kinesiatrics. The exclusion criteria were those aged under 18 years, cervical pain of any origin, shoulder pain caused by radicular, vascular or neoplasia disorder, inability to complete the Quick DASH questionnaire due to cognitive alterations or language difficulties. The study was approved in accordance with the Helsinki Declaration principles and by the Pontifical Catholic University of Chile Medicine Faculty Ethics Committee. All the patients handed in their informed consent form before participating, and were free to leave the study whenever they wished (Table 1).

Data collecting procedure

The patients completed the Quick DASH questionnaire at 3 different times (T1, T2 and T3). T1, following a visit to the doctor treating the patient, T2, upon starting the kinesics rehabilitation, and T3, after completing 10 kinesiotherapy sessions. The patients were invited to participate by the doctor treating them. Upon accepting, they were given a questionnaire (T1) with written instructions and the informed consent to be completed and handed in to the kinesiologist treating them on the first session. In this session, patients completed the Quick DASH questionnaire for the second time (T2), and demographic data and medical diagnoses were obtained. All patients were also asked the first key question: How do you feel your symptoms have changed since the first time you filled out the questionnaire? The response options are: much better, quite better, somewhat better, the same, somewhat worse, quite worse, and much worse. With patients who answered the key question with "the same", the test-retest reliability of the Quick DASH and the minimal detectable change (Minimum detectable change (MDC) were analysed. In T3, when the patient completed session 10 of kinesiotherapy, the questionnaire was handed in again. When comparing T1 and T3, the instrument sensitivity was obtained via the size of effect. All patients were also asked the second key question: How do you feel your symptoms have changed since the first time you filled out the questionnaire? The response options are: much better, quite better, somewhat better, the same, somewhat worse, quite worse, and much worse. For patients that replied to the key question with "somewhat better", the minimal important change (MIC) in the questionnaire was also established, and those who replied with "quite better", the relevant clinical change (RCC) of the Quick DASH was established.

Data collection instrument

Quick DASH

Each item is scored on a categoric scale with 5 response options, ranging from "no difficulty" or "no symptoms" (1 point) to "severe disability" or "severe symptoms" (5 points). The questions address the degree of difficulty experienced when performing physical activities due to the shoulder, elbow or hand problems (items 1-6), the effects of the upper extremity problems on social, work or sleep activity (items 7, 8, 11), the severity of the painful symptoms (item 8), and the severity of the tingling symptoms (item 10) in the shoulder, elbow and hand (items 9, 11). These items provide the Quick DASH disability/symptoms score, ranging from zero (no disability) to 100 (severe disability). This score is obtained using the following equation:

$$\text{Quick DASH} = [(\text{sum of n responses}/n) - 1] \times 25,$$

in which n is the number of completed responses. The Quick DASH scoring cannot be calculated if there is more than 1 missing item[9].

Statistical analysis

For research linked to the study of the psychometric properties of instruments, a sample size of 5-10 patients is recommended for each item in the questionnaire. As the questionnaire comprises 11 items, and taking into account a 20% desertion rate, a sample size of 80 participants is suggested[10].

The following psychometric properties were analysed:

Internal consistency

This was established using Cronbach alpha coefficient identified in T1. This coefficient assesses if there is a correlation in each item of the questionnaire with the Quick DASH total score. A value recommended for a health questionnaire is around 0.7[11].

Reliability Test-Retest

This was established by comparing the Quick DASH score in T1 and T2 in patients that reported no changes in the key question consulted in T2. An instrument is considered reliable if it delivers similar results over time to each patient under the same conditions. To qualify the test-retest reliability, the intraclass correlation coefficient (ICC) was used. An ICC between 0.0 and 0.39 is classified as poor; between 0.40 and 0.59 as regular; between 0.60 and 0.74 as good, and between 0.75 and 1.00 as excellent reliability10. It is recommended for health-related questionnaires to have a score of at least 0.711. To obtain the ICC, the 2 channel model with random effects was used. The Bland-Altman[12] graph was used to analyse the distribution of the difference between the average scores from the T1 and T2 Quick DASH.

Minimum detectable change (MDC)

This is the minimal measurable change between the scores that represents a statistically significant difference over the margin of error of the measurement instrument under similar conditions[13]. To obtain it, the questionnaire scores from T1 and T2 in which patients responded to the first key question with "the same" were compared. First the standard error of measurement (SEMMDC) was calculated by dividing the standard deviation of the differences between T1 and T2 by the root of 2[12]. SEM is considered an absolute measure of error of measurement[11]. The MDC is calculated using the following formula: MDC=1.96*√2*SEM (considering SEM= standard deviation of the differences between the Quick DASH scores in T1 and T2/√2).

The MDC establishes the interval which is within the margin of error of the instrument with 95% reliability and thereby the minimum variation in the questionnaire score that must be present to be considered statistically significant[14]. The difference in the Quick DASH score between T1 and T2 was contrasted with the average scores in the questionnaire using the Bland-Altman graph.

To express SEM as a percentage (SEM%), the following formula was used:

(Differences between the Quick DASH in T1 and T2/Quick DASH score in T1)*100.

To express MDC as a percentage (MDC%), the following formula was used:

MDC%=1.96*√2*SEM%.

Minimum important change (MIC)

This is the minimal measurable change for the subject assessed to consider a slight improvement[14]. The calculation was performed with subjects that replied to the second key question with "somewhat better". First the standard error of measurement (SEMMIC) was calculated by dividing the standard deviation of the differences between T1 and T3 by the root of 2[12]. The MIC was calculated using the formula MIC=1.96*√2*SEM, in which MIC is the minimum change present in an individual's Quick DASH score to ensure 95% reliability that this change is linked to the patient's perceived improvement.

To express SEM as a percentage (SEM%), the following formula was used:

(Differences between the Quick DASH in T1 and T3/Quick DASH score in T1)*100.

To express MIC as a percentage (MIC%), the following formula was used:

MIC%=1.96*√2*SEM%..

Relevant clinical change (RCC)

This is the minimal possible change in the Quick DASH score for the subject assessed to consider a relevant improvement[15]. The calculation was only performed with subjects that replied to the second key question with "quite better". First the standard error of measurement (SEMRCC) was calculated by dividing the standard deviation of the differences between T1 and T3 by the root of 2[13]. The RCC was calculated using the formula RCC=1.96*√2*SEM, in which RCC is the minimum change present in an individual's Quick DASH score to ensure 95% reliability that this change is linked to the patient's perceived relevant improvement.

To express SEM* as a percentage (SEM%), the following formula was used:

(Differences between the Quick DASH in T1 and T3/Quick DASH score in T1)*100

To express RCC as a percentage (RCC%), the following formula was used:

RCC%=1.96*√2*SEM%

Sensitivity

Sensitivity was expressed using the size of the effect (difference in the Quick DASH score between T1 and T3/standard deviation of the differences between T1 and T3). If the size of the effect is near 0.2, it is considered small, 0.5 is considered moderate, and around 0.8 high[15].

An analysis of the patients' demographic characteristics and data regarding the different psychometric properties of the Quick DASH questionnaire was performed using the SPSS 25 statistics programme.

Results

81 patients participated in the study and completed the Quick DASH in T1. Their demographic characteristics are displayed in Table 1 and the psychometric properties of the Quick DASH are in Table 2. Figure 1 displays the Bland Altman graph to contrast the difference between T1 and T2 Quick DASH scores with the average of the questionnaire scores.

Table 1. Demographic characteristics and medical diagnosis of the participants.

	n	Age (years)	Weight (kgs)	Height (cms)
Total	81	47 (4)	71 (6)	168 (9)
	41 M 40 F	41 (18) 53 (16)	78 (13) 60 (8)	175 (6) 161 (7)
Rotator cuff tendinopathy*	11M 12 F	62 (17) 59 (14)	79 (9) 66 (8)	174 (5) 160 (7)
Operated broken rotator cuff	6 M 6 F	68 (4) 65 (8)	75 (4) 60 (6)	175 (5) 158 (6)
Instability non-operated shoulder	3 M 3 F	23 (5) 22 (5)	68 (8) 60 (5)	173 (6) 165 (5)
Instability operated shoulder	8 M 2 F	22 (4) 20 (5)	66 (10) 60 (2)	175 (8) 165 (2)
Acromioclavicular disjunction	6 M 2 F	24 (4) 23 (2)	80 (20) 60 (2)	178 (10) 160 (2)
Prosthesis	1F	60	65	160
Sub-acromial bursitis	5 M 8 F	40 (10) 50 (10)	75 (12) 62 (4)	175 (4) 162 (2)
Humerus fracture	1F	40	70	170
Adhesive capsulitis	2 M 5 F	45 (3) 52 (6)	82 (5) 65 (6)	176 (4) 162 (6)

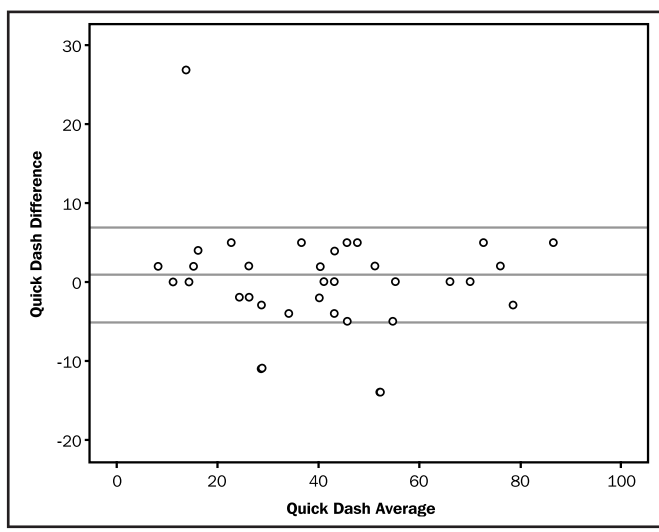
M: Male; F: Female.
*Rotator cuff tendinopathy groups the diagnoses of tendinosis, tendinitis and rotator cuff breakage, or some of its components. Operated and non-operated shoulder instability group SLAP, Bankart and Latarjet injuries.

Table 2. Psychometric properties of the Quick DASH questionnaire.

Internal consistence (n=81)	
Cronbach's Alpha	0.92
Reliability Test-Retest (n=37)	
ICC	0.95
95% ICC	0.91-0.97
MDC (n=37)	
SEM_{MDC}	2.2
SEM%	6.9
MDC	6.9
MDC%	19.6
MIC (n=23)	
SEM_{MDC}	3.4
SEM%	9.6
MIC	9.0
MIC%	25.5
RCC (n=34)	
SEM_{RCC}	8.3
SEM%	22.4
RCC	13.2
RCC%	37.1
Sensitivity (n=64)	1.1

ICC: test-retest reliability; SEMMDC: standard error of measurement for the calculation of the MDC; SEMMIC: standard error of measurement for the calculation of the MIC; SEMRCC: standard error of measurement for the calculation of the RCC; SEM% value of the SEM expressed as a percentage; MDC: minimal detectable change; MIC: minimal important change; RCC: relevant clinical change.

Figure 1. Bland Altman graph to contrast the difference between T1 and T2 Quick DASH scores with the average of the questionnaire scores.



Discussion

The psychometric properties described reveal that the Quick DASH questionnaire can be used reliably in both clinical and research contexts on Chilean patients with shoulder-related pathologies. According to the Munro[11] classification, the ICC reported reveals excellent inter-assessment reliability. The narrow confidence interval ensures a high level of reliability in determining the ICC. The high internal consistency reveals an extremely good correlation between each of the 11 items in terms of the questionnaire as a whole. The sensitivity established by the size of effect reveals that the questionnaire has an excellent capacity for detecting relevant changes over time. This was determined between the start and finish of 10 kinesiotherapy sessions, which patients generally took between 3 and 4 weeks to complete. When expressed as a percentage, the MDC gives a value of 20%. This means that for a specific intervention to be considered as minimally relevant for Chilean patients with shoulder-related pathologies, the reduction in the score obtained in the questionnaire after intervention must be at least 20%. In this study, significant clinical improvement was considered to be a variation in the questionnaire score associated to the subjective perception of being "quite better". The RCC discovered is 37%, and can be an important reference value when it comes to classifying a surgical or conservative intervention as successful, taking into account the Quick DASH score as the outcome. Other studies[13,15,16] use the MIC to consider the success or failure of an intervention. However, this variation is associated with the perception of a slight improvement, which cannot be considered a success for the patient or for the medical team. Upon comparing the psychometric properties with those of other PROM, the internal consistency is similar to that found by the American Shoulder and Elbow Surgeons (ASES) with a Cronbach's Alpha of 0.94 and a size of effect of 1.33[17]. Cronbach's Alpha (0.94) and ICC (0.96) are reported[18] for the Western Ontario Rotator Cuff Index (WORC) questionnaire. A similar MDC to that found in this study of 6 points, is reported[19] by the Western Ontario Shoulder Instability Index (WOSI), and of 12 points by the Oxford Shoulder Instability Score (OSIS).

The relevance of the study is the knowledge of the psychometric properties of a PROM validated for the Chilean population. This enables the Quick DASH to constitute an alternative in assessing the dysfunctions associated with shoulder pathologies, and the patient's perceived outcome following surgical or conservative intervention, and for this to be interpreted correctly from a methodological perspective. The study strengths are its sample size and the homogeneity of the demographic assessed. Its limitations include the factor that the gender and age of the patients may play an influencing factor in subjective perspective, and that the results were not standardised for these variables.

Conclusion

The psychometric properties of the Quick DASH questionnaire reveal that it constitutes an excellent alternative for reliable use in both clinical and research contexts on Chilean patients with shoulder-related pathologies.

Conflict of interests

The authors claim to have received no economic support or conflict of interest in the undertaking of this article.

The undersigning authors of the articles accept responsibility as stipulated by the World Association of Medical Editors http://www.wame.org/

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Original article

Anthropometric characteristics and cardiorespiratory capacity of male and female trail runners

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Summary

Introduction: The objectives of this study were to compare the anthropometric characteristics and the cardiorespiratory capacity of male and female mountain runners, and to examine the association between anthropometric and cardiorespiratory variables.
Material and method: A total of 48 mountain runners, 16 women and 32 men take part in the study. Anthropometric parameters were measured and a maximum incremental test was performed on treadmill with ramp for cardiorespiratory assessment.
Results: The results showed significant differences in the anthropometric characteristics corresponding to mass, height, body mass index (BMI), perimeters, diameters, % muscle mass, % residual mass and mesomorphic component between men and women, being significantly higher in the male group ($p < 0.05$). The female group obtained significantly higher values ($p < 0.05$) in tricipital, thigh, leg and ilecorestal skinfolds, as well as in 8 folds sum, % fat, fat mass, bone mass and in the endomorphic component. With respect to cardiorespiratory capacity, the male group obtained significantly higher values in the initial systolic volume ($p < 0.01$, TE = -1.45, large), oxygen consumption at the second ventilatory threshold (VO_2VT2) ($p < 0.01$, TE = -1.66, large) and maximum oxygen consumption (VO_2max) ($p < 0.05$, TE = -1.78, large). A large correlation was obtained between the sum of 8 skinfolds and the VO_2max ($r = -0.79$, $p < 0.01$), between the % fat and the VO_2max ($r = -0.81$, $p < 0.01$) and between % fat and VO_2VT2 ($r = -0.79$, $p < 0.01$).
Conclusions: The results seem to show differences between male and female mountain runners in anthropometric characteristics and cardiorespiratory capacity and that anthropometric characteristics can influence the cardiorespiratory performance of mountain runners.

Key words:
Trail running. Morphology. Body composition. Cardiovascular capacity. Endurance. High performance.

Características antropométricas y capacidad cardiorrespiratoria de corredores de montaña masculinos y femeninos

Resumen

Introducción: Los objetivos de este estudio fueron comparar las características antropométricas y cardiorrespiratorias de corredores de montaña masculinos y femeninos, y examinar la asociación entre las variables antropométricas y cardiorrespiratorias.
Material y métodos: Un total de 48 corredores de montaña, 16 mujeres y 32 hombres participaron en el estudio. Se midieron los parámetros antropométricos y se realizó un test incremental máximo en tapiz rodante con rampa para la valoración cardiorrespiratoria.
Resultados: Los resultados arrojaron diferencias significativas en las características antropométricas correspondientes a masa, talla, índice de masa corporal (IMC), perímetros, diámetros, % masa muscular, % masa residual y componente mesomórfico entre hombres y mujeres, siendo significativamente superiores en el grupo masculino ($p < 0,05$). El grupo femenino obtuvo en pliegues tricipital, muslo, pierna e ilecorestal, así como en la suma Σ 8 pliegues, % de grasa, en la masa grasa, masa ósea y en el componente endomórfico valores significativamente mayores ($p < 0,05$). Con respecto a la capacidad cardiorrespiratoria, el grupo masculino obtuvo valores significativamente mayores en el volumen sistólico inicial ($p < 0,01$, TE = -1,45, alto), consumo de oxígeno en el segundo umbral ventilatorio (VO_2VT2) ($p < 0,01$, TE = -1,66, alto) y consumo de oxígeno máximo ($VO_{2máx}$) ($p < 0,05$, TE = -1,78, alto). Se obtuvo una correlación alta entre la suma de 8 pliegues y el $VO_{2máx}$ ($r = -0,79$, $p < 0,01$), entre el % de grasa y el $VO_{2máx}$ ($r = -0,81$, $p < 0,01$) y entre el % de grasa y el VO_2VT2 ($r = -0,79$, $p < 0,01$).
Conclusiones: Los resultados parecen evidenciar diferencias entre corredores de montaña hombres y mujeres en las características antropométricas y en la capacidad cardiorrespiratoria y que las características antropométricas pueden influir en el rendimiento cardiorrespiratorio de los corredores de montaña.

Palabras clave:
Carreras de montaña. Morfología. Composición corporal. Capacidad cardiovascular. Resistencia. Alto rendimiento.

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Introduction

Trail running is a speciality that takes place in a natural setting[1], regulated by the International Trail Running Association (ITRA). Participants compete on irregular terrain, subject to different climatology and at variable altitudes oscillating between 0m and over 4,000m. Despite trail runs today covering different distances, the most common are competitions ranging from 42 km to over 100 km, lasting a single day or with stages spanning over various days[2]. Due to the characteristics of competition, athletes specialising in this modality are subject to high physical and physiological demands and require low fat percentages, a suitable percentage of muscle mass, major joint stability, and correct metabolic efficiency that enables them to maintain good running economy to ensure optimum performance[3,4]. Despite the major increase in popularity of trail races, to date there are few scientific studies that analyse these specific variables among this sporting demographic[5].

Due to the high physical demands of competition, anthropometry has been described as a relevant factor in sporting performance for this modality[6]. Specifically, in trail runners, a study carried out by Ramírez-Vélez *et al.*[5] analysed the body composition and somatotype of elite category runners, observing that participants had 13.3±3.2% body fat, a sum of the six folds of 67.8±32.0 mm and an endomorphic (3.6±1.5), mesomorphic (4.0±1.4) and ectomorphic (2.1±1.1) somatotype. Another recent study[7] analysed the differences in the anthropometric characteristics of trail runners of different sex, concluding that there were differences in the anthropometric characteristics of men and women (body mass index: 71.9±5.6 *vs.* 59.9±4.8 kg.m^{-2}, body fat percentage: 12.4±3.0 *vs*. 20.0±4.9%, total lean mass: 60.9±5.7 *vs*. 46.2±2.6%, total lean mass of legs: 20.1±1.9 *vs*. 15.5 ± 2.0). Despite there being anthropometric studies on trail runners in different contexts, as well as before and after competition[8], there are few studies that analyse the differences between male and female trail runners, as there have been in other resistance modalities[9-11]. Therefore, studies should be carried out that characterise and identify whether there are differences in the anthropometric characteristics and in the somatotype of trail runners depending on sex.

On the other hand, descriptions have claimed that as well as requiring a suitable body composition, cardiorespiratory capacity is another crucial factor in trail running performance[6]. This assessment is of interest to athletes and coaches in order to plan customised training sessions and as a sporting performance predictor[12]. For example, Hausswirth *et al.* (2015), analysed the cardiorespiratory capacity [maximum oxygen consumption (VO_2max), the maximum aerobic velocity and ventilatory thresholds (VT1 and VT2)] of trail runners. Along the same line, recent studies have also analysed oxygen consumption (VO_2), ventilatory thresholds (VT1 and VT2), lactate thresholds, running economy, mechanical and maximum power[14] or maximum heart rate (maxHR), the (VO_2max), ventilatory threshold (VT), the lactate concentration in the blood and rate of perceived exertion (RPE)[15]. Specifically, these authors[13] observed how the participating trail runners had a VO_2max of 62.0±63.9 ml.min^{-1}.kg^{-1}, maximum aerobic velocity (MAV) of 18.7±1.1 km.h^{-1}, a VT1 at 14.2±60.7 km.h^{-1} and a VT2 at 16.7±61.2 km.h^{-1}. Despite there being studies that assess trail runners' cardiorespiratory capacity[14,15], there are few examples of studies that analyse differences by sex. More studies may be required to analyse whether there are differences in the cardiorespiratory capacity of female and male trail runners.

Therefore, the aims of this study were: 1) to compare the anthropometric and cardiorespiratory characteristics of male and female trail runners, and 2) to examine the link between anthropometric characteristics and cardiorespiratory capacity.

Material and method

Participants

Forty-eight top level Colombian trail runners (30.81±2.56 years; 165.2±7.5 cm; 61.04±8.47 kg; 22.29±2.50 kg.m^{-2}) participated in this study. Of the total number of participants, 16 were female (29.5±7.6 years; 158.9±6.2 cm; 53.37±3.64 kg; 20.89±1.53 kg.m^{-2}) and 32 were male (32.0±12.7 years; 168.1±6.2 cm; 64.87±7.53 kg; 22.99±2.61 kg.m^{2}). All the participants competed frequently at national (Colombia) and international level, and actively trained between 4 to 6 days a week, with 1 or 2 days of rest or recovery. The training sessions held comprised 1 or 2 days of recovery running, 1 day of extensive running, 1 day of intensive running, 1 day of sets, 1 day of intervals, with participants performing 1 or 2 days of strength training. The relationship with the amount of training hours carried out by the athletes varied between 10 and 20 hours a week, reaching totals of between 5,000 and 10,000 m of accumulated gradient each week. The inclusion criteria for participation in this study were for the trail runners to be following structured and consecutive training, to have participated in official competitions during the previous quarter to the tests, and to have no injury, metabolic or inflammatory disease of any kind that may risk the athletes' health. Before performing the research, each of the athletes were informed about the protocols and the study test that would be carried out, and they all signed an informed consent form. The study was reviewed and approved by the Sports Science and Physical Education Faculty of the University of Cundinamarca (Fusagasugá, Colombia), which adhered to the ethical guidelines stipulated in the Helsinki Declaration (2013).

Procedure

The tests and measurements were performed in the University of Cundinamarca physiology department (Fusagasugá, Colombia), located approximately 1,700 m above sea level. In a single session, the participants attended the laboratory where their anthropometric measurements were taken, then a maximal incremental test was carried out on a treadmill. The laboratory conditions were between 21-23ºC in temperature and with 70-75% relative humidity. All participants indicated that they had refrained from performing intense exercise or taking caffeinated or alcoholic drinks in the 48 hours prior to the test session.

Measurements

Anthropometric assessment, body composition and somatotype: All the measurements were performed in adherence with the rules standardised by the International Society for the Advancement of Kinanthropometry (ISAK)[16]. Measurements were taken of the subscapularis, tricipital, abdominal, suprailiac, thigh, leg, bicipital and iliac crest folds with skin fold callipers (Harpenden, West Sussex, United Kingdom). Next, the participants' height was measured with a height rod (Seca 217, Hamburg, Germany), and body mass with a mechanical weighing scale (Detecto Scale, Missouri, USA). The perimeters of the neutral thorax, abdomen, hips, extended arm, bent arm, thigh and leg of each participant were measured with an anthropometric tape (Mabis Healthcare 35-780-010, Alabama, USA). The biachromial, transverse thorax, bi-ileocrestal and elbow measurements were taken with an anthropometer (GPM Siber Hegner Maschinery type Martin, Zurich, Switzerland). Finally, to establish the body composition and somatotype, the Spanish Group of Kineanthropometry (GREC, Spanish acronym), from which the percentage of muscle mass, bone mass and remaining were established, as well as mesomorphy, ectomorphy and endomorphy[17].

A maximum incremental test was performed on a treadmill with a ramp: all participants performed a maximum incremental test, adhering to the findings made by Davis *et al.*[18] to determine cardiorespiratory capacity. The test started with a warm-up at a constant speed of 3 km.h^{-1} for 3 minutes. Next, the speed was increased to 9 km.h^{-1}, the test commencing with an initial gradient of 0°. The slope progressively increased by 2º every minute, to eventually reach 15º. From this point, the speed was increased by 1 km.h^{-1} a minute, to reach volemia extenuation. Before starting the test, the resting heart rate (RHR) of each athlete was recorded using a band (Polar H_3, Kempele, Finland) and a heart rate monitor (Polar RCX_3, Kempele, Finland). Both before and after finishing the test, blood pressure (systolic and diastolic) was taken using an automatic blood pressure metre (Omrom M6, Kyoto, Japan), and the blood oxygen saturation (SpO_2) was measured with a finger pulse oximeter (Deluxe SM-110, Tustin, USA). Throughout the entire test, the heart rate (HR) of each athlete was recorded constantly using a band (Polar H_3, Kempele, Finland) and a heart rate monitor (Polar RCX_3, Kempele, Finland). Furthermore, during the test the athletes wore a portable system for measuring ventilatory parameters (Cortex Biophysik, MetaMax 3B portable CPX System, Leipzig, Germany) with an oronasal silicone mask (Hans Rudolph, Shawnee, USA). Data was analysed using specific software (Cortex Biophysik MetaSoft® Studio (MSS) CPX, Leipzig, Germany). To establish the ventilatory threshold 1 (VT1) and 2 (VT2), the method proposed by López-Chicarro J, *et al.*[19] was used. At the end of the test, all the athletes stated their reported perception of exertion (final RPE) using the Abellán Alemán[20] scale of 6-20.

Statistical analysis

The results are displayed as average±typical deviation (TD). The data normality test was performed using the Shapiro-Wilk test, and the homogeneity of variance (homoscedasticity) was measured using the Levene test. The variables followed a normal distribution, therefore parametric tests were performed. The differences between the male and female groups in anthropometric characteristics, body composition, somatotype and in cardiorespiratory capacity, were established using a t-test for independent samples. The difference of averages was also calculated (Dif. %) as well as the size of effect (SE)[21] to discover the differences between the groups for practical purposes. SE below (0.2), between (0.2 and 0.5), between (0.5 and 0.8), greater than (0.8) were considered trivial, low, moderate and high, respectively[21]. The associations between variables were calculated using the Pearson correlation coefficient (r). To interpret the correlation magnitudes, the following scale was used: lower than (0.1) trivial; from (0.1 to 0.3) low; from (0.3 to 0.5) moderate; from (0.5 to 0.7) high; (0.7 to 0.9) very high; more than (0.9) almost perfect. The data was analysed using the Statistical Package for Social Science (SPSS® Inc, version 23.0 for Windows, Chicago, IL, USA). The significance level was established at $p < 0.05$. In cases in which despite the differences not being significant, the sizes of effect were high (SE > 0.8), these were considered differences for practical purposes.

Results

The results corresponding to the anthropometric characteristics, the body composition and the somatotype of the trail runners that participated in this study, as well as the comparison by sex, are displayed in Table 1, Figure 1 and Figure 2. The results obtained in this study reveal significant differences between the male and female groups, with values corresponding to mass, height, BMI, perimeters, diameters, muscle mass percentage, remaining percentage and mesomorphic component being higher in the male group ($p< 0.01$ or $p < 0.05$). However, the group of female runners obtained significantly higher values ($p< 0.01$ or $p < 0.05$) in the tricipital, thigh, leg and ileocrestal folds, as well as in the sum of folds, in fat percentage, in fat mass, bone mass and in the endomorphic component. No significant differences were observed in terms of subscapularis, abdominal, suprailiac and ileocrestal folds, in the perimeter of the hips and thighs, and in the ectomorphic component.

Table 2 displays the results of the maximum incremental test obtained in the laboratory for the trail runners. The male group obtained significantly higher values in the initial systolic value ($p < 0.01$, SE = -1.45, high), in the VO_2VT2 ($p < 0.01$, SE = -1.66 high) and in the VO_2max ($p < 0.05$, SE = -1.78 low). However, the female group of runners obtained significantly higher values than the male group in $SaPO_2$ Max. exercise ($p < 0.01$, SE = 1.16, high).

With regards to the association between the age of the runners and anthropometric characteristics, a low correlation was observed (r = 0.37 to 0.49, $p < 0.01$) between age and weight, abdominal fold, BMI, neutral thorax perimeter, bent arm perimeter, transverse thorax diameter, bone mass percentage, remaining percentage and weight index, and a moderate correlation (r = 0.51 to -0.65, $p < 0.01$) between age and the abdominal perimeter, extended arm perimeter and the peak HR in the maximum incremental test.

Finally, regarding the link between the anthropometric or body composition characteristics and the incremental test results in the

Table 1. Results of the anthropometric characteristics, body composition and somatotype of all participants and differences depending on the sex of mountain runners.

Variable	All	Males	Females	Dif. (%)	SE	*P*
General						
Mass (Kg)	61.04±8.47	64.87±7.53	53.37±3.64	-17.72	-2.06	**
Height (cm)	165.02±7.49	168.06±6.15	158.94±6.18	-5.43	-1.48	**
BMI (kg.m^{-2})	22.29±2.50	22.99±2.61	20.89±1.53	-9.11	-1.01	*
Folds						
Subscapularis (mm)	13.21±5.16	12.85±5.20	13.98±5.18	8.34	0.21	
Tricipital (mm)	9.53±3.04	7.86±1.61	12.86±2.42	63.58	2.48	**
Abdominal (mm)	17.43±6.67	16.66±7.10	18.97±5.61	13.88	0.36	
Suprailiac (mm)	10.72±5.24	9.78±5.70	12.59±3.64	28.80	0.60	
Thigh (mm)	11.98±6.01	9.10±3.03	17.74±6.40	94.98	1.83	**
Leg (mm)	8.04±4.67	5.90±1.96	12.32±5.57	108.68	1.70	**
Bicipital (mm)	4.64±2.43	3.59±1.31	6.73±2.80	87.47	1.53	**
Iliocrestal (mm)	11.65±5.92	10.77±6.48	13.39±4.27	24.34	0.49	
Σ 8 Folds (mm)	87.19±29.88	76.51±26.37	108.53±24.14	41.85	1.24	**
Perimeters						
Neutral thorax (cm)	92.13±7.27	95.38±5.79	85.64±5.37	-10.21	-1.75	**
Abdomen 2 (cm)	79.15±6.92	81.32±6.44	74.80±5.84	-8.02	-1.06	**
Hips (cm)	92.71±3.97	92.74±4.41	92.66±3.03	-0.09	-0.02	
Extended arm (cm)	28.15±2.37	28.85±2.27	26.73±1.94	-7.35	-1.01	*
Bent arm (cm)	29.26±2.80	30.51±2.42	26.78±1.61	-12.21	-1.85	**
Forearm (cm)	24.50±1.95	25.51±1.38	22.49±1.22	-11.82	-2.31	**
Thigh (cm)	55.15±7.00	55.66±8.00	54.13±2.51	-2.75	-0.28	
Leg (cm)	34.50±2.26	35.06±2.33	33.48±1.75	-4.50	-0.77	*
Diameters						
Biachromial (cm)	37.22±2.72	38.23±2.63	35.22±1.57	-7.86	-1.43	**
Transverse thorax (cm)	27.43±2.25	28.43±1.93	25.43±1.34	-10.56	-1.84	**
Bi-iliocrestal (cm)	27.99±2.91	27.90±1.36	28.16±4.75	0.92	0.08	
Elbow (cm)	6.38±0.54	6.62±0.40	5.89±0.46	-11.04	-1.70	**
Body composition						
Waist and hips index	22.29±2.50	0.54±0.20	0.78±0.34	44.69	0.89	*
Fat mass %	14.36±5.13	12.45±4.75	18.18±3.52	46.02	1.38	**
Bone mass %	26.57±5.31	24.87±5.34	29.97±3.29	20.49	1.18	**
Muscle mass %	42.92±4.05	44.41±3.57	39.93±3.31	-10.08	-1.30	**
Rest %	16.86±6.95	19.64±6.46	11.29±3.95	-42.51	-1.60	**
Somatotype						
Endo	4.28±5.40	3.17±1.14	6.50±8.99	105.49	0.66	*
Meso	4.28±5.40	4.81±1.29	4.07±0.80	-15.35	-0.71	*
Ecto	4.56±1.19	2.19±1.14	2.33±0.92	6.06	0.13	
Weight index	42.02±1.60	41.91±1.75	42.23±1.27	0.77	0.21	

Dif. (%): difference of averages in percentage; SE: size of effect; BMI: Body Mass Index; Σ: sum of the 8 folds; Endo: endomorphic component; Meso: mesomorphic component; Ecto: ectomorphic component. **(p<0.01) *(p<0.05) significant difference between the male and female group.

laboratory, a moderate correlation was observed between most of the skin folds or the body composition variables with the results obtained in the incremental test. A high correlation between the Σ folds and the VO_2max was obtained (r = -0.79, p < 0.01) (Figure 3A), between the fat % and the VO_2max (r = -0.81, $p < 0.01$) (Figure 3B) and between the fat % and the VO_2VT2 (r = -0.79, $p < 0.01$) (Figure 3C).

Discussion

The aims of this study were to compare the anthropometric and cardiorespiratory characteristics of male and female trail runners, and to examine the link between anthropometric characteristics and cardiorespiratory capacity. Despite there being studies that analyse the anthropometric characteristics and cardiorespiratory performance of trail runners[5,7,12,14], few studies analyse the differences between male and female trail runners. Despite differences in sex being well established in terms of physical, physiological and metabolic factors in the different sporting modalities, study protocols are heterogeneous and continue to be far from conclusive among highly-trained or elite athletes, as the differences between male and female athletes are fewer the higher the level of performance compared to amateur athletes[23].

Knowing the anthropometric characteristics of resistance runners could be relevant as an important link has been described with competi-

Table 2. Results of the maximum incremental test in the laboratory obtained from the total of all participants and differentiated by the sex of the trail runners.

Variable	All	Males	Females	Dif. (%)	SE	*P*
Resting values						
Resting HR (beats min^{-1})	64.50±11.55	63.63±11.52	66.25±11.78	4.12	0.22	
Reserve HR (beats min^{-1})	118.67±13.96	122.03±14.57	111.94±9.96	-8.27	-0.82	
Initial systole (mmHg)	123.15±14.39	128.88±12.17	111.69±11.53	-13.34	-1.45	**
Initial diastole (mmHg)	76.56±9.31	77.38±10.10	74.94±7.52	-3.15	-0.27	
Resting $SaPO_2$ (mmHg)	96.88±1.41	96.72±1.51	97.19±1.17	0.48	0.35	
Resting VO_2 (ml·kg^{-1}·min^{-1})	8.23±4.57	7.28±1.42	10.13±3.46	39.05	0.64	
VT1 values						
VO_2 VT1 (ml·kg^{-1}·min^{-1})	42.33±7.80	44.19±8.34	38.63±5.00	-12.58	-0.83	
VT1% Peak VO_2 (ml·kg^{-1}·min^{-1})	74.59±12.09	72.06±11.27	79.66±12.45	10.55	0.64	
VT2 values						
VO_2 VT2 (ml·kg^{-1}·min^{-1})	52.33±8.50	55.94±7.50	45.13±5.51	-19.32	-1.66*	
VT2% Peak VO_2 (ml·kg^{-1}·min^{-1})	91.43±5.63	91.04±5.38	92.22±6.21	1.28	0.20	
Peak values						
Peak HR (beats min^{-1})	182.60±11.19	185.66±10.92	176.50±9.30	-4.93	-0.90	
Peak systolic value (mmHg)	149.60±25.16	156.81±24.96	135.19±19.04	-13.79	-0.98	
Peak diastolic value (mmHg)	82.85±9.34	84.03±8.60	80.50±10.57	-4.20	-0.36	
VO_2max (ml·kg^{-1}·min^{-1})	64.24±10.90	66.56±8.81	51.62±7.92	-22.45	-1.78**	
Peak $SaPO_2$ (mmHg)	91.52±3.87	90.31±3.96	93.94±2.16	4.01	1.16	*
Final RPE	15.69±2.43	16.13±1.72	14.81±3.35	-8.13	-0.51	

% Dif: percentage of difference; SE: size of effect; $SaPO_2$: Saturation of oxygen in the blood, HR: Heart rate; VO_2: oxygen volume; VT1: Ventilatory threshold 1; VT2: Ventilatory threshold 2, RPE: reported perception of exertion.
**(p<0.01) *(p<0.05) significant difference between the male and female group

Figure 1. Somato-chart females.

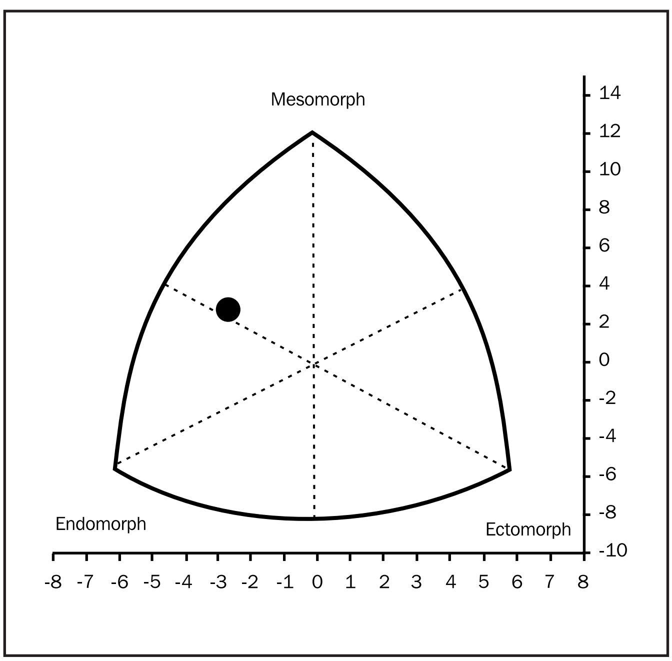
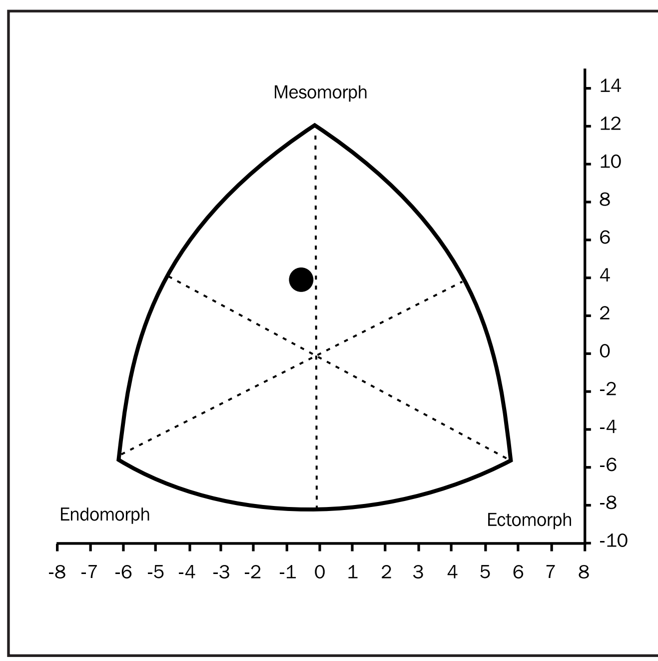
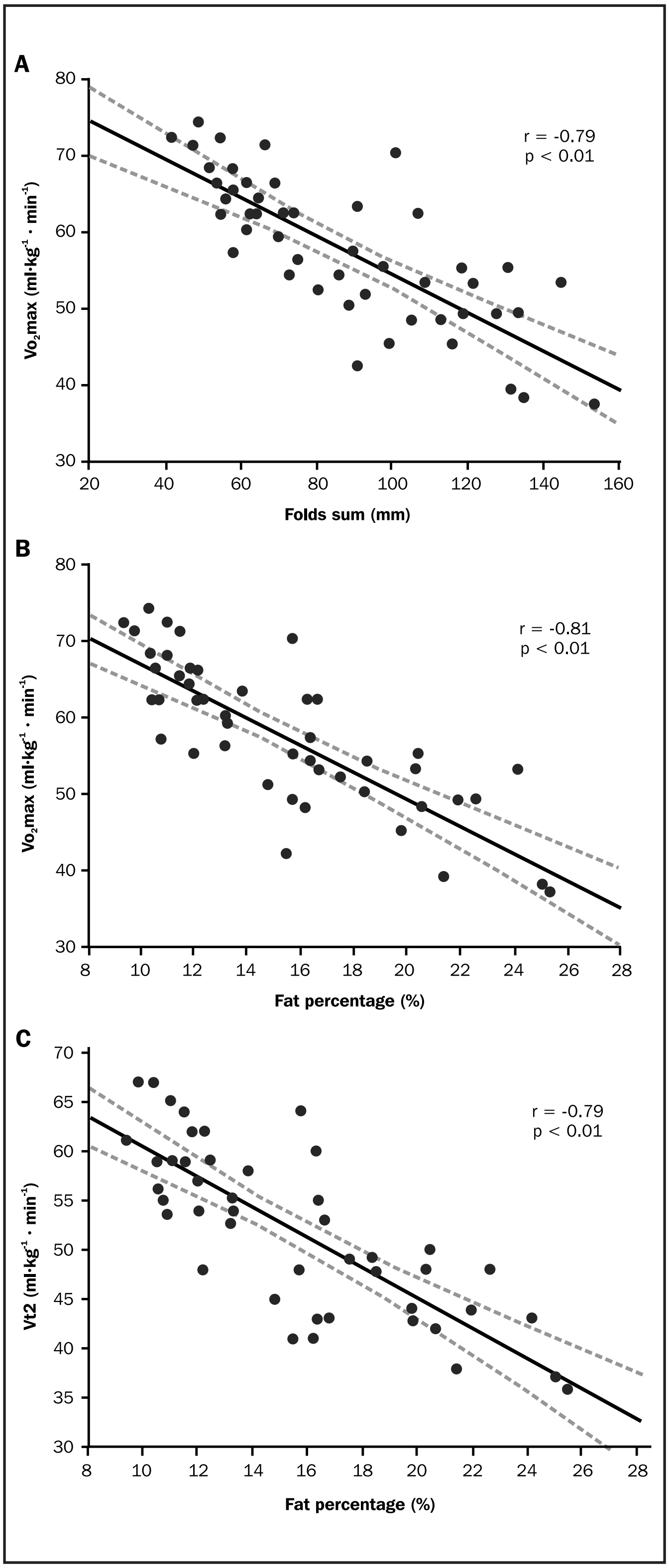


Figure 2. Somato-chart males.



tion performance[24]. Previous studies have revealed that anthropometric characteristics tend to be different in male and female athletes, due principally to sexual dimorphism[25]. However, it is not known whether these anthropometric characteristic differences by sex also occur in highly trained trail runners. A study performed on half marathon road runners claims that the females studied had higher skin fold values, a higher body fat percentage and lower muscle mass compared to the males[9]. Along the same lines, another study observes that among

Figure 3. Results of the correlation between the sum of folds and the VO_2max (3A), the fat percentage and the VO_2max (3B) and between the fat percentage and the VO_2VT2 (3C). In trail runners. VO_2max maximum oxygen consumption; VO_2VT2: oxygen consumption in the ventilatory threshold 2.



IronMan triathletes, there are significant differences between the fat percentage of male and female triathletes[11]. In a study performed on regional and national competing cyclists, it was observed that leg volume was significantly lower for female cyclists compared to male cyclists, a parameter linked to raw efficiency[10]. Most of the studies published in the literature that analyse the anthropometric characteristic differences by sex in other sporting modalities, coincide with the results obtained in our study - performed upon highly-trained trail runners - as in this study it has been observed that the females had a greater sum of the 8 skin folds and a higher fat mass than the males, whilst the group of male trail runners had a higher BMI and more muscle mass. It would appear that - as in other sporting modalities - sex can also condition the anthropometric characteristics of trail runners and can be a factor to consider due to the link this has with performance in resistance running[26]. It has been described how the anthropometric characteristics of runners depends significantly upon the nature of the competition, the energy requirements needed, the competition level, and the training and dietary habits of the athletes[27,28]. Considering that equally male and female runners must overcome major uneven ground in competition and in training, they may require suitable anthropometric characteristics that can enhance their performance[7].

In terms of cardiorespiratory capacity in other resistance sporting modalities, previous studies have been performed upon the difference between males and females[25-27]. For example, a previous study performed on mountaineer skiers observed that the males reached higher final speeds (17%) than the females in an incremental test, as well as higher VO_2max values (7-14%)[30]. Along the same lines, another study performed upon top-level competition cyclists, observed that male cyclists had a significantly higher VO_2max than the females, though these results varied depending on their level of training[10]. On the other hand, another study performed upon trained swimmers, who carried out a discontinuous incremental test, revealed higher VO_2max values in males, though the females displayed higher oxygen consumption linked to the first ventilatory threshold (VO_2VT1) and in the HR[32]. Most of the study results published coincide with those obtained in this study, in which the males displayed higher values than the females in reserve HR, in the initial systolic value, in the VO_2 VT2, in the peak HR and in the VO_2max. It appears that, just as in other resistance modality sports, cardiorespiratory capacity can be the determining factor in expressing the difference between highly trained male and female trail runners. These differences can be due to aspects such as body composition, biological potential, haemoglobin levels (Hb) and red blood cells, or the systolic volume factor (assiduously lower among females)[33], including other factors. However, considering that cardiorespiratory capacity can be improved with training[34], it could be particularly relevant for trail running training sessions to focus on improving this capacity.

Scientific literature includes a host of studies in different resistance sport modalities, analysing the link between anthropometric characteristics and cardiorespiratory parameters or sporting performance[31,32]. For example, a study performed on triathletes participating in the IronMan trial, reveals that BMI is linked to the total trial time and with the time achieved in the running sector[37]. Along the same lines, specifically among trail runners, a study of participants in the Western States Endurance Run, covering 161 km, reveals a significant correlation between BMI and race speed[38]. The results obtained in previous studies are consistent with the results from this study, in which a high and negative correlation was found between the Σ folds and the VO_2max (r = -0.79, $p < 0.01$), between fat % and the VO_2max (r = -0.81, $p < 0.01$) and between the fat % and the VO_2VT2 (r = -0.79, $p < 0.01$) among trail runners. Despite this study observing that a higher Σ folds or higher fat% had a detrimental effect on performance in an incremental laboratory test, it did not analyse the link between anthropometric characteristics and competition performance. Some studies have revealed that the anthropometric characteristics and body composition of athletes depend on the state of training, the distance covered in the trials, and the type of modality[39], therefore it would be interesting to analyse the influence that anthropometric characteristics could have on the competition performance of trail runners. However, in accordance with the results obtained in this study, the conclusion can be drawn that a lower fat % and a lower sum of folds, can be linked to improved cardiorespiratory capacity. Therefore, one of the training targets of trail runners could be geared towards reducing their fat % and the sum of folds.

This study is not exempt from limitations. The main constraint lies in the limited number of participants, and a lower number of female athletes. Future studies should perform assessments with a higher number of trail runners. On the other hand, this study analysed cardiorespiratory capacity in a laboratory test, thereby for later studies a field test could be suggested, a competition trial, or protocols that simulate competition. Finally, the incremental test was performed at approximately 1,726 m above sea level. This aspect may have considerably conditioned the results obtained by the runners.

Conclusions

The results obtained in this study highlight the existing differences between male and female trail runners in terms of anthropometric characteristics and cardiorespiratory capacity. The sum of skin folds and the fat percentage were higher among the females. In terms of the VO_2max the group of male runners obtained higher values than the group of females in reserve HR, in initial systolic volume, in the VO_2VT2, in peak HR and in the VO_2max. On the other hand, the results obtained in this study display a significant and high inverse correlation between the fat percentage and the VO_2max and the fat percentage and the VO_2VT2, highlighting how anthropometric characteristics may influence the cardiorespiratory performance of trail runners.

Conflict of interests

The authors claim to have no conflict of interest whatsoever.

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Effects of oophorectomy on the lumbar spine of Wistar rats submitted to vibratory platform treatment

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Summary

Introduction: This study evaluated the effect of the vibratory platform on the lumbar spine morphology of Wistar rats submitted to hormonal deprivation by oophorectomy.
Material and method: Twenty rats were divided into four groups: Pseudo-oophorectomy (GP), Pseudo-oophorectomy Treated (GPT), Oophorectomy (GO), Oophorectomy Treated (GOT). After 60 days of postoperative, the treated groups started exercises on a vibratory platform in the frequency parameters of 60 Hz, alternating vibration with amplitude of 2 mm, acceleration of 57.6 m/s^2 and time of 10 minutes, three times in the during the four week period. Following the euthanasia of the animals, the lumbar spine was dissected and processed for analysis of the anterior and posterior longitudinal ligaments, intervertebral disc height (IVD), nucleus pulposus (NP) height, radiographic density of the lumbar vertebrae and IVD morphology.
Results: The results showed a decrease in the height of the NP associated to the GO and morphological alterations such as lamellar disorganization and presence of rifts in the fibrous ring, alterations of the structural limits and decrease of the NP cavity; while the other groups presented organized lamellae, peripheral and nuclear region well delimited and wide cavity of the nucleus.
Conclusion: In this way, it can be concluded that the oophorectomy protocol promoted a decrease in the height of the NP of the IVD and that the exercise in the vibratory platform did not generate lesions in the analyzed tissues, as well as preserved the structural organization of the IVD and the height of the NP of oophorectomized rats.

Key words:
Osteoporosis. Menopause. Vibration. Exercise therapy. Physical therapy modalities.

Efectos de la ooforectomía en la columna lumbar de las ratas de Wistar sometidas a tratamiento con plataforma vibratoria

Resumen

Introducción: Este estudio evaluó el efecto de la plataforma vibratoria sobre la morfología de la columna lumbar de las ratas de Wistar sometidas a privación hormonal por ooforectomía.
Material y método: Veinte ratas fueron divididas en cuatro grupos: Pseudo-ooforectomía (GP), Pseudo-ooforectomía tratada (GPT), Ooforectomía (GO), Ooforectomía tratada (GOT). Después de 60 días de postoperatorio, los grupos tratados iniciaron los ejercicios en una plataforma vibratoria en los parámetros de frecuencia de 60 Hz, alternando vibración con amplitud de 2 mm, aceleración de 57,6 m/s^2 y tiempo de 10 minutos, tres veces en el período de cuatro semanas. Tras la eutanasia de los animales, la columna lumbar fue diseccionada y procesada para el análisis de los ligamentos longitudinales anteriores y posteriores, la altura del disco intervertebral (DIV), la altura del núcleo pulposo (NP), la densidad radiográfica de las vértebras lumbares y la morfología del DIV.
Resultados: Los resultados mostraron una disminución en la altura del PN asociado al GO y alteraciones morfológicas tales como desorganización laminar y presencia de fisuras en el anillo fibroso, alteraciones de los límites estructurales y disminución de la cavidad del PN; mientras que los otros grupos presentaron láminas organizadas, región periférica y nuclear bien delimitadas y amplia cavidad del núcleo.
Conclusión: De esta manera, se puede concluir que el protocolo de ooforectomía promovió una disminución en la altura del PN de la IVD y que el ejercicio en la plataforma vibratoria no generó lesiones en los tejidos analizados, además de preservar la organización estructural de la IVD y la altura de la NP de las ratas ooforectomizadas.

Palabras clave:
Osteoporosis. Menopausia. Vibración. Terapia por ejercicio. Modalidades de fisioterapia.

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Introduction

The increase in life expectancy has led to the growth of the world's aging population and, therefore, there has been an increase in the number of age-related degenerative diseases that modify vital body structures, such as the bone system[1]. Osteoporosis is a systemic disease that manifests itself strongly in the skeletal system, restricting the process of bone mineralization, which contributes to the destruction of its microarchitecture[2,3]. The individual develops a greater risk of suffering fractures with consequent complications, putting their functionality at risk[4,5].

The clinical manifestation of osteoporosis in women correlates with the menopausal period, a female physiological condition characterized by a decrease in the production of estrogen and progesterone, substantial hormones of skeletal maintenance[6]. In these circumstances, the bone resorption process becomes more pronounced than the one of synthesis, generating a structural imbalance, an important factor in the characterization of the disease[7,8]. There is an expected that 50% of women after 50 years of age will suffer from a fracture caused by low bone mineral density, with vertebral injuries being more frequent[9]. After the initial episode, which in turn may be asymptomatic, the individual is exposed to the risk of developing additional fractures[10].

Among the forms of treatment and prevention of this skeletal dysfunction is the use of pharmacological therapy with medications, supplements or even replacement therapy with synthetic hormones[11,12]. However, these methods sometimes require the need for daily injections and are related to ineffectiveness or acute side effects, such as musculoskeletal pain and gastrointestinal symptoms, and in the long term by vascular diseases or even breast carcinoma[13,14].

Thus, it is recommended to practice physical exercises for bone maintenance, this being the main non-pharmacological intervention for the prevention and symptomatic treatment of osteoporosis, assisting in bone structural repair as well as gain of strength and resistance and adjacent structures stability, providing systemic benefits[15,16]. Physical exercise directly interferes with modifiable risk factors, such as obesity, sedentary lifestyle, and consequent hormonal disturbances that favors the establishment of osteoporosis, acting on the maintenance of lean mass and bone mineral density[17,18].

Among the treatment modalities, the whole-body vibration (WBV) presents as an alternative to developing better cardiovascular (increase in blood flow, vasodilation due to NO release and reduction of endothelin-1 levels, sympathetic-vagal balance) and neuromuscular conditions, due to the vibration tonic reflex causing excitement of muscle spindles, generating slow and fast fiber hypertrophy, increased hormone secretion and proprioceptive stimulation[19,20]. By means of oscillations generated by the equipment, mechanical stimuli are produced in the body, which in turn, generate a mechanical load that promotes an anabolic bone response by the action of the piezoelectric effect, making the bone structure more resistant to loads[20,21].

However, biodynamic responses to vibration depend on pre-set treatment parameters. Since the vibration magnitude varies as the oscillation attenuation occurs and also diverges according to the tissue location and composition[19,22]. Studies have shown that vibration can cause deleterious effects on the vertebral column, reproductive, visual and auditory systems due to the resonance phenomenon, then careful evaluation is necessary for the safe use of WBV[23,24].

In view of the above, there is necessary to have research that ensures treatment resources for the most susceptible individuals, among them, the population with osteoporosis. The literature also presents an extensive range of treatment parameters, making bone synthesis and muscle anabolism possible, but the WBV action on the connective tissue is still not well elucidated. The aims of the study were to verify the effects of hormonal deprivation and treatment with WBV on the histomorphometry of the anterior and posterior longitudinal ligaments, intervertebral discs of the lumbar spine and the radiographic density of the lumbar vertebrae of Wistar rats submitted to oophorectomy.

Material and method

This study characterizes as experimental, transversal and quantitative. It was developing at the Universidade Estadual do Oeste do Paraná (UNIOESTE). The project was conducted according to the international standards of ethics in animal experimentation and approved by the Ethics Committee in Animal Use of UNIOESTE.

Sample

The sample group consisted of 20 female Wistar rats, nulliparous, with mean weight and initial age of 177.20±16.32 g and 8±1 weeks, respectively, kept in standard polypropylene boxes in temperature of 23±1ºC, with a photoperiod of 12 hours, receiving water and feed ad libitum.

The animals were randomly distributed in the pseudo-oophorectomy (PGs) and oophorectomy (OGs) groups, both with 10 animals each. These two groups were further subdivided into two other groups:

- PGs (n=10) – pseudo-oophorectomized and subdivided animals:
  - PG (n=5) – only submitted to pseudo-oophorectomy, euthanized at 4 weeks.
  - VPG (n=5) – undergoing pseudo-oophorectomy and vibrating platform for 4 weeks.
- OGs (n=10) – animals submitted to oophorectomy and subdivided:
  - OG (n=5) – only submitted to oophorectomy, euthanized at 4 weeks.
  - VOG (n=5) – undergoing oophorectomy and vibrating platform for 4 weeks.

The development process of the study occurred after performing oophorectomy and pseudo-oophorectomy surgery, followed by the vibratory platform training for four weeks, ending with euthanasia and collection of the biological material for histological analysis.

Protocol of oophorectomy and pseudo-oophorectomy

For oophorectomy, pseudo-oophorectomy and euthanasia, the rats were weighed and submitted to an anesthesia protocol consisting of intraperitoneal injection with ketamine hydrochloride (Dopalen – Ceva, Brazil) 100mg/kg) and xylazine (Anasedan – Ceva, Brazil) 50mg/kg).

Oophorectomy and pseudo-oophorectomy procedures were done in the 8th week of life of the animals. After anesthesia, the trichotomy and asepsis were performed with iodinated alcohol in the lower belly, then a longitudinal surgical incision was performed with a scalpel blade number 11. After accessing the peritoneal cavity, the adipose tissue was removed until the uterine tubes and ovaries. Then, a simple catgut 4.0 wire sutures was performed in the uterine horn area, promoting the resection of the ovaries bilateral. At the end of the procedure, internal suture was performed with a single catgut 4.0 resorbable wire and external ones with nylon 4.0. This protocol is the most suitable for performing oophorectomy in experimental models because it is easy, fast and allows a better recovery for the animals[25].

Pseudo-oophorectomy consisted of performing all surgical steps similar to oophorectomy, with the exception of removal of the ovaries. It was considered the control group, but its purpose is to subject the animals to the same stress caused by the surgery. Subsequent to this process, the rats remained for 60 days without any intervention, free in the cage for the induction period of the effects of the hormonal deficit.

Vibratory platform protocol

For WBV treatment, the Arktus® (Brazil) professional tri-plane oscillating vibration platform was used. The protocol used was adapted from Butezloff *et al.*[26], using 60 Hz frequency and indirect alternating sine-wave vibrations, with an amplitude of 2 millimeters, acceleration of 57.6 m/s^2, for 10 minutes, three times a week. The treatment started from the 9th to the 12th postoperative week with a duration of 4 weeks.

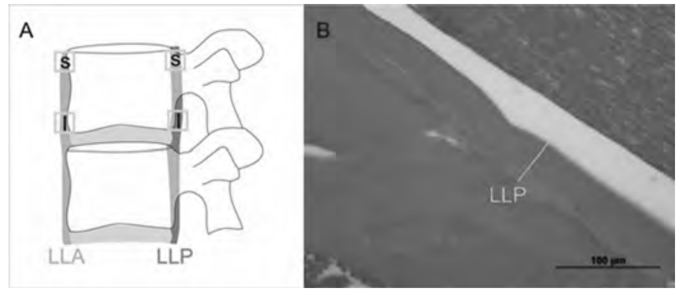
A support developed by the researchers was utilized according to the dimensions of the vibration platform. The use of support was designed to contain the animal during the treatment with the vibration and to allow the accomplishment of the training with several animals simultaneously. This support was made with MDF wood, white, and allowed to position eight animals concomitantly in the stalls (13 cm wide, 19 cm long and 25 cm high). Also, in order to minimize a possible bias regarding the positioning of the animals on different areas of the platform, a rotation between the bays was made, causing the animals to alternate their place of residence during training. The apparatus was put on the platform avoiding contact with the surface so as not to hinder the amplitude. Groups PG and OG were also positioned on the apparatus without the vibration.

Histomorphometric analysis

Thickness of anterior and posterior longitudinal ligament

For histomorphometric analysis the lumbar vertebrae were dissected, fixed at 10% buffered formalin for 24 hours, at room temperature. Subsequently, decalcification was performed with 5% trichloroacetic acid for 25 days. After that time, the bones were washed for 1 hour with running water and 1 hour with distilled water, cleaved longitudinally, dehydrated, diaphanized and embedded in histological paraffin to be sectioned at a thickness of 7 micrometers (μm), using a histological microtome. After the blades were prepared, they were stained with hematoxylin-eosin (HE). The images were obtained by photomicrography and analyzed in the program Image-Pro Plus version 6.0 (Media Cybernetics, USA).

Figure 1. (A) Illustrative representation of the quadrants selected for analysis of anterior (ALL) and posterior (PLL) longitudinal ligaments in the upper (U) and lower (L) quadrants. (B) Photomicrography of the posterior longitudinal Wistar rat ligament, hematoxylin and eosin staining. Demonstration of thickness measurement (yellow line).



Each lumbar vertebra was analyzed in four quadrants: two quadrants for Anterior Longitudinal Ligament (ALL – upper and lower) and two in the Posterior Longitudinal Ligament (PLL – upper and lower). The images were obtained at a magnification of 400x, just below the articular cartilage and the apparent chondrocyte concentration, for the superior images, and immediately above the articular cartilage and the apparent chondrocyte concentration, for the inferior images[27]. The thickness of the longitudinal ligaments was obtained from the 5th lumbar vertebrae, drawing a line to measure its width in micrometers (μm) and positioned at the center of the image at 50% zoom using Image Pro Plus 6.0 program (Media Cybernetics, USA). In the presence of space in the ligaments, resulting from the artifacts, was not considered in the total measure. Morphological analysis was performed in each quadrant, observing the presence of chondrocytes between connective tissue fibers and the presence of neovascularization (Figure 1).

Intervertebral disc and nucleus pulposus

The visual fields of interest in the slides were selected and the images were recorded in the 10x magnification for the intervertebral disc (IVD) and the nucleus pulposus (NP). The images obtained was recorded in JPEG format and analyzed in the Image-Pro-Plus version 6.0® (Media Cybernetics, USA).

In the measurement of the IVD, a distance of 1.0 mm from the PLL was used for standardization to measure H1 (height of the posterior portion of the disc); and from the ALL for the measurement of H3 (height of the anterior portion of the disc); H2 and H4 were measured from the midpoint of the width of the DIV for the verification of the disk height and the NP height and area, respectively (Figure 2).

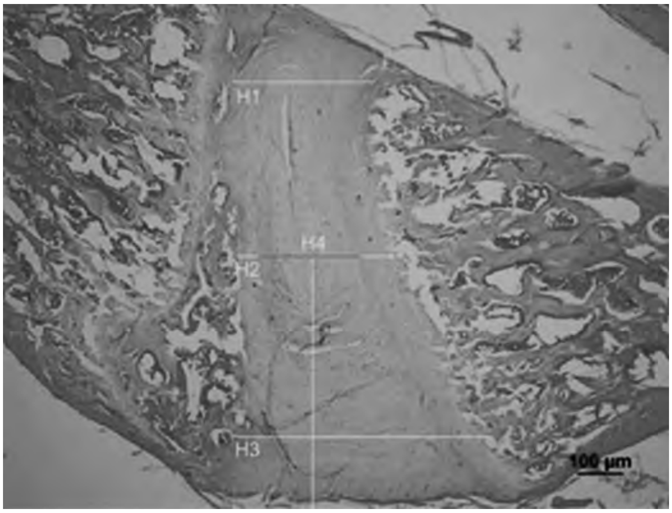
Histology of the intervertebral disc

For the analysis of the IVD, the morphology of the fibrous ring (FR) and NP were assessed in relation to the histologic pattern of normality or presence of alterations[28].

Radiographic density analysis

Radiographies measurements were performed on the lumbar spine segment after collection of the lumbar segment using a x-ray equipment

Figure 2. Photomicrograph representing the analysis of the height of the intervertebral disc, in the anterior (H1), middle (H2), posterior (H3) and nucleus pulposus (H4).



(Gnatus, Brazil), 70 kVp-7 mA, with a focus-sensor distance of 250 mm, for an exposure time of 0.6 seconds and axis positioned perpendicular to the material. The direct digital image was generated using the Kodak Dental Imaging Software - 6.12.32.0 (Kodak, USA), connected to a sensor where the lumbar segment was positioned on its surface. For the densitometry reference, an eight-step aluminum penetrometer was placed parallel to the vertebrae also on the sensor, towards the main X-ray beam. The digitized images were saved with TIFF extension for better resolution during subsequent analysis.

The evaluation of bone radiopacity was performed using the Image J (National Institute of Health, USA) program, calibrated to select 8 aluminum steps for measuring the amount of gray tone of the material[29]. A region of interest of 60 x 20 pixels was delimited for quantification of gray tone present in the body of the fifth lumbar vertebra of animals in all groups[30]. The results were tabulated using the Microsoft Excel 2013® (Microsoft, USA) program and, therefore, the radiographic density was expressed in millimeters of aluminum (mmAl) after subsequent conversion[31].

Statistical analysis

The SPSS 20.0 (IBM, USA) program was used to perform the statistical analysis. The data were presented in mean and standard error, in addition to the confidence interval (95%), the inferential analysis was performed with the Generalized Linear Models test and the LSD post-test (Fisher's Least Significant Difference), in both cases the accepted significance level was 5%.

Results

By means of the averages of the ligament thicknesses of the upper and lower regions, it was observed that for the LLA in its upper portion, the VPG showed values higher than VOG. In the mean values, there was also a difference in LLA, and VOG showed lower values than the other groups (Table 1).

Table 1. Mean and standard error (below confidence interval) of the thicknesses of the anterior and posterior longitudinal ligaments of upper and lower vertebral region and total means by ligament.

	PG	VPG	OG	VOG
ALL Upp	237.45 ± 24.09 194.63 – 289.70	304.67 ± 30.92 249.72 – 371.71	238.00 ± 24.15 195.08 – 290.37	195.38 ± 19.83 a 160.14 – 290.37
p-value		0.022		
ALL Low	245.92 ± 36.21 184.28 – 328.19	234.65 ± 34.55 184.28 – 328.19	253.25 ± 37.29 189.77 – 337.96	177.29 ± 26.10 184.28 – 328.19
p-value		0.295		
PLL Upp	269.84 ± 30.51 216.20 – 336.78	232.12 ± 26.25 185.98 – 289.71	256.84 ± 29.04 205.79 – 320.56	252.71 ± 28.57 202.48 – 315.41
p-value		0.820		
PLL Low	253.49 ± 27.27 205.30 – 312.98	254.03 ± 27.33 205.30 – 312.98	283.97± 30.55 229.99 – 350.62	215.87 ± 23.22 174.84 – 266.53
p-value		0.347		
ALL	241.69 ± 21.35 203.27 – 287.37	269.66 ± 23.82 226.79 – 320.64	245.62 ± 21.70 206.58 – 292.05	186.34 ± 16.46 b 156.71 – 221.56
p-value		0.022		
PLL	261.66 ± 24.25 218.03 – 314.03	243.08 ± 22.62 202.55 – 291.73	270.41 ± 25.17 225.32 – 324.52	234.29 ± 21.81 195.22 – 281.18
p-value		0.682		

a - difference between VGP and VOG ALL Upp (p=0.003). b - difference between VOG with PG (0.040), VPG (0.004) and OG (p=0.029).

Table 2. Means and standard error (below confidence interval) of the measurement of the intervertebral disc height, as to the height of the nucleus pulposus and the intervertebral disc: an anterior portion, middle portion and a posterior portion.

	PG	VPG	OG	VOG
NP	13.62 ± 1.31 11.28 – 16.45	15.01 ± 1.45 12.43 – 18.13	13.42 ± 1.29 11.11 – 16.21	18.57 ± 1.79 15.37 – 22.43
p-value	0.065			
Anterior	54.60 ± 4.72 45.34 – 63.86	58.82 ± 4.72 49.56 – 68.08	53.12 ± 4.72 43.86 – 62.38	59.03 ± 4.72 49.77 – 68.28
p-value	0.753			
Medium	34.99 ± 2.22 30.89 – 39.64	39.12 ± 2.49 34.53 – 44.31	37.12 ± 2.36 32.77 – 42.05	34.44 ± 2.19 30.40 – 39.01
p-value	0.471			
Posterior	29.56 ± 2.64 24.81 – 35.21	29.87 ± 2.67 25.07 – 35.59	26.14 ± 2.34 21.94 – 31.14	35.52 ± 3.18 29.81 – 42.32
p-value	0.113			

Table 3. Means and standard error (below confidence interval) for radiographic density (RD) analysis.

	PG	VPG	OG	VOG
RD	13.36 ± 0.63 12.19 – 14.64	13.43 ± 0.63 12.26 – 14.73	12.69 ± 0.59 11.57 – 13.91	12.81 ± 0.60 11.69 – 14.04
p-value	0.766			

No morphological alterations were identified in the ligaments, such as neovascularization, chondrocyte invasion or the presence of other cells in the connective tissue medium in the analyzed regions.

For an analysis of the intervertebral disc height, there was no statistical difference (Table 2).

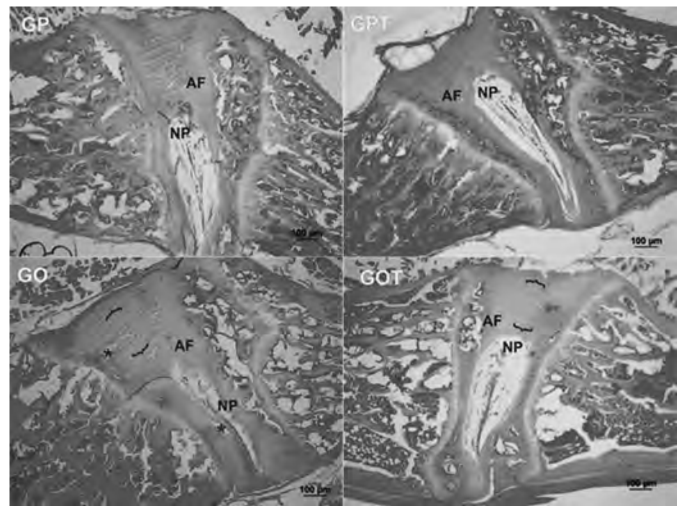
Regarding the radiographic density analysis, there were no differences between groups as showed in Table 3.

In the morphological analysis of the PG and VPG, the IVD presented normal appearance, with concentric layers of fibrocartilage forming the FR around the NP, discrete lamellae with bulging and without folds. NP formed by cells immersed in viscous extracellular matrix with large bulging of the central cavity, which represents about two-thirds of the height of the IVD (Figure 3).

The OG, presented alteration of the FR in the presence of opposite lamellae and the presence of small folds and oblique and transverse gaps. In the NP, it was verified a reduction of the cavity size, being smaller than one third of the height of the IVD, and alteration of the structural borders, which were poorly delimited.

The VOG also presented alterations in the organization of the connective tissue of the FR with slightly opposing lamellas, but better organizational structure. NP maintained a nuclear aspect similar to that of groups without hormonal deprivation.

Figure 3. Photomicrographs of the lumbar spine intervertebral disc of Wistars rats, longitudinal section, 7 µm, hematoxylin and eosin staining. In PG, pseudo-oophorectomized rats and VPG, treated pseudo-oophorectomized rats, with presence of the nucleus pulposus (NP), with due bulging, height and sharpness of the morphological limits, and fibrous ring (AF), with well disposed lamellae, without folds and presence of gaps. In OG, oophorectomized rats, presenting opposite lamellae, oblique and transverse gaps (asterisks) and cavity of the nucleus pulposus decreased and, in VOG, oophorectomized rats submitted to the vibratory treatment with the presence of slight lamellar opposition, but better disposed with preservation of the nuclear cavity.



Discussion

The present study indicated that there were alterations at the ALL thickness of the animals submitted to hormonal deprivation and WBV. Research shows that individuals with metabolic disorders present osteoporosis, with stiffening and thickening of the posterior longitudinal ligament from the age of forty years. However, there are not any data to indicate the onset of morphological changes after instilling injury[32,33]. Since changes have been observed that point to a reduction in the thickness of the ALL, it may be an indication of positive effects, i.e. although it decreases in thickness, it does not mean that it is less resistant, mainly because other characteristics have not been observed that worsen mechanical stress.

In the Simas *et al.*[7] study, rats with 60 days of oophorectomy, presented deleterious effects of the upper cartilage tissue of the tibia with a decrease in their thickness, proving that this period of hormonal deprivation is efficient to assign negative alterations to the connective tissue. The same postoperative period was also used in the present study, but just metrical differences were observed in the ALL. In a degenerative process such as osteoporosis, spinal-related ligaments and IVD suffer a chondrocyte invasion and proliferation of fibroblast-like spindle cells that make the connective structures more rigid, thicker and less resistant to mechanical stress[34,35].

For an analysis of the IVD height, no statistical differences between the groups were observed. Studies show that in osteoporotic individuals, who develop lumbar fractures, presents a narrowing of the IVD space, which is a risk factor for spinal biomechanics, due to the increase in vertical load on the peduncle and the articular facets[21,36].

At age-related changes or in the presence of degenerative diseases, IVD dehydration occurs where FR gets cracks in the boundaries between the periphery and the nucleus and the NP becomes more rigid. NP is a more susceptible structure because its anatomical location gives it greater exposure to stress when opposed to other connective tissues[37]. In this research, it was possible to observe that the OG obtained a lower NP height when compared to the other groups. On the other hand, VOG did not show statistical differences and this was a sign that the WBV preserved the central region more susceptible to IVD.

It has been hypothesized that exercise has not only favored the maintenance of the disc collagen. But also produced benefits to the stabilizing structures of the lumbar spine, since vibratory stimulations generate excitation of the primary muscle spindle through afferent type Ia neurons, producing a vibration tonic reflex[38]. This, contributes to the maintenance of muscle strength as well as maintenance of joint positioning[39].

McCann *et al.*[40] subjected mice to 45 Hz WBV, 74 µm peak-to-peak amplitude, 30 minutes a day, 5 days a week for 2, 4, and 8 weeks. The results showed that the group submitted to the 4-week treatment obtained a decrease in the height of the IVD. In a comparison of the parameters used, treatment protocol presented similar duration, but with less frequency and with only two days of interval between weeks of treatment. Thus, the frequency of 60 Hz associated with the treatment with a 1-day rest interval and in the period of only 10 minutes of time did not promote detrimental changes at the time of IVD, demonstrating at a safe range for the VPG and VOG.

Recently the therapeutic vibratory treatment demonstrated anabolic effects on several tissues, especially muscles and bones[34]. Due to its low biomechanical impact and the lack of great mobility and strength of the individual being treated. This resource presents itself as an exercise modality, pertinent to the aging population, individuals with neurological alterations and metabolic syndromes that commonly have difficulties to perform physical activities or impact[41,42].

The WBV is gaining more and more interest, due to the benefits that the modality presents to the varied corporeal tissues, of these it is emphasized the proprioceptive improvement, modulation of the reflex excitability of the vertebral column and modification of the excitability of the motor cortex. Vibration is also a form of dynamic mechanical loading, being a potent stimulation of osteogenesis[43].

Regarding the results obtained regarding radiographic density, no differences were observed between the analyzed groups. In the research conducted by Lam *et al.*[44], a WBV protocol of 32 to 37 Hz, amplitude of 0.085 mm, during 20 minutes/day, 5 times/week, at 52 weeks, resulted in an increase in the bone mineral density of the lumbar spine, whose vibration was used to treat adolescents with osteopenia and idiopathic scoliosis, demonstrating a positive effect over a longer treatment period compared to the present study.

Pasqualini *et al.*[24] compared the frequencies of 8 Hz, 52 Hz and 90 Hz, 10 minutes, 5 days a week for 28 days on the bone tissue, analyzing the second lumbar vertebra and the tibia. Research has shown that the lower frequency produced deleterious effects on bone mineral density, 52 Hz optimized vertebral microarchitecture and finally, the higher frequency provided benefits for both structures. The present study demonstrated that no deleterious effects were observed on the bone segments analyzed, since the radiographic density did not vary between groups.

In the present study, it was observed that OGs showed morphological alterations of lamellar structure disorganization and limits, presence of cracks as well as noticiable reduction of internal nuclear cavities. IVD is composed of FR externally with approximately 60% collagen and internally by NP, which in addition to collagen (20%) also presents proteoglycan proteins responsible for giving resistance and attracting water to the internal environment. The composition between collagen and proteoglycans confers the characteristic of resistance, elasticity and compressibility of articular cartilage, damping and dissipating the incoming forces and reducing friction during movements[45,46]. Metabolic syndromes or even aging lead to a decrease in proteoglycans associated with lower mechanical resistance of cartilage due to decreased hydration and increased susceptibility to collagenase[47], which are the possible causes of the alterations present in the OG.

For the treated groups, the morphology of the IVD did undergo major structural modifications. Only VOG showed subtle lamellar alterations due to the probable hormonal deprivation. But with better appearance when compared to the OG. The mechanical stress on the tissue generates a piezoelectric effect that, through compression of the bone matrix, cilia and membrane receptors present in the osteocytes detect the stimulus and promote bone synthesis[48].

Genetic and socioeconomic factors can act by modifying the composition of the IVD, for example, by decreasing the expression of type IX collagen that forms the support matrix. This can lead to annular fissures and promote structural and biomechanical changes favoring a degenerative process[23,49,50].

McCann *et al.*[51] performed a search on mice, submitted to 45 Hz vibrations, 30 minutes a day for 4 weeks. The histological analysis of a lumbar segment revealed signs of IVD degeneration, such as loss of the border between NP and FR, alterations in the disposition and disorganization of lamellar collagen and the presence of interlamellar spaces. Thus, the treatment parameters used in the present study ensured a better morphological pattern for oophorectomized rats.

There is a need for further studies that present the effect of vibratory therapy on osteoporosis as well as more extensive investigations that evaluate other tissues besides the bone and ligament, such as muscles, nerves and adjacent ligaments, in order to develop safe protocols that aid in efficient physiotherapeutic treatments as well as in the improvement of the patient quality of life. We suggest the implementation of protocols that contemplate long-term vibration in a model of hormonal deprivation, which is a limitation of this study.

Conclusion

The present study concluded that the hormonal deprivation model was an effect to promote structural alterations, added to WBV alter the

ALL thickness at the lumbar level, but not in the radiographic density of their respective vertebrae and height of the NP, but promoted morphological alterations in the IVD.

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Conflict of interest

The authors declare no conflict of interest.

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Obesity and physical exercise in adults
Obesidad y ejercicio físico en adultos

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Introduction

This paper analyses the scientific evidence on exercise in adults with overweight or obesity, and represents a consensus position of the Sociedad Española de Medicina del Deporte (Spanish Society of Sports Medicine).

It is clear that both the prevention and treatment of obesity requires a multifaceted approach focussing, to a considerable extent, on lifestyle. Within lifestyle, diet and combining it with exercise plays a key role.

In this paper, we will focus exclusively on physical exercise, given that significant changes have been witnessed in this field in recent years and a growing number of, sometimes contradictory, recommendations have been voiced.

First of all, we will address the existence of evidence regarding the role played by exercise (in its various forms) in the treatment and prevention of obesity. We will then analyse what is currently known about the functional assessment data which can be used to programme exercise in obese subjects. Finally, we will make an in-depth analysis of the evidence existing at this time regarding the role of both continuous and interval training in losing weight (and maintaining such loss) in obese subjects.

Current evidence on the role of exercise in weight loss in obese adults

Obesity is one of the biggest health problems currently faced by society and the most common factor of comorbidity in subjects with conditions which include metabolic disorders (metabolic syndrome, hypertension, dyslipidaemia, type 2 diabetes, fatty liver disease, cholelithiasis, tumours, polycystic ovaries, etc.), cardiovascular problems (hypertension, coronary heart disease and atrial fibrillation, among others), mechanical disorders (chiefly, hypoventilation, sleep apnoea and osteoarthritis) and psychosocial disorders (depression, social/job discrimination, low self-esteem, eating disorders)[1-3]. The prevalence of obesity in adults over 18 years of age in Spain is 18.2% for men and 16.7% women[4], with a sedentary population of 31.9% in men and 40.0% in women[5]. The WHO recently presented its Global Action Plan on Physical Activity 2018-2030 in an attempt at reducing physical inactivity and promoting health in the face of the worrying fact that non-communicable diseases (NCDs) are responsible for 71% of all deaths worldwide, including 15 million people aged between 30 and 70 every year[6].

Weight loss is associated with improvement in comorbidity, evidence of which has been cited for many years and has been corroborated more recently by various scientific studies, systematic reviews and meta-analyses conducted up to 2020, especially with regard the normalisation of the blood sugar level in type 2 diabetes and dyslipidaemia, decreased blood pressure[7-10] and symptoms of osteoarthritis[11], principally in obese individuals.

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Review of the most recent meta-analyses on the effect of exercise on weight loss

One way to lose weight within the field of lifestyle changes and keeping healthy is to do regular exercise[10,12-16], even before and after bariatric surgery[17].

Moderate-intensity physical activity for 150 to 250 min/week expending energy equivalent to 1,200 to 2,000 kcal/week would seem sufficient to prevent and avoid increases in weight of more than 3% in most adults, and can lead to moderate weight losses of 2 to 3 kg, the figure reaching 5 and 7.5 kg when exercise is performed for 225-420 min/week. The same recommendation applies to individuals with overweight and obesity, in which case a better response, involving greater weight loss and better weight maintenance, is likely[12].

The search for systematic reviews and/or meta-analyses until 2020 throws up several showing that exercise alone is not very effective for initial weight loss in people with obesity and that those who diet and exercise maintain their weight loss better than those who just diet, demonstrating that diet and exercise favour loss which can be maintained for at least 6 months[9,18,19].

It is important to remember that the WHO[6] defines "insufficient physical activity" in adults over 18 as moderate-intensity activity for less than 150 minutes per week and the American College of Sports Medicine (ACSM) states that there is a dose-response relationship with the prevention of weight gain, which is more pronounced when exposure to moderate-to-vigorous physical activity (≥3 METs, similar to brisk walking) exceeds 150 minutes per week[15]. There is insufficient evidence available to determine whether there is any association between light-intensity activity (<3 METs) and attenuated weight gain in adults, and moderate evidence to indicate that the relationship between greater amounts of physical activity and attenuated weight gain in adults does not appear to vary by sex.

Walking is not consistently associated with change in weight or BMI, or with the incidence of developing obesity. However, it is reported that walking 10,000 steps or more per day attenuates weight gain compared with not hitting 10,000 steps per day, which may suggest that high volumes of walking need to be achieved to attenuate weight gain[15]. In a similar vein, a significant inverse relationship has been observed between physical activity and weight gain, encompassing a broad age range including young, middle-aged and older adults of both sexes[15].

As for the amount of time per day devoted to exercise, according to the systematic review conducted by Jakicic *et al* on the relationship between the duration of physical activity and health[10], there is still reason to support the idea that physical activity accumulated in sessions lasting 10 minutes or more can improve a variety of health-related results, including the incidence of obesity, with reductions in weight, fat weight and BMI. Supplementary evidence also exists from cross-sectional and prospective cohort studies to suggest that physical activity accumulated in sessions lasting less than 10 minutes is also associated with favourable results, including mortality from all causes. With respect to the incidence of obesity, however, only one cross-sectional study indicates that sessions of less than 10 minutes are effective in terms of reducing BMI and fat weight[10].

Review of the evidence on different types of exercises and/or programmes

To improve the physical and mental health and/or fitness of most adults, the ACSM[20] recommends a comprehensive programme of exercise including cardiorespiratory, resistance, flexibility and neuromotor exercise of sufficient volume and quality (stretching, warm-up stages and gradual increase in intensity, duration and frequency), recommending effective strategies to reduce the musculoskeletal and CVD risks of exercise, including screening for and educating about prodromal signs and symptoms of cardiovascular disease[20]. In this vein, the American College of Cardiology/American Heart Association recommends a stress test for asymptomatic subjects with diabetes mellitus, men over 45 and women over 55 before starting a vigorous exercise programme to rule out cardiovascular risk factors and provide a guide for additional diagnostic tests[21].

The application of this comprehensive approach in obese adults is more complex given their sedentary lifestyle, making it essential to carry out a preliminary assessment of their functional capacity, limitations (mobility, overload/impact, impaired balance and response to heat, poorly controlled sleep apnoea, dyspnoea) and the risks to which they could be subjected (cardiovascular risks, joint and musculoskeletal injuries) in order to guide and prescribe individualised exercise[10,12-18,20-22].

When choosing the type of exercise, intensity, time and programme, recent meta-analyses to 2020 of obese adults of both sexes (chiefly, 18-to-65-year-olds) provide the following evidence:

- Resistance exercise would not appear to be an effective way to lose weight on its own, but it is associated with many other health benefits, including lowering different risk factors for chronic diseases (high blood pressure, dyslipidaemia, hyperglycaemia), increasing lean mass and decreasing fat mass, especially abdominal fat mass[10,18].
- 18 studies of exercise of up to 30 min/day lasting an average of 16 weeks (range 4-7 weeks) show a significant drop in body weight, BMI and fat weight, proving more effective when exceeding 10 weeks ($p < 0.05$) than in shorter intervention periods of ≤10 weeks[23].
- Short-term (5-16 weeks) moderate-intensity continuous training (MICT) and high-intensity interval training (HIIT) can lead to significant improvements ($p<0.05$) in body composition in people who are overweight and obese (fat weight and waist circumference), but no changes in body weight. There are no significant differences between HIIT and MICT for any body composition measurement, their proving similarly effective. HIIT, however, requires 40% less time, which may be an advantage in terms of efficiency in weight management programmes[24].

Takeaways

- Moderate-intensity activity from 150 to 250 min/week, expending energy equivalent to 1,200-2,000 kcal/week seems sufficient to prevent and avoid weight gain.
- Exercise alone is not very effective for initial weight loss in obese individuals. Those who both diet and do exercise hold their weight loss better than those who only diet or only do exercise.
- There is a dose-response relationship between exercise and weight gain prevention, which is more pronounced for moderate-to vigorous-intensity physical activity.
- Physical activity accumulated in sessions of 10 minutes or more can improve a range of health-related results, including the incidence of obesity, lowering weight, fat weight and BMI.
- Exercise of up to 30 min/day leads to a significant reduction in body weight, fat weight and BMI. This is more effective when done for more than 10 weeks.
- Interval exercise and continuous exercise do not seem to have very different effects. In the short term (5-16 weeks), both lead to significant improvements in body composition in people with overweight and obesity (fat weight and waist circumference).

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The contribution of stress tests when prescribing exercise in obese patients

Stress tests on overweight or obese people provide us with the same functional and health information as they do with any other subject, but with these patients they also supply extremely valuable information on energy expenditure.

We know that carbohydrates are the most important metabolic substrate during prolonged moderate-to-high-intensity exercise[1] and that the muscle glycogen stores are emptied when the exercise is sufficiently intense and lasts long enough[2,3]. Humans store an average of 740 g of glycogen, which represents about 3,000 kcal at 4 kcal/g[4].

However, fat reserves in humans are virtually limitless when exercising. A subject weighing 70 kg with a fat component of only 10% (7 kg) stores 68,250 kcal at 9.75 kcal/g[5].

Optimising fat oxidation, therefore, is not only of paramount interest for long-distance competitions and military operations, but also for health, its relationship with markers such as insulin sensitivity or weight gain having been demonstrated[6].

Indirect calorimetry

A subject's energy expenditure can be studied at rest or during exercise at a specific intensity or at different intensities. In constant load tests, the intensity is kept at a set value while, fundamentally, the effects of duration are explored.

Whichever the case, energy expenditure is studied by indirect calorimetry. Indirect calorimetry is the method by which the type and rate of substrate utilisation and energy metabolism are estimated *in vivo* based on gas exchange measurements[7]. This technique provides unique information, is not invasive and can be advantageously combined with other experimental methods to investigate numerous aspects of nutrient assimilation, thermogenesis, exercise energy and the pathogenesis of metabolic diseases.

Indirect calorimetry was fundamentally a research method until the 1980s and '90s, when it began to be used for clinical applications of interest[8]. It is now an essential tool to understand the mechanisms underlying overweight and obesity[9], and is considered the "gold standard" method to determine energy expenditure, by measuring lung gas exchange[10,11].

Different attempts have been made at predicting energy expenditure without any need for calorimetry (e.g. using HR), but to date none of these has been successful[12], meaning that for the time being indirect calorimetry is still indispensable in order to determine it.

First ventilatory threshold and Fatmax

In an incremental test, the fat oxidation rate (FOR) for each load, expressed in g/min, is obtained using the equation $1{,}695 \cdot VO_2 - 1{,}701\ VCO_2 - 1.77n$ for any intensity[13]. In this equation, "n" represents the urinary nitrogen excretion, which we assume is negligible and, therefore, n = 014. With the data from this equation, a 2nd order polynomial fit between FOR and exercise intensity is constructed. The highest FOR value in the entire intensity range is called Maximal Fat Oxidation (MFO) and the exercise intensity at which MFO occurs is called Fatmax. The range of intensities 10% either side of Fatmax is called the "Fatmax zone"[13].

The first ventilatory threshold (VT1) was first described by Hollmann at the Third Pan-American Congress for Sports Medicine held in Chicago in 1959[15], although the concept (and its implications) was subsequently consolidated by Wasserman and McIlroy in 1964[16]. The technique used today was definitively described by Reinhardt, Müller and Schmülling in 1979[17]. Since these pioneering studies, we have known that the metabolic meaning of the first ventilatory threshold lies precisely in the fact that it is the exercise intensity as of which glycolysis is activated (and, therefore, blood lactate accumulation begins) due to a drop in the fat oxidation rate. Therefore, VT1 and Fatmax conceptually describe very proximate, if not the same, intensities.

In fact, in obese subjects there is no significant difference between the intensities at which VT1 and Fatmax appear[13] ,and so the VT1 intensity is also a good indicator for exercise prescription in this population[13,18].

There are, however, methodological differences. While VT1 calls for incremental ramp tests or incremental step tests with very small steps, for Fatmax, each load needs to reach a steady state in order to establish the respiratory exchange ratio (RER).

In 2002, Achten *et al.*[19] developed a test to determine the intensity at which maximal fat oxidation (Fatmax) occurs. They proposed 5-min stages with 35-W increments until RER = 1 was reached. However, it is very likely that sedentary subjects need more time to reach a steady state[6.]

Since then, the protocols have evolved and different adaptations and variations for both cycle and treadmill have appeared[6].

It should be borne in mind that the measurement of Fatmax can be influenced by the protocol used in the test[20,21] and other circumstances which cause great variability. These include[6,22]: sex, level of training, nutritional status (acute and chronic), type of exercise and other data related to performance of the test, such as ignorance of the muscle glycogen content beforehand. Different studies have also shown that the fat oxidation rate determined with short stages does not correlate with that occurring with longer exercises (for example, 1 hour)[23].

As for the values that we could expect in obese subjects, Maunder's review[6] reveals percentiles of normality for different situations. In men, Fatmax appears at a mean intensity of 43% VO_2max, with a FOR of 0.28 g/min (p50) and a range between 0.16 (p20) and 0.39 g/min (p80). In women, however, the mean is at an intensity of 61% VO2max, with a FOR of 0.16 g/min (p50) and a range between 0.12 (p20) and 0.20 g/min (p80).

Regarding modification depending on training, the authors of this review have found that:

- MFO increases in response to specific training while Fatmax remains unchanged;
- MFO changes are observed in sedentary populations, but not in previously active populations;
- MFO changes occur both with interval training and continuous training at moderate intensity, and are independent of body mass.

It has also been described that Fatmax-intensity training in class II and III obese subjects gives the same results as high-intensity interval training (HIIT), with the difference that during Fatmax training a reduction in insulin resistance is detected which does not appear with HIIT[24].

As for changes in VT1, we know that the intensity at which it appears improves with training not only in athletes, but also in subjects with obesity[25].

Takeaways

- Indirect calorimetry is the methodology for determining the energy variables to use when prescribing or controlling physical activity in overweight subjects.
- VT1 and Fatmax conceptually describe very proximate, if not the same, intensities and both can be used to prescribe and control exercise in this population.
- VT1 is determined with incremental ramp tests or incremental step tests with small steps.
- Fatmax is determined using incremental tests with longer loads.
- Fatmax does not seem to change with training, but the maximal fat oxidation (MFO) rate does.
- VT1 intensity changes with training.
- The changes in MFO and VT1 occur with both continuous and high-intensity interval training.

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The usefulness of interval training to lose weight

We do exercise when we repeat structured movements specifically designed to improve our fitness, performance and/or health[1]. Although it cannot be outrightly stated that this alone can help reduce and/or maintain our weight without complementary strategies such as nutritional management or education in healthy habits[2,3], there is solid evidence on the benefits of continuous exercise in maintaining body composition, physical function and mental health[1,2,4-6], on the close relationship between these variables, and the importance their interactions have in achieving holistic health[1,3].

Understanding continuous exercise as uninterrupted exercise performed long enough to produce responses and, therefore, positive adaptations, the literature on the subject raises no doubts about its contribution to reducing obesity, medical conditions, chronic disease, mortality and morbimortality [1,3]. Nevertheless, the increasing application and effectiveness of interval-based methodologies means that it is necessary to review the effectiveness of programmes based on such sustained exertion, their substantiation, strategies and rules of prescription.

Substantiation

For obese people, continuous exercise is performed at intensities beneath or in the vicinity of the second ventilatory threshold (VT2)[1,7]: Light-intensity activity (1-3 METs), moderate-intensity activity (3-6 METs) and vigorous-intensity activity (>6 METs). The exertion is lengthened enough time to bring about improvements despite its low intensity, provided that this can be done safely and independently of previous fitness level[1]. Given that the use of accelerometers and other recent technologies offers conflicting evidence on the benefits of light physical activity[1,5], work with obese individuals should focus on intensities above 3 METs, where the evidence is solid[1,2,4-6,8].

The metabolic demand or mechanical load at these intensities may seem low compared to those of normal weight individuals (in absolute values), but is sufficiently demanding in terms of relative intensity and perceived exertion[1]. Among other things, it should be borne in mind that these individuals' likely rejection of physical exercise[3,8], usual lack of experience -or even bad experiences in the past[3]-, poor fitness level at the start of exercise programmes[3,9] and augmented perception of exertion[1,3] will mean that the demands, both perceived and real, will actually be high[1,3].

Although it is generally accepted that the benefits of exercise or the reduction of the risks attributed to exercise can be obtained within a range of 500 to 1.00 MET x minutes per week (equivalent to 150-300 minutes per week of moderate exercise or 75-150 minutes when working at twice the intensity)[1], there is strong evidence suggesting that this prescription is insufficient in the obese population, both when it comes to losing weight and when it comes to keeping it stable once lost[1,2,4,5,10]. Having observed the relationship between a longer physical activity time and reducing and maintaining body weight (dose-response)[1-5,10], if food intake remains unchanged, the time should exceed 150 min, with the objective of up to 7 hours per week[1,2,4,5,6,8,10].

From the review conducted by Donnelly *et al.*[4], it can be taken as a certainty that >150 min/week of moderate-to-vigorous-intensity activity can reduce weight by about 2-3 kg, but increasing this to 225-420 min/week leads to greater losses (5-7.5 kg). Similarly, 150-220 min/week (1,200-2,000 kcal per week) are sufficient to prevent weight gain of more than 3% (evidence statement I), but it is necessary to keep doing at least 200-300 min/week in order not to regain weight previously lost (regain; evidence statement IIa) [4]. These guidelines are reflected in subsequent health guides[1, 3, 6, 8, 10] but other widely referenced proposals are also worthy of note beside them, such as: Ross *et al.*[11] with a drop of 7.5 kg (7%) in men with BMI >27kg/m^2 after 12 weeks of exercise at a constant intensity <70% VO_2 until 700 kcal per day (about 60 minutes per day) without nutritional control; or Chiu *et al.*[12] with similar weight losses (6.72 kg, 7%) also after 12 weeks, but with three weekly training which lasted 60 min, progressing in intensity from 40 to 80% HRR.

So, it is advisable to start continuous exercise at very low relative intensities (40-50%) to increase gradually and shift the thresholds to within a range of 60-70%, and up to 80-90% for capable or more trained subjects[2,3,6,7,9,13]. The progression in intensity which both continuous exercise itself and interventions of greater longitudinal duration permit when compared to high-intensity exercise[1,6,9] ensures an enhanced oxidative capacity, preserving muscle mass and protecting joints[9,13], while technique and efficiency at higher intensities are also improved. This is important because many obese patients also present other medical conditions which can also benefit from moderate exercise and progressive loads[3,13]. Additionally, training every or almost every day of the week favours the habit and contributes to the stability of progress achieved in the long term[2,3,9], even in children[14].

As for the duration of the sessions, although continuous moderate exercise lasting more than 10 minutes is considered effective in normal weight subjects (e.g. 40-60% HR or VO_2 reserve)[1,6], in the obese population more than 30 minutes[3,15], even approaching 60 minutes[1-4,13,15], of moderate exercise are needed.

In any event, it is important to remember, as Arad *et al.*[15] state, that in the exercise-based treatment of the obese population, we find responders and non-responders [2,13]; there are inconsistencies in the research methodology; and individualised longitudinal monitoring is needed if we want to get the prescription (dose and type of exercise) right, regardless of the strategy prescribed.

Strategies

Each specific case can benefit from a specific strategy. Success lies in analysing the characteristics and needs of subjects in depth, choosing and specifying the goals well, hitting on the ideal prescription and monitoring it continuously to make adjustments. We are not only faced with responders and non-responders[2,13,15] ;human beings are complex systems[1] and even the best stimuli tend to stagnate and lose potential with time[16]. Consequently, although different strategies share certain points or can be combined within a single programme, some preliminary considerations would seem advisable, at least in 3 main areas:

Specifying objectives

Focussing on improving body composition in general is not the same as specifying weight loss and/or reducing the fat percentage, or even achieving an improvement in general health based on lowering body weight and fat mass when this is not the main goal pursued. Be that as it may, it is always possible to concomitantly (or not) seek to improve the physical function and/or fitness and/or quality of life and/or psychosocial well-being.

The parameters which determine the intensity at which we burn fat are not the same as those that condition our ability to work at VO_2max[15,16]. Moderate-to-high-intensity exercise is recommended to focus more on reducing fat[8,12,13,16] -particularly visceral fat and waist circumference[1,4,12]-, greater weight gain[4,8] or cardiorespiratory fitness -increased VO_2max[1,6,8]. It is also recommended when less time is available. Conversely, moderate continuous exercise is sufficient to reduce the risk of metabolic syndrome -body composition, insulin resistance and glycosylated haemoglobin[1,6,8,12]- or improve physical fitness, endothelial function, lipid profile, and blood glucose control[6,8], among other things, but affects weight and fat reduction and general body composition less[4,8,13].

Since some 11-12 kcal/kg per day are needed to maintain the changes in body composition after a weight loss intervention[2], it would seem that conservative strategies (moderate exercise) are more easily extended over time, minimise rejection from the obese population, generate good habits and lead to significant improvements in health, with good long-term results[2,3,6,8,13,14,17]. As already noted, they are also less traumatic than high-intensity-based strategies[1], avoiding the risks of increased pain and bone/ligament/muscle problems[2,13,16], and reducing anxiety and state changes[2,8]. They also avoid the reduction of calories in daily activities which can accompany strategies based on more intense exertion [2].

Focus and type of exercise in the programme

Both what is traditionally known as aerobic exercise[1,6,18] -large muscle mass mobilised in patterns of continuous exercise, with a cardiovascular and respiratory focus- and strength training involving loads or counter-resistance[1,6,18] have led to proven improvements in body composition in adults[1,4,10] and children[1,16,18]. It should be borne in mind that strength training can be given a metabolic focus by combining initially neuromuscular exercises and incorporating them in wide-ranging circuits to generate metabolic demands not dissimilar to aerobic exercise. Likewise, multicomponent training programmes, which work on several aspects at the same time, can be presented as a form of moderate-to-vigorous-intensity continuous exercise, with improvements in body composition, which has proved effective in both elderly adults with overweight[19,20] and children[14,16,21].

And so we differentiate between strategies with focuses which are distinctly metabolic (cardiovascular and respiratory), distinctly neuromuscular (related to strength and/or function) and combinations of these. Similarly, and regardless of the focus, we should select the main type of muscle contraction to stress: concentric, eccentric or, once again, a combination of the two.

For example, and although the evidence cannot yet be considered solid because it is still so new, it seems that eccentric work -such as backpedalling or braking forward-driven pedal movement- favours the use of greater muscle mass, with increased neural activation and mechanical production for lower absolute intensity, meaning that, once learned, it is ideal in programmes with obese subjects and allows them to exercise for longer [22-26]. If, in general, cycling and forms of exercise where the subject does not bear their own weight (swimming, exercise on elliptical trainers, rowing) are ideal because they eliminate the osteoarticular stress which can be demanding for this population, minimising pain and the risk of injury[13,26], continuous reverse exercise using these same methods would be a good alternative for doing long sets and consuming fat in obese populations. We will probably find out more about this kind of exercise in the next few years.

Dose format (one long session vs. several shorter sessions)

In brief, here we are talking about the way to administer the programme: if we are interested in one longer session on training days or doing the same amount of exercise but in numerous micro-sessions lasting less time.

More than 10 minutes is considered an effective amount of time for moderate-to-vigorous exercise and such sessions can be accumulated over the length of the day to meet the daily recommendations and provide obese individuals with the same benefits[1,3,6]. However, it seems that these benefits are influenced by the type of exercise performed, as well as by the parameters evaluated, even for the same volumes, at least in elderly overweight adults[19]. For example, with the oft-cited prescription of walking at least 10,000 steps per day, we find that there is no solid evidence to back walking in itself when distributed over the day without any programming or periodisation of rhythms[1,5]. It appears that dose distribution can affect body composition outcomes when less metabolically demanding tasks are performed[19].

Indication

No matter what strategy is used, it is essential to define the load properly, trying to individualise the parameters as much as possible. This individualisation needs to be dynamic in order to remain efficient as initial goals and changes are achieved. Individualising exercise is like tailoring a suit and, in the case of obesity, continuous adjustments need to be made to the size and seams of the suit as the programme progresses. More than a one-off, unchanging measure, exercise programmes and training in general should be seen as an individualised process dependent on the results achieved.

To complete a minimum of 7 hours of physical activity at the lower end of moderate activity, representing about 2,200 kcal/week extra for a subject weighing 100 kg (420 min at 3 METs; 1 MET = 0.0175 kcal/min/kg body weight), it is necessary to have a wide range of options and progress in all the parameters of training depending on the motivations and possibilities of the subject. It is better to raise first the volume of exercise than its intensity, progressing more slowly with older people[1]. Once this initial load has been assimilated (a couple of week could suffice), the different parameters can be adjusted, the recommendation being not to drop beneath 12 weeks (according to most of the literature). We are focussing only on the continuous exercise here, but, professionally, the recommendation would be to gradually include forms of interval training and greater intensities, as well as actual strength work, in the event of extending the intervention[6].

Intensity

With moderate exercise (3-6 METs), progress from relatively very low intensities (40-50%), in the vicinity of the first threshold, up to a range of 60-70%, seeking longer durations and the use of fat. With moderate-to-intense exercise (>6 METs), progress from these low intensities up to 75-80%, even 85-90% with more capable or trained subjects, in order to mobilise glucose and increase the metabolic rate and cardio-vascular demands. The METs of the most common types of activities can be consulted in the prescribing guidelines or on specialised websites (such as the *Compendium of Physical Activities,* extracted from: https://sites.google.com/site/compendiumofphysicalactivities/home)

Duration and frequency

These variables are necessarily linked to establish the total volume of exercise. Depending on the intensity and very much depending on the strategy/strategies selected, achieving at least 30 min/day, 5 days per week, or increasing this to 60 min, 3 days/week, can be set as an objective in the first stage to reach 150-220 min/week (1,200-2,000 kcal/week; ≥300 kcal/session). Remember that these volumes can be reduced in proportion to an increase in intensity, provided the subject does not fall beneath the target calories. In the next stages, the number of training days and/or the duration of the sessions can be gradually increased to more than 7 hours per week (225-420 min/week), or at least leaving no more than one day without training[8]. The first option would seem simpler in obese individuals [8]. When the strategy involves several short sessions per day, this is achieved by starting with micro-sessions lasting at least 10 minutes to accumulate 30 min/day and then increasing the length or intensity of the micro-sessions to complete 60 min/day or about 300 kcal/day. But remember that higher intensities are needed in each micro-session when this strategy is applied to obese individuals.

When the strategy is based on eccentric exercise, it is possible to exercise at lower intensities, as already noted, or increase the intensity as the individual masters the technique, achieving benefit at lower volumes, in order to accomplish the same overall metabolic expenditure objectives. In all events, the subject should start very low and get used to the type of muscle contraction beforehand.

Takeaways

- 150 min of moderate- or vigorous-intensity exercise can reduce weight by about 2-3 kg, but increasing this to 225-420 min per week leads to greater loss (between 5 and 7.5 kg).
- 150-220 min per week (1,200-2,000 kcal) are sufficient to prevent weight gain of more than 3% (evidence statement I), but it is necessary to keep doing at least 200-300 min per week in order not to regain weight previously lost (evidence statement IIa).
- It is advisable to start continuous exercise at very low relative intensities (40-50%) to increase gradually and shift the thresholds to within a range of 60-70%, and up to 80-90% for capable subjects.
- Both what is traditionally known as aerobic exercise (large muscle mass mobilised in patterns of continuous exercise) and strength training involving loads or counter-resistance (aimed at improving strength or functionality) have led to proven improvements in body composition.
- Mixed work in continuous protocols has less impact on reducing fat than aerobic exercise. However, people with metabolic syndrome or who are overweight with type II diabetes benefit from the combination of both focuses in a programme.

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Usefulness of interval training to lose weight

Interval training comes from the world of sport. Gerschler-Reindell called it Interval Training in mid-1950. In 1960, the first scientific studies in which Åstrand, Christensen & Hedman concluded that the intervals which led to the greatest cardiorespiratory adaptations were those lasting 2-3 minutes appeared1. In sport, the aim of high-intensity interval training (HIIT or HIT) is to improve VO_2max, working on the basis that the total time of the work bouts in HIIT means it is possible to exercise longer than it is with continuous exercise at the same intensity until exhaustion. This work takes place at the stage known as metabolic instability, when the maximal lactate steady state or ventilatory threshold 2 has been passed, at about 80-90% VO_2max[2].

In recent years, great interest has been shown in HIIT in the exercise and health world, and its use with people with all kinds of medical conditions, obesity for instance. It should be borne very much in mind that to apply HIIT with such populations, most of the forms used will be adaptations of those applied in performance sports and, generally, will not comply with either the intensity or duration of the work intervals used in them. Untrained subjects (and even more so if they suffer medical conditions) will barely be able to withstand or even reach such high intensity loads or maintain the steady VO_2max plateau, which in most cases will coincide with their exhaustion peak, meaning they will only be able to exercise at these intensities for a very short period of time. HIIT in these populations involves lowering the intensity considerably, increasing its range and decreasing the length of the work intervals.

Substantiation

There is no universal definition of HIIT. We will use the one quoted by Campbell in 2019[3] which defines it as "episodic short bouts of high intensity exercise separated by short periods of recovery at a lower intensity" or complete rest, but understanding that "high intensity" can be as low as 65% VO_2max or as high as maximal exertion, and that the length of the work bouts can range from 30 seconds to 4 minutes, or even more. For it to be considered HIIT, there must be at least two exercise bouts[3].

The protocols of interval training are usually divided by intensity into 3:

– High Intensity Interval Training (HIIT). Approximately 80%-100% MHR, 65-100% VO_2max. This is also known as aerobic interval training and interval lengths are differentiated.
  - Short, up to 1 min, at approximately 90%-100% MHR, VO_2max.
  - Long, 1 to 4 min, at approximately 60-89% MHR, 65%-89% VO_2max (vigorous aerobic intensity), which can be within the individual's aerobic capacity. For most people, this can be equated with an intensity that, if done without rest, could be maintained for 5 to 10 min before fatigue.
– Sprint Interval Training (SIT).

Short exercise reps, 8 to 30 seconds at 100% VO_2max or more and recovery periods lasting between 1 and 4 min.

Repeated Sprint Training (RST), characteristic of team sports, is also distinguished from Speed Strength Training, for sprinters, in the world of sport[1]. Technically, SIT, RST and Speed Strength Training do not come under HIIT because the goal is not to improve VO_2max and their intensity is above 90% VO_2max.

Exercise beneath the intensities defined, 55-69% MHR, 40-59% VO_2max., is called Moderate-Intensity Continuous Training (MICT). " "Traditional" stable-state exercise for a set period of time (usually 20-60 min)[4].

Systematic reviews with meta-analysis[3-6] compare the effects of MICT with HIIT/SIT to measure their effectiveness in adults regarding the management of overweight and/or obesity. Although the adjustments are always specific to the training method used and the variables that define the load applied, they generally conclude that HIIT/SIT is more effective than MICT when it comes to losing weight and has two big advantages: a) up to 40% less time dedicated to training can yield the same benefits as a similar time commitment and/or similar energy expenditure. b) it leads to greater activation of all the factors needed to consume fat as an energy substrate during exercise (hormone secretion, transportation through the bloodstream and greater mitochondrial consumption) and after exercise (through excess post-exercise oxygen consumption - EPOC), favouring lipolysis.

The intensity of exercise plays an important role in regulating body composition and local fat consumption in obese people, who often suffer some kind of lipid metabolism disorder. If the exercise is not sufficiently intense, the effect of regulating insulin and improving exercise resistance is significantly weakened or may even disappear. Improving insulin sensitivity lies in muscle contraction and HIIT/SIT involves a larger proportion of muscle fibres than MICT. HIIT/SIT produces an oxidative increase in skeletal muscle, improving insulin sensitivity and glycaemic control, proving more effective than MICT[5,7].

Strategies

Epidemiological data show that most adults do not meet the recommended exercise guidelines to prevent weight gain[4], the main reason being a lack of time[8]. Some authors attribute the rapid acceptance of HIIT/SIT at sports centres to the shorter time spent in each session to achieve the same results[9].

General considerations

The application of HIIT as a way of reducing body fat is the primary objective of millions of people. Its actual effectiveness when it comes to solving a social problem in most cases caused by an inadequate diet is another question[2]. Exercise, be it MICT or HIIT/SIT, unless applied in very high volumes, does not by itself lead to significant changes in weight loss and should, therefore, be a component of a long-term obesity management programme[4,5,10]. The effects of exercise on blood lipid levels in overweight or obese individuals depend on their blood lipid levels prior to exercise, the intensity of the exercise, the duration of the exercise, body composition, caloric intake, metabolic rate and lifestyle, so all these factors must be taken into account.

Total energy consumption plays a more critical role in weight loss than exercise intensity[5], so effective body weight loss should be associated with caloric restriction and the other aspects mentioned10. These factors are generally poorly controlled or not controlled at all. In the review published by Keating et al. in 2017[4], which includes 31 studies with meta-analysis comparing MICT with HIIT (n=17) and SIT (n=14), only 26% took them into account.

An important aspect to bear in mind is the influence of exercise on intake habits. HIIT reduces energy intake and, because it involves higher energy expenditure during exercise, fosters a greater negative energy balance. HIIT has been shown to produce more beneficial changes in appetite regulation than MICT and SIT, has been found to suppress energy intake after exercise to a greater extent in overweight men and reduces perceptions of hunger and satiety after exercise significantly more than MICT. To date, there has been no long-term research into the impact of MICT and HIIT/SIT on changes in sedentary behaviour, usual levels of physical activity, diet or energy expenditure, examining the adoption of and long term compliance with HIIT/SIT and MICT protocols in real-world settings, or compliance with the intensities established[4].

Meanwhile, the variable that most affects daily energy consumption is Non-Exercise Activity Thermogenesis (NEAT), which spans all the activities of daily life not associated with programmed exercise. NEAT could account for between 15 and 50% of energy expenditure. Obesity is currently associated with decreased NEAT, mainly in the workplace, obese people being more prone to low NEAT and sedentary lifestyles. Therefore, regardless of the exercise intensity, obesity management programmes should be accompanied by increased NEAT. Adapting the workplace so that subjects can walk at a leisurely pace for 2.5 hours per day leads to a weight loss of 20 to 30 kg/year in obese individuals without inducing compensation or a decrease in activity or energy expenditure during non-working hours[11].

Adaptation and tolerance level

Is it appropriate and/or possible to do long-term high-intensity work with obese adult populations who are usually sedentary? SIT protocols are extremely difficult and are unlikely to be tolerated or enjoyed by previously inactive individuals or populations with obesity[4,12,13]. Non-athletes with obesity/overweight will have trouble tolerating HIIT/SIT without getting their bodies used to MICT beforehand. HIIT/SIT can cause not only musculoskeletal disorders (injuries), but also psychological disorders leading to the abandonment of such exercise[4]. However, some authors advocate training aerobic capacity from the outset via HIIT before MICT in individuals presenting low fitness levels, obesity, cardiovascular disease or metabolic disorders[2].

Motivation and adherence

The American College of Sports Medicine has published annual worldwide trends in fitness in *ACSM's Health & Fitness Journal* for the last 14 years. The instrument used is a survey covering 38 possible trends in order to establish and be able to compare the fitness trends in Europe, North and South America, and Asia. In its latest study[14], 56,746 surveys were sent electronically to professionals from the sector and 3,037 of these from more than 40 countries responded. HIIT has featured among the top 3 trends since 2014 and in the latest survey (2020), while it was not even in the top 20 in Asia, held first place in North America, second in Europe and seventh in South America. In the "exercise for weight loss" trend (involving diet and an exercise programme) for 2020, it held first place in South America and Asia, sixth in Europe and eleventh in North America. There can be little doubt that these data demonstrate the wide acceptance of HIIT and weight loss programmes for a population which exercises regularly. Are these trends just as motivating for the obese adult population?

As previously stated, there have been no long-term studies on HIIT in obese populations, with most lasting between 2 and 14 weeks[4], barely sufficient time to generate true adherence to exercise and not enough to produce stable adaptations in the body. The results speak of weight loss in the short term, but none of them talks about the long term. How long will the outcomes last when training is no longer continued? Weight loss maintenance is defined as losing at least 5% of body weight or reducing the body mass index by at least 1 unit and keeping weight below this minimum amount for at least 1 year[5].

How long can an obese individual keep up HIIT as a training method and lifelong habit? The willingness and ability of people to adhere to HIIT/SIT on a long-term basis is currently unknown. More research is needed into the scope of psychological responses to HIIT/SIT and MICT in obese populations compared to regular intensity-independent exercise[4].

It is important to note that failing to achieve the desired results is strongly associated with the stress generated by the need to lose weight, feelings of guilt and failure leading to non-attendance[15,16]. Considering that adherence to exercise is the key to long-term weight loss, it may be more appropriate to propose a long-term programme which is progressive in terms of intensity, thereby decreasing the likelihood of having to stop constantly due to musculoskeletal problems or psychological factors, relieving the stress produced by the need to lose weight in the short term and seeking to mobilise and activate the metabolism every day, increasing energy expenditure. The aim should be for the subject to switch from their initial extrinsic motivation (weight loss by prescription) to intrinsic motivation (enjoyment of the activity itself), key to adherence to and continuity with the programme. To this end, it is essential to take into account such important aspects as socialisation (group activities with the same objectives), positive feedback (directed by an empathetic monitor with motivational skills) and activities that, as far as possible, are pleasant for the subjects.

Indication

Safety

Individuals must pass a medical examination beforehand showing that they are fit to train at the intensities set for HIIT/SIT. Existing scientific evidence shows that HIIT is safe and effective for almost all types of populations and conditions[3-6]. The safety of SIT for clinical populations, including the obese, has not been established[4]. In the review published by Jelleyman *et al.*[17], only 34% of the studies report adverse events. Out of a total of 18 musculoskeletal injuries incurred in exercise, 14 occurred with HIIT.

Load variables

These must be established, but in a general manner, without being too strict and adapting to the specific needs of each type of population. Such a broad range of intensities can make it difficult for health professionals to plan suitable programmes in an optimal manner. Further research should be conducted into suitable, optimal doses for each type of population, because these have not been established[3].

The fundamental goal of HIIT in obese populations is to reduce the body fat percentage. The variables to determine for general application are:

- *Type of exercise:* dynamic, involving a large proportion of the muscle chains in the body. Activities like running, swimming, cycling, rowing, boxing and others based on running and multi-joint exercises and on multiple planes. With obese populations, assessment is required to determine the appropriacy of complex exercises (Olympic exercises and variants, burpees, jump training, suspended push-ups, etc.) and equipment (dumbbells, Olympic bars, high bars, thick ropes, etc .) that cause great stress to the joints and/or may result in injuries due to, for example, lack of strength and technique, speed of movement, eccentric work, etc..
- *Weekly frequency:* Reducing the physiological profile (intensity

and duration) of HIIT for obese populations makes more frequent sessions possible. The goal should be to exercise every day.

- *Intensity:* approximately 80-100% MHR, 65-100% VO_2max.
- *Volume:* the total working time in HIIT is usually between 4 and 20-30 min, plus 10 min of warm-up-activation and 5-10 min to cool down, making for a total time of between 24 and 50 min per session.
- *Work-to-rest ratio and rest between repetitions and/or sets:* Work-to-rest ratio of 1:4 to 2:1. Beginner 1: 2; Intermediate 1: 1; Advanced 2: 1. With obese populations, short intervals of up to 60 seconds are the most common, while those of 30 seconds are considered more acceptable because they are less stressful. When working with intervals of 30 seconds, progressively increase to 4 sets of 6 intervals with rest between intervals of 1:1 and between sets of 1 to 2 minutes. When working with intervals of up to 60 seconds, progressively increase up to 2 sets of 6 intervals with rest between intervals of 1:1 and between sets of 2 minutes. When working by intervals, sets are not necessary[2].
- *Intensity indicators and session assessment:* The new technology on the equipment at sports centres and/or the user's own devices can be used to programme and control exercise. The simplest way is probably by %MHR with submaximal HIIT. To minimise the HR error, the heart rate reserve or Karvonen formula should be used. Another simple way is to use the Borg rating of perceived exertion scale (RPE). Its ease of use makes it well suited for all protocols and it also takes into account psychophysical aspects such as rest and a number of subjective factors. The scale is specific for each type of exercise and, once the subject is familiar with how to use it, is highly valid and reliable. Values of 17-18 (6-20 scale) and 7-8 (1-10 scale) for obese populations are a good indication that HIIT has been carried out at the right intensity [2].

As for the time of day for HIIT, it should be borne in mind that this type of training greatly activates the sympathetic nervous system and causes a significant decrease in parasympathetic reactivation afterwards. It is, therefore, not recommended at the end of the day so that the subject can rest properly[1].

Takeaways

- Both long-term HIIT and MICT lead to clinically significant fat loss.
- Existing scientific evidence shows that HIIT is safe and effective for almost all types of populations and conditions.
- Dynamic exercises involving a large proportion of the muscle chains in the body should be performed.
- Start with intervals of 30 to 60 seconds and a 1:2 work-to-rest ratio.
- In order to be effective managing obesity, such exercise should be complemented with dietary intervention and the adoption of healthy habits, increasing total daily energy expenditure.

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Review

Impact of aquatic exercise on persons with kidney disease: a systematic review and meta-analysis

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Summary

Objective: Analyze the scientific evidence on the effects that aquatic physical exercise has on people with chronic kidney disease (CKD).
Material and method: A systematic review was carried out following the checklist Preferred Reporting Items for Systematic review and Meta-Analysis Protocols, with the objective of locating the largest number of investigations that aimed to identify the effects of the practice of aquatic physical exercise in people with ERC. A search of the PubMed, PEDro, Scopus and Cochrane databases were carried out until March 2019, using the PEDro, CERT, MINORS and NIH scales to determine the methodological quality of the same.
Results: Five investigations were located, two of them were randomized control trials, another two studies comparatives and one was uncontrolled. The mean score and the median obtained after applying PEDro scale were 4 and 4 respectively. All the interventions proposed aerobic exercise programs, being generally of short duration and highly supervised, without any adverse effects arising from their practice. In a large part of the studies, significant effects were observed in physical condition, physiological parameters and quality of life, to a lesser extent. The practice of exercise had no significant impact on either the activity of the disease or the perceived pain in patients.
Conclusions: Practice of aquatic exercise is beneficial in people with ERC. More longitudinal studies are needed to assess the impact of aquatic exercises as well as its effect and quality of life in long term.

Key words:
Aquatic exercise. Chronic kidney disease. Review. Meta-analysis.

Efectos del ejercicio acuático en personas con enfermedad renal: revisión sistemática y metaanálisis

Resumen

Objetivo: Analizar la evidencia científica existente sobre los efectos que el ejercicio físico acuatico tiene en personas con enfermedad renal crónica (ERC).
Material y método: Se realizó una revisión sistemática siguiendo la lista de verificación *Preferred Reporting Items for Systematic review and Meta-Analysis Protocols*, con el objetivo de localizar el mayor número de investigaciones que tuviesen como objetivo identificar los efectos de la práctica de ejercicio físico acuático en personas con ERC. Se realizó una búsqueda en las bases de datos PubMed, PEDro, Scopus y Cochrane, hasta marzo de 2019, empleándose las escalas PEDro, CERT, MINORS y NIH para determinar la calidad metodológica de las mismas.
Resultados: Se localizaron cinco investigaciones, dos fueron estudios aleatorizados controlados, dos estudios comparativos y uno no controlado. La puntuación media y la mediana obtenida tras aplicar la escala PEDro fue de 4 y de 4 respectivamente. Todas las intervenciones plantearon programas de ejercicio aeróbico, siendo por lo general de corta duración y altamente supervisadas, sin que se registraran efectos adversos derivados de su práctica. En gran parte de los estudios, se observaron efectos significativos en la condición física, parámetros fisiológicos y la calidad de vida, en menor medida. La práctica de ejercicio no tuvo impacto significativo ni en la actividad de la enfermedad ni en el dolor percibido en los pacientes.
Conclusiones: La práctica de ejercicio físico acuático es beneficiosa para las personas con ERC. Son necesarios estudios longitudinales que valoren el impacto de programas de ejercicio, así como su efecto sobre la calidad de vida a largo plazo.

Palabras clave:
Ejercicio acuático. Enfermedad renal crónica. Revisión. Metá-análisis.

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Introduction

Chronic kidney disease (CKD) is a health problem of particular importance to society[1,2]. CKD is defined as a disease in which there is a decreased functioning of the kidneys, expressed in values of FG < 60 ml/min/1.73 m^2 or with damage persisting for three months or more. In Spain, the prevalence of CKD is 9.16% in the adult population aged over 20 years and increases to 23.7% in adults aged over 65 years[3] with the main reasons for developing this condition being ageing itself, arterial hypertension (AHT), diabetes and vascular disease[4]. On the other hand, risk situations that increase the probability of CKD encompass smoking, diabetes, cardiovascular disease, family history, being an African American, and anaemia among others[2].

In terms of cost, the Spanish state spends around EUR 800 million as stated in the SEN-semFYC (Spanish Society of Nephrology - Spanish Society of Family and Community Medicine) Consensus Document on chronic disease[5]. A large part of the budget is allocated to treatment, although there is no definitive solution, the most common palliative care is dialysis[6]. The disease, together with the appropriate treatment may have adverse effects, including a lower quality of life[7,8]. Moreover, this pathology is frequently associated with other diagnoses, such as a reduction in bone density[9], cardiovascular risk[10], hypertension and type II diabetes[11].

Given that there is no definitive treatment for CKD, exercise stands as an aid to prevention and to the improvement of those aspects affected by pain. It is known that patients undergoing haemodialysis show hypertension or anaemia, lowering their quality of life. Exercise is therefore considered to be an option for slowing down the disease[12,13]. In turn, the association between physical inactivity and mortality has been studied by a number of authors in earlier investigations, in which they indicate the high importance of physical exercise as a means to reduce the mortality rate in persons with kidney disease[14,15].

In most of the studies, aerobic or strength exercises were performed[16,17], in a land-based environment in which gravity has a negative effect on the patient's physical abilities. Together with this, a number of problems can be found, including protocol assistance and adherence. The reasons are manifold, ranging from inability to go to the centre due to illness, admission to hospital or lack of transport[18]. On the other hand, physical factors also come into play, such as a limited range of movement, difficulty in getting around, lack of physical strength or fear of falling over, all indicating that this environment is not ideal for working with this population[19].

Taking the above into account, aquatic exercise stands as an interesting alternative. Water offers a pleasant sensation and, thanks to its buoyancy, it is possible to take regular exercise and to conduct dynamic and motivating training programmes. In fact, aquatic exercise has been used in pathologies in which the patient's mobility and physical condition are seriously affected[20].

It would therefore appear necessary to determine the impact of aquatic exercise programmes on persons with CKD and to provide guidelines for the prescription and control of such programmes. This information would facilitate the task of those rehabilitation professionals who are treating this population. This objective can be achieved by making systematic reviews in order to synthesize the scientific evidence available on a topic and to offer a critical interpretation of the quality and validity of such evidence.

The purpose of this investigation is therefore to conduct a systematic review of any existing investigations whose objective was to analyse the impact of aquatic exercise on persons with CKD.

Material and method

A systematic review was designed, based on the P*referred Reporting Items for Systematic review and Meta-Analysis Protocols* (PRISMA-P) checklist, which is recommended for studies of this type[21].

Search strategy

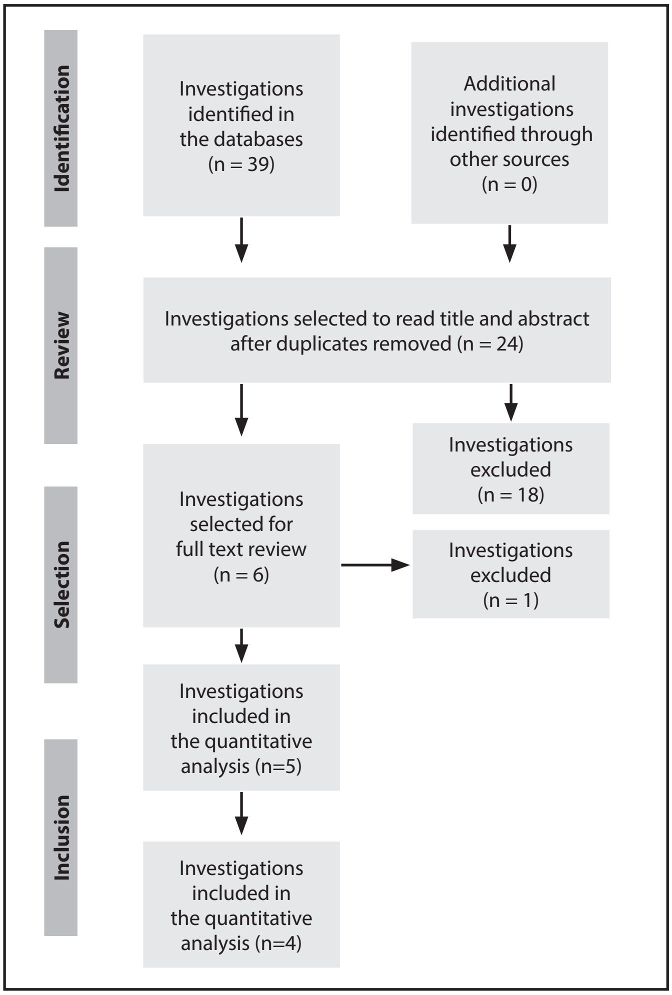
The search strategy was designed to find the greatest number of investigations whose objective was to identify the effects of aquatic exercise on persons with renal failure. For this purpose, a search was made with no time limit in the databases of PubMed, PEDro, Scopus and Cochrane, up to March 2019, combining the keywords and Boolean Operators as follows: *"Kidney Disease" OR "Dialysis" OR "Renal Failure" AND "Water Based Exercise" OR "Aquatic Exercise".*The search was repeated in May 2019, including the "Dialnet" database in order to update the results of the search and to locate any possible investigations in Spanish (Figure 1).

The search excluded all investigations that: a) included a sample comprising participants with different diseases and that did not provide separate information for persons with renal insufficiency; b) did not describe the physical training programme performed; c) analysed the effects of a single physical exercise session; d) used aquatic exercise in combination with other rehabilitation therapies; e) were written in a language other than Spanish, Portuguese or English.

Selection procedure

The information included in the title and/or abstract of each of the studies identified by the search strategy was screened independently, classifying the studies either as "valid *(a priori)*" or "not valid" based on the aforementioned selection criteria. Those studies whose title or abstract did not provide sufficient information to decide on their inclusion/exclusion were initially considered to be "valid *(a priori)*". Once this initial selection stage had been completed, both authors compared their respective classifications in order to reach a consensus. For any doubts as to whether a particular study should be considered as "valid *(a priori)*" or "not valid", the opinion of the supervising professor was requested. The next step was to read the full text of the studies finally classified as "valid *(a priori)*".

Figure 1. Diagram summarising the search.



Data extraction

The information extracted from the "valid" studies was made by reading the studies, making it possible to independently identify and organise the relevant data in evidence tables. For each study, the following was considered: design type; sample characteristics; aquatic exercise programme; study variables; the respective evaluation tools; and the most significant results described in the investigation. The data extraction procedure was not blinded to the names of the authors of the studies selected nor to the names of the journals in which the studies were published.

Evaluation of the methodological quality

LThe methodological quality of the studies considered to be randomised controlled clinical trials (CKD) was determined through the use of the PEDro scale[22].

The PEDro scale is a list of eleven criteria or points. The first one is not added to the total, the other ten are used to assess each article and to rate its methodological quality. The quality of the investigations was either taken directly from the PEDro database or directly assessed by the author of this study, for those investigations with no prior assessment.

The total score obtained served to differentiate the quality of the investigations between high (6 or more points) or low (5 or less points)[23]. For the comparative investigations, the "MINORS"[24] methodological index for non-randomised studies was used for the evaluation. The MINORS scale is a list containing 8 key points that is extended to 12 points for comparative studies. Each section is rated with a score from 0 to 2, based on the quality obtained for each point. An acceptable score would be half the total points of the evaluation (8 or 12 depending on the type of study). For the full articles, the *Consensus on Exercise Reporting Template* (CERT) scale was used. This scale contains the minimum and sufficient recommendations to conduct an effective intervention[25]. The scale has a list of 16 points and the scores vary from 1 to 19, considering a score of less than 9 to be low quality and a score of 9 or more to be good quality. For those articles with a single pre-post intervention group, the *Quality Assessment Tool for Before-After* (Pre-post) *Studies With No Control Group* (NIH) was used, whereby the reviewer is the one to decide on the reliability of the study.

Meta-analysis

A meta-analysis was made of those studies that, including an intervention group and a comparison group, provided information on the effect of the aquatic programme using similar variables in at least two investigations.

To do so, the standardised mean differences (SMD) were calculated and the confidence interval (CI) of 95% to measure the changes in the pre- and post-intervention control group, a comparison was also made across each variable. The SMD is the mean divided by the standard deviation (SD). This was calculated by taking the sizes of the samples of the pre- and post-intervention test groups for physical condition and blood, together with their means and their standard deviations for each one of the variables. Higgins *et al*[26]. To obtain the combined effects, a fixed effects model was made[27], selecting the most suitable models for each analysis in relation to the level of heterogeneity. The analysis used effects diagrams showing SMD and CI of 95%, serving to compare the effects between pre- and post-intervention control groups. The SMD is significant when 95% of its CI excludes zero, while the SMD values of less than ± 0.2, or ranges between ± 0.2 to 0.8, or greater than ± 0.8 indicate the existence of a small, medium or large effect respectively. All the analyses were made with the *Comprehensive Meta-Analysis* version 2.2.064.

Results

Designs and samples

A total of 39 articles were located from the search. After reading the title, 24 articles were initially selected. Of these, 18 articles were rejected due to the fact that their subject matter did not meet the criteria, leaving a total of 6 articles. After reading the full text of the articles, 5 studies were selected to make this present study (2 studies with randomized clinical trials, 1 study with no control group, and 2 comparative studies). A total of 119 participants were included (62.2% male and 37.8% female) with a mean age of 50.1± 12.6 years and a disease duration of between 3 months and 42 months (0.25-3.5 years).

Methodological quality

The 2 randomised clinical trial articles were both given a score of 4 points (Table 1)[28,29]. The two comparative studies[30,31] obtained a score of 17 and 20 points respectively (Table 2). The methodological quality of the study with no control group32 was considered to be good (Table 3). The quality with which the characteristics of the studies were detailed was considered to be low for articles[28,29-32] based on the CERT scale (Table 4).

Table 1. Methodological quality of the randomised controlled studies (PEDro Scale)

Criterion	Petchter *et al*. 2014 [28]	Anastasia *et al.* 2016[29]
1. Random allocation	1	1
2. Concealed allocation	0	0
3. Comparison of baseline data	1	1
4. Blinding of subjects	0	0
5. Blinding of therapists	0	0
6. Blinding of assessors	0	0
7. Outcome 85% subjects	0	0
8. Intention to treat analysis	0	1
9. Comparative analysis of groups	1	1
10. Estimate and variability points	1	0
Total score	4/10	4/10

Interventions

All the physical activity programmes were conducted in shallow pools with a water temperature of between 32 and 24 degrees. The duration of the interventions ranged from 12 to 16 weeks, with sessions of between 30 to 60 minutes and with a frequency of between 1 to 3

Table 3. Methodological quality of the study with no control group (*NHLBI* Scale).

Criterion	Dziubek *et al*. 2015[32]
1. Is the study objective clearly stated?	Yes
2. Clear selection criteria	Yes
3. Are the participants in this study representative of those that would be selected to replicate the intervention?	Yes
4. Did those participants who met the criterion complete the study?	Yes
5.Was the sample sufficiently large to provide significant results?	Yes
6. Was the intervention clearly described and applied to the entire sample?	Yes
7. Were the measures of the variables clearly defined, valid, replicable and consistently assessed?	Yes
8. Were the persons assessed blinded to the interventions of the participants' variables?	NR
9. Was the drop-out rate of the initial group less than 20%? Was this 20% accounted for in the analysis?	Yes
10. Were changes shown in the measures of the variables before and after the intervention? Did the statistical tests give a p-value?	Yes
11. Were the variables collected at different times before the intervention and after the intervention?	NR
12. Did the statistical analysis determine individually and at a group level the effect of the intervention?	Yes
Quality score	**Good**

Table 2. Methodological quality of the comparative studies (*MINORS* Scale).

Study	1 Clearly stated aim	2 Inclusion of consecutive patients	3 Prospective collection of data	4 Evaluations appropriate to study aim	5 Unbiased evaluations made	6 Follow-up period appropriate to study aim	7 Loss rate in follow-up less than 5%	8 Prospective estimate of sample size	9 Adequate control group	10 Simultaneous groups	11 Homogeneous baseline groups	12 Adequate statistical analysis	SCORE
Petcher *et al.* 2003a[30]	2	2	1	2	0	0	2	2	1	2	2	1	17/24
Petcher *et al,* 2003b[31]	2	1	2	2	0	2	2	2	2	2	1	2	20/24

Table 4. Quality of the information reported in relation to the characteristics of the proposed intervention (*CERT* Scale)

Study	Petchter *et al.* 2003a[30]	Petchter *et al.* 2003b[31]	Petchter *et al.* 2014[28]	Anastasia *et al.* 2015[29]	Dziubek *et al.* 2015[32]
1. Sports equipment	1	0	0	1	1
2 Instructor's experience	0	1	1	1	0
3. Individual or group exercise	1	1	1	1	1
4. Supervision mechanism	0	1	1	1	1
5. Adherence to exercise	0	0	0	1	0
6. Motivation strategies	0	0	0	0	0
7 a. Determining progression	1	1	1	1	1
7b. Progression of exercise	1	1	1	1	1
8. Description of the exercise	0	0	0	0	0
9. Exercise at home	0	0	0	0	0
10. Non-exercise components	0	0	0	0	0
11. Adverse events	0	0	0	1	1
12. Setting	1	0	0	0	1
13. Intervention	0	0	0	0	0
14a. Generic/tailored exercise	0	0	0	0	0
14b. How was it tailored?	0	0	0	0	0
15. Starting level	1	1	1	1	1
16a. Adherence to programme	0	0	0	0	0
16b. Success of programme	1	0	0	0	1
Points/19	**7/19**	**6/19**	**6/19**	**9/19**	**8/19**

days/week[28-32]. All studies were based on aerobic resistance exercises[28-32]. There were no reports of drop-outs or adverse effects resulting from the intervention.

Effects of the programmes

The studies analysed the effects of the aquatic programmes on physiological variables related to the physical condition. The main findings are summarised below and are also shown in Table 5.

Physiological effects

Two studies analysed the effects of the programme on physiological parameters[30,31] finding significant improvements in systolic and diastolic pressures, as well as in different renal markers.

Physical condition

Four investigations provided information on the effects of the programme on different aspects of physical condition. Two found improvements in aerobic capacity[30,31]. Two in strength and flexibility[32,29].

Quality of life

Only one investigation[29] included quality of life as a study variable, finding that aquatic exercise exerted a positive impact on this.

Results of the meta-analysis

The meta-analysis conducted on the physiological variables[30,31] (n=2) (Figure 2) showed a significantly high statistical effect for cysteine (SMD=1.84, 95% CI–1.06 – 2.62), diastolic pressure (SMD=1.23, 95% CI–0.66 – 1.93) and systolic pressure (SMD=1.43, 95% CI–0.76 – 2.11) with positive significance and high heterogeneity (P=0.01>; P=0.01>; P=0.01>; P=0.05>; I2=76.9%).

The meta-analysis of the physical condition variables[32,29] (n=2) (Figure 3) showed a significantly high statistical effect for cardiorespiratory capacity (SMD=0.61, 95% CI–0.09–1.12; SMD=0.71, 95% CI–0.20 – 1.29), strength (SMD=0.93, 95% CI–0.04 – 1.39), flexibility and balance (SMD=0.57, 95% CI–0.12 – 0.63) with a significance level and high heterogeneity (P=0.01>; P=0.01>; P=0.05>; I2=67.1%).

Figure 2. Meta-analysis - physiological variables.

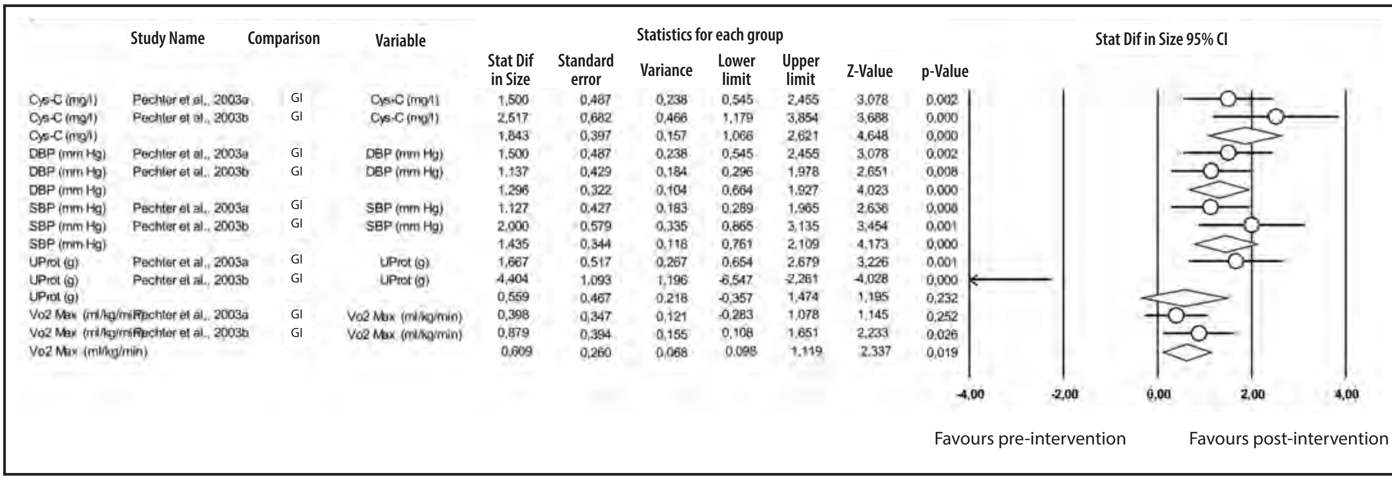


Table 5. Results of the articles analysed.

Authors (year)	Objective	Participants	Intervention	Variables (test)	Significant results (p <0.05).
Dziubek *et al.* 2015[32]	The study objective was to measure the impact of a 3-month programme of physical activity in an aquatic environment on patients with renal disease and the effect on the physical function of the knee joint muscles.	Sample (n; %men/women): *n*=20 (16 women and 4 men) Distribution and age: Mean group age 64.2 ± 13.1 years T diagnosed (years): 3.5 ± 0.5 years	Duration: 12 weeks IG (intervention group): Frequency: 1 day/week Duration: 60 min. Type of exercise: Aquatic exercise. Intensity: Non-specific. .	Physical fitness: Fullerton Functional Fitness Test by Rikli and Jones (Senior Fitness Test): - Eight foot up and go - Arm curl - Chair stand - Back scratch - Chair seat and reach - 6-minute walk test [m] Muscle Strength of the Lower Extremities in Isokinetic Conditions: - Peak torque (60º) flexors - Peak torque (180º) flexors - Peak torque (300º) flexors - Peak torque (60º) extensors - Peak torque (180º) extensors - Peak torque (300º) extensors	Drop-outs (n): 8 women Rate: 40% Adverse effects: NR Significant differences: - Intra-group (pre-post): - Eight foot up and go [s]* - Arm curl [Reps] ** - Chair stand [Reps] ** - Back scratch [cm] * - Chair seat and reach [cm] ** - 6-minute walk test [m] * - Peak torque flexors: - 60/s: Right* - 180/s:Right* left* - 300/s:left* - Peak torque extensors: - 60/s: right** - 180/s:right** - 300/s:right** left*
Petchter *et al.* 2003a[3]	The study objective was to determine the effectiveness of 12 weeks of regular aquatic exercise, training the cardiorespiratory resistance and functional parameters in patients with chronic renal failure	Sample (n; %men/women): *n*=20 (IG: *n*: 11. 66% men, 33% women) (CG (control group): n: 9. 66.7% men, 32.3% women) Distribution and age: IG: 45 ± 3.5 years (6 men and 5 women (n=11) (CG: 47.9 ± 3.8 years (6 men and 3 women (n=9) T diagnosed (years): NI	Duration: 12 weeks IG: Frequency: 2 day/week Duration: 30 min. Type of exercise: Aquatic exercise. Intensity: 40–50% of VO_2max CG: maintain normal activity.	Physical condition: - VO_2max - BMI Biochemical and/or haematological - Cysteine C - Glomerular filtration rate - Proteinuria - Blood pressure	Drop-outs (n): 0 Rate: 0% Adverse effects: NE Significant differences: Intervention group: - VO2max* - SBP ** - DBP * - UProtV** - CysC *
Petchter *et al.* 2014[28]	The study objective was to analyse the association of 10 years of regularly performed aquatic exercise with the study endpoint, all-cause death or start of dialysis.	Sample (n; %men/women): *n*=16 (IG: n: 3 % 42.9 men 4 57.1 % women.) (CG: n= 6, % 66.7 men, 3, 32.3 % women) Distribution and age: IG: 47 ± 16 years CG: 50±15 T diagnosed (years): NI	Duration: 12 weeks IG: Frequency: 2 day/week Duration: 30 min. Type of exercise: Aquatic exercise. Intensity: 40–50% of VO2max CG: maintain its normal activity.	Biochemical and/or haematological - Glomerular filtration rate - Proteinuria	Drop-outs (n): 10 Rate: 35.6% Adverse effects: NR Significant differences: NR

(continúa)

Authors (year)	Objective	Participants	Intervention	Variables (test)	Significant results (p <0.05).
Anastasia *et al.* 2015[29]	The study objective was to assess the effects of an aquatic programme on the functional capacity and quality of life of patients on haemodialysis.	Sample (n; %men/women): *n*=27 (IG: n= 15 ,86.67% men 13, %13.33 women 2) (CG: *n*= 12, 91.67% men 11, 8.33% women 1) Distribution and age: IG: 48.0 ± 11.3 CG: 48.6 ± 15.4 T diagnosed (years): 0.25> years minimum.	Duration: 16 weeks IG: Frequency: 3 day/week Duration: 60 min. Type of exercise: Aquatic exercise. Intensity: Borg Scale of Perceived Exertion (6-20) with effort 40-50% of VO_2max CG: maintain normal activity.	Physical fitness assessment-exercise testing - 6 min walk test(m) - Sit to stand (s) - Handgrip (kg) - Sit and reach (cm) - Timed up and go - (s) Health-related quality of life assessment. - Short Form-36 Questionnaire	Drop-outs (n): 2 Rate: 6.9% Adverse effects: NR Significant differences: Intervention group: SF-36: PCS** MCS** Physical fitness assessment-exercise testing - 6 min walk test** - Sit to stand ** - Handgrip ** - Sit and reach ** - Timed up and go** Control group: Physical fitness assessment-exercise testing - 6 min walk test** - Handgrip * - Sit and reach * Comparing groups: SF-36: MCS** Physical fitness assessment-exercise testing - 6 min walk test** - Sit to stand * - Sit and reach ** - Timed up and go*
Petchter *et al.* 2003[31]	The study objective was to determine whether a regular aquatic programme with 12 weeks of low intensity exercise could have an improving effect on patients with moderate CRF and to compare the outcome with data from the sedentary control group.	Sample (n; %men/women): *n*=26 (IG: *n* =17, 41.2% men 7, 58.8% women10)(CG: n =9, 6, 66.7% MEN , 3, 33.3% WOMEN) Distribution and age: IG: 50 ± 15 years CG: 51.5 ± 20.5 years T diagnosed (years): NI	Duration: 12 weeks IG: Frequency: 2 day/week Duration: 30 min. Type of exercise: Aquatic exercise. Intensity: 40–50% of VO_2max CG: NE	Physical condition: - VO_2max - BMI Biochemical and/or haematological - Cystatin-C - Glomerular filtration rate - Proteinuria - Blood pressure	Drop-outs (n): 0 Rate: 0 Adverse effects: NR Significant differences: Intervention group: - Peak 02 pulse* - Peak ventilation* - Peak load* - Cys-C* - U-Prot* - SBP* - DBP* - LPO*

Discussion

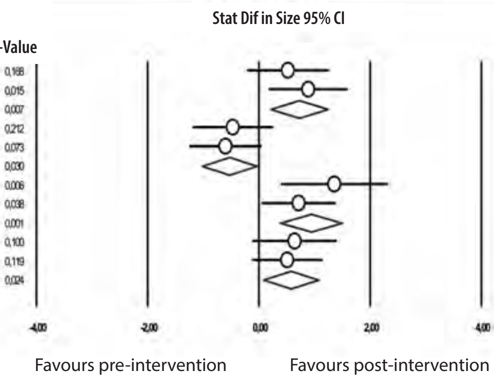
In this investigation, the evidence of the effectiveness of physical exercise programmes in an aquatic environment as a strategy to improve the physical condition of patients with CKD was examined and critically reviewed. It is important to emphasise that, in addition to randomised clinical trials, this study also included other trials. This was done for the following reasons. Firstly, the lack of studies that lead to a substantiated conclusion. It was therefore decided to include in this investigation articles that were not randomised clinical trials in order to obtain a more general view of the evidence available and to report it for future scientific investigations[33]. Secondly, the inclusion of more studies gives greater robustness to the meta-analyses conducted, making it possible to substantiate each statistical test with better judgement[27].

Although a limited number of investigations were located and their methodological quality was poor, it was still possible to extract important information that can be used for future studies.

After presenting the data on exercise programmes in an aquatic environment in this review, it can be said that these programmes had a beneficial and significant impact on the physical condition parameters (strength in lower limbs, stability), physiological parameters (cardiorespiratory capacity, renal function, diastolic pressure, systolic pressure) as well as emotional parameters (quality of life) for persons with CKD. Generally speaking, the results of the meta-analyses give greater weight to the benefits of this aquatic environment to those suffering from this disease. The effects of these aquatic programmes indicate that the physical condition, the physiology together with the patient's state of mind, can improve significantly more than with other physical exercise programmes.

Figure 3. Meta-analysis - physical condition variables.

	Study Name	Comparison	Variable	Statistics for each group						
				Stat Dif in Size	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
6 min walk test	Anastasia et al., 2016	GI	6 min walk test	0,523	0,379	0,144	-0,221	1,266	1,378	0,168
6 min walk test	Dziubek et al., 2014	GI	6 min walk test	0,889	0,364	0,133	0,175	1,602	2,441	0,015
6 min walk test				0,713	0,263	0,069	0,198	1,228	2,715	0,007
Sit and reach (cm)	Anastasia et al., 2016	GI	Sit and reach (cm)	-0,468	0,375	0,141	-1,203	0,267	-1,249	0,212
Sit and reach (cm)	Dziubek et al., 2014	GI	Sit and reach (cm)	-0,599	0,335	0,112	-1,256	0,057	-1,791	0,073
Sit and reach (cm)				-0,541	0,250	0,062	-1,031	-0,052	-2,167	0,030
Sit to stand (sec)	Anastasia et al., 2016	GI	Sit to stand (sec)	1,356	0,493	0,243	0,390	2,323	2,750	0,006
Sit to stand (sec)	Dziubek et al., 2014	GI	Sit to stand (sec)	0,717	0,346	0,119	0,039	1,394	2,074	0,038
Sit to stand (sec)				0,927	0,283	0,080	0,373	1,482	3,277	0,001
Timed up and go (sec)	Anastasia et al., 2016	GI	Timed up and go (sec)	0,643	0,391	0,153	-0,123	1,409	1,644	0,100
Timed up and go (sec)	Dziubek et al., 2014	GI	Timed up and go (sec)	0,510	0,328	0,107	-0,132	1,152	1,557	0,119
Timed up and go (sec)				0,565	0,251	0,063	0,073	1,057	2,250	0,024



Likewise, it should be emphasised that, in the course of these aquatic exercise programmes, no drop-outs or adverse effects were reported. In land-based programmes, the principal cause of drop-out or the failure to complete the programmes is a lack of physical strength[19], making it difficult for patients to continue the study, giving even more support to studies in an aquatic environment. Those suffering from CKD may have a poor quality of life, making it difficult to attend programmes to improve their physical condition. With regard to this, it should be pointed out that complete attendance in the studies shows a high frequency (91.6%). These results make it more feasible to propose interventions in an aquatic environment as the way to improve physical condition. Moreover, diseases such as arthrosis, diabetes *mellitus* or Parkinson's, have found benefits in aquatic exercise[19,20,34]. Finally, it should be highlighted that these patients come with quality of life problems that affect the disease. In this review, only one study[29], made an investigation to show the results with regard to the quality of life of these patients. Although the study considers a number of variables for these patients, both emotional and physical, these are insufficient to provide clear and accurate information to the review. This therefore represents an opportunity to conduct a more detailed study on the exercise programmes and the quality of life of patients suffering from this disease in order to consider the potential benefits that this could bring.

The study is not without limitations. Firstly, the methodological quality of the studies was poor, requiring better quality in order to conduct a more accurate investigation, indicating that future investigations should be made. Secondly, although the statistical tests show a positive correlation, men and women are included within the sample, forming a non-heterogeneous group. Furthermore, the groups in the studies do not contain the same sample for each group, giving more weight to one study compared to another. Thirdly, considering the limited number of studies included in the meta-analysis and its high heterogeneity, the assertions made in the study must be taken with caution, given the limitation in the application of the statistical tests in the actual studies analysed and in the meta-analysis subsequently conducted in this article.

Conclusion

The results of this review indicate that aquatic exercise is a safe and feasible option for persons with CKD. Although there is limited scientific evidence with regard to its benefits, doing aquatic exercise could improve the physical health of patients. However, further studies on this subject are necessary, particularly those focussing on the impact of this type of physical therapy.

Conflict of interests

The authors have no conflict of interest at all.

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Systemic iron homeostasis in female athletes: hepcidin, exercise and sex influence

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Summary

Iron is necessary for adequate deliver oxygen to the tissues since it is an essential component of the haemoglobin. However, iron deficiency remains a common problem among athletes, particularly for women experiencing the menstrual bleeding every month. The iron losses through menstrual blood loss during the early follicular phase (or menses) and an inadequate dietary intake of iron are two important factors contributing to this disease. Furthermore, the large hormonal changes that women experience along the menstrual cycle, especially in oestrogen and progesterone may influence on the optimization of iron absorption. Iron absorption is mainly mediated by hepcidin hormone, which seems to be affected by several stimulus and factors such as oestrogen and progesterone concentrations. Moreover, the regular practice of exercise is another important modulator of this hormone. Therefore, premenopausal active females are the most susceptible population to develop an iron deficiency or iron deficiency anemia, affecting their health and performance due to the less iron availability within the body and consequently a reduction of haemoglobin which compromise the oxygen transport. To date, most studies have not explored the acute post-exercise hepcidin response taking endogenous and exogenous sexual hormones influence into account.
This narrative review will focus on how iron homeostasis is modulated by different factors mainly influenced by exercise and female sexual hormones.

Key words:
Iron deficieny. Oestrogen. Progesterone. Female athlete. Iron absorption.

Homeostasis sistémica del hierro en mujeres deportistas: hepcidina, ejercicio y la influencia del sexo

Resumen

El hierro es necesario para suministrar adecuadamente el oxígeno a los tejidos, ya que es un componente esencial de la hemoglobina. Sin embargo, la deficiencia de hierro sigue siendo un problema común entre los atletas, particularmente para las mujeres que experimentan el sangrado menstrual cada mes. Las pérdidas de hierro producidas tras la el sangrado menstrual durante la fase folicular temprana (o menstruación), además de una ingesta dietética inadecuada de hierro son dos factores importantes que contribuyen a esta enfermedad. Además, los grandes cambios hormonales que experimentan las mujeres a lo largo del ciclo menstrual, especialmente en el estrógeno y la progesterona, pueden influir en la optimización de la absorción de hierro. La absorción de hierro está mediada principalmente por la hormona hepcidina, que parece verse afectada por varios estímulos y factores como las concentraciones de estrógeno y progesterona. Además, la práctica regular de ejercicio es otro importante modulador de esta hormona. Por lo tanto, las mujeres activas premenopáusicas son la población más susceptible de desarrollar una deficiencia de hierro o anemia ferropénica, lo que afecta a su salud y rendimiento debido a la menor disponibilidad de hierro en el cuerpo y en consecuencia, a la reducción de la hemoglobina que compromete el transporte de oxígeno. Hasta la fecha, la mayoría de los estudios no han explorado la respuesta aguda de la hepcidina después del ejercicio teniendo en cuenta la influencia de las hormonas sexuales endógenas y exógenas.
Esta revisión narrativa se centrará en cómo la homeostasis del hierro es modulada por diferentes factores influenciados principalmente por el ejercicio y las hormonas sexuales femeninas.

Palabras clave:
Hierro. Estrógeno. Progesterona. Mujer atleta. Anemia.

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Introduction

Iron deficiency (ID) is the most common nutritional deficiency worldwide, affecting specially to premenopausal women, around 40% in the developing world due to the additional iron demands of menstruation and pregnancy[1].

Athletes present a higher prevalence of ID than common population[1], particularly those involved in endurance sports[2]. In women, the regular exercise practice combined with menstrual blood losses, and in occasions accompanied by inadequate iron intake may compromise iron status[3,4]. Therefore, the prevention of ID, especially in females participating in endurance-type exercise are of major importance in order to avoid this event.

Additionally, oral contraceptive (OC) agents have been reported to improve iron parameters levels[5–7], since lesser quantity and shorter duration of menstrual blood loss is reported in OC users[7]. However, OC comes in a variety of formulations that contain several concentrations of synthetic ethinyl estradiol and progesterone, and the potential side effects in the measurement variables may be different due to the differences in dosages.

Systemic iron homeostasis depends primarily on the hepcidin hormone, which is the master regulator of iron homeostasis. Hepcidin inhibit the cellular efflux of iron to plasma by binding to ferroportin and inducing its internalization and degradation, therefore this fact modulates duodenal iron absorption and recycling in macrophages[8].

The regulation of hepcidin hormone occurs via many different stimuli including iron status, increments of interleukin-6 (IL-6), inflammation or hypoxia, among others, causing a future increase or decrease in iron absorption[8]. Furthermore, female sex hormones have been reported to exert a potential effect on hepcidin expression, specially oestrogen and progesterone[9,10], which may consequently influence on iron absorption.

This narrative review aims to address the different stimuli and mechanisms regulating hepcidin hormone, considering exercise and female sex hormones influence and their corresponding consequences on iron homeostasis.

Hepcidin-ferroportin system

Hepcidin is synthesized and released mainly by the liver and it acts controlling the membrane expression of ferroportin[11]. Hepcidin binds to ferroportin and degrades it, so the hepcidin-ferroportin interaction controls the flux of iron into the plasma and consequently the iron used by tissues[11]. The liver becomes a sensor and an effector regarding the control of iron metabolism, recognizing different stimuli: intra and extracellular iron concentrations (hepatic and plasma iron), the erythropoietic needs and the increase in concentrations of inflammatory markers[12].

Increased hepcidin expression in hepatocytes occurs when iron levels are abundant in the body, which reduce its absorption and release from stores (mainly ferritin within hepatocytes and macrophages). When iron is deficient, hepatocytes decrease hepcidin synthesis, inhibiting ferroportin degradation and allowing iron entrance into plasma[13].

Aforementioned stimulus can be activated or supressed by different mechanisms. One of them is exercise, which has influence over several pathways regulating hepcidin production[14–16]. Nevertheless, this influence need more research to provide evidence, especially in female athletes, where exercising is combined with sex hormones variations along the menstrual cycle.

Mechanisms regulating hepcidin-ferroportin system

Several mechanisms regulate hepcidin-ferroportin axis in order to maintain iron homeostasis. These mechanisms are not isolated, as they interact and cooperate between them in order to adjust iron levels *in vivo*.

Intra and extracellular iron homeostasis

Basically, iron-dependent mechanisms in the body, mainly erythropoiesis, are addressed by a tight coordination between iron absorption from the diet by enterocytes, iron recycling by macrophages following degradation of senescent erythrocytes, and iron release to plasma from ferritin reserves[17]. Most of the iron in the body is found as part of the haemoglobin contained in erythrocytes[11]. Erythropoiesis occurs in the bone marrow to compensate the daily destruction of senescent erythrocytes (lifespan of ~90 days). From the aforementioned iron supply processes, erythrocytes recycling by macrophages account approximately for 90% of the iron requirements[18]. The other 10%-20% depends on the iron absorption from the small intestine according to the body´s needs.

Furthermore, the excess of iron in relation to the requirements is stored in liver, spleen, bone marrow, duodenum, skeletal muscle and other anatomical zones by means of two extraordinary proteins: ferritin and hemosiderin[17]. Within normal limits, the transformation of ferritin to hemosiderin could be the best evolutionary step to reduce iron toxicity, due to the low iron elimination capacity of the human body[19].

Hepatocytes have the largest capacity for iron storage and when iron absorption and recycling do not meet the body iron demands, these iron stores supply the necessary iron to plasma, addressing the needs[19]. To export iron from cells (duodenal enterocytes, iron-storing hepatocytes and iron-recycling macrophages) into plasma, ferroportin is required[20].

The aforementioned mechanisms cooperate together over the control of plasma iron concentration. Once in the plasma, iron is oxidized to join transferrin protein (the main iron transporter in plasma) which is mainly destined to supply iron to the bone marrow[20].

When all of the above results in an increase in plasma iron, there is a decrease in iron absorption mediated by increments in hepcidin levels and subsequent ferroportin degradation[21].

Interleukin-6

IL-6 as Myokine: IL-6 is produced locally in working skeletal muscle and can account for the increase in plasma IL-6 during exercise[22]. The intensity and duration of the exercise are determinants in the IL-6 production[23] as well as the low muscle glycogen contents. In addition, IL-6 levels depend on the amount of muscle mass involved during exercise[24].

According to some authors, cytokines and other peptides that are produced and released by muscle fibres and exert effects on metabolism (paracrine or endocrine) should be classified as "myokines"[22]. Muscle-derived IL-6 is released into the circulation during exercise participating in the maintenance of the glucose homeostasis and exercise-induce lipolysis due to the effect exerted on the liver and adipose tissue, respectively[25].

Several authors have demonstrated a clear disassociation between IL-6 and tumor necrosis factor alpha (TNF-α)[26]. These authors showed that although intramuscular IL-6 gene expression and protein release was remarkable during continuous contractile activity, no response for TNF-α was evident, indicating that IL-6 is not always related or released after an inflammation process.

IL-6 as Cytokine: Infection or tissue injury are related to the production of cytokines that are released at the site of inflammation. The local inflammatory response is accompanied by a systemic response known as the acute phase response, in which several hepatocyte derived acute phase proteins are produced (C-reactive protein, transferrin, alpha-2 macroglobulin)[27].

Strenuous exercise has been reported in several occasions to develop inflammation in the body due to elevations in plasma of TNF-α and IL-6, but only as a result of marathon running[28,29]. Concurrently, exercise seems to have strong anti-inflammatory effects[26,30]. During exercise, IL-6 is the first cytokine present in the circulation. High circulating levels of IL-6 are followed by an increase of the anti-inflammatory cytokines IL-1ra and IL-10 and inhibit the production of the proinflammatory cytokine TNF-α as shown *in vitro*[31] and animal studies[32,33].

Furthermore, according to some authors[34], IL-6 stimulates the production of C-reactive protein, which has an important function in the induction of anti-inflammatory cytokines in circulating monocytes and in the suppression of the synthesis of proinflammatory cytokines in tissue macrophages.

According to Pedersen and Hoffman-Goetz[27], IL-6 should be classified as an "inflammation-responsive" cytokine, since IL-6 does not directly induce inflammation. Therefore, to confirm an inflammatory state, IL-6 should not be considered in isolation, but together with the expression of other pro-inflammatory cytokines such as TNF-α.

Exercise influence on mechanisms regulating hepcidin

The aforementioned mechanisims are affected acutely or chronically by exercise. The following sections describe the interaction of exercise with each of them.

Exercise and iron status

Body iron status has been reported to influence the previously described response of hepcidin to exercise. Healthy individuals have shown a positive association between ferritin and hepcidin concentrations, being higher the hepcidin levels in those subjects with higher ferritin reserves[35]. Two studies have described that the higher the ferritin reserves before exercise, the higher the hepcidin levels 3 hours post-exercise in response to IL-6 increase just post-exercise[36,37]. Curiously, when ferritin reserves are insufficient (below 30 micrograms/ml), hepcidin does not seem to experiment a significant increase after exercise, instead of the IL-6 peak produced at 0h post-exercise36. This fact points out that the body iron stores could act as a defensive mechanism against the hepcidin increase induced by exercise. This mechanism would be focused on allowing the body to restore the iron reserves and supply enough iron to plasma in order to meet the requirements for iron-dependent processes.

In addition, exercise produces iron losses that could affect body iron stores by several mechanisms, such as haemolysis, gastrointestinal bleeding, haematuria and/or sweating[38]. Exercise produces haemolysis during exercise, consisting on the destruction of red blood cells. Curiously, running exercise shows the most severe haemolysis, since the foot strikes repeatedly occurring in this discipline produce the destruction of erythrocytes[39]. As a result of haemolysis, haemoglobin and iron are released into the plasma.

Gastrointestinal bleeding and iron losses via the urinary tract and sweating are less usual or not powerfull enough to produce significant iron losses. However, considered as a whole, they can substantially contribute to the daily iron losses and reduce the athlete´s iron status.

Exercise and interleukin-6

Most studies have identified IL-6 as an important upstream mediator of hepcidin induction by post-exercise inflammation[14,37,38,40], however this does not necessarily imply an inflammatory state, since IL-6 is produced during exercise in absence of inflammation.

Some studies have reported that hepcidin levels seem to peak between 3-6 h subsequent to the peak in IL-6 elevation after an exercise bout[41,42].

Since endurance exercise is able to significantly increase IL-6 levels, several studies have already investigated this time-course after exercise. A research study[43] investigated in women the effects of two treadmill runs (60 and 120 min) at 65% of VO_2max, finding that both runs resulted in significant increases in hepcidin 3 hours after exercise and preceded by significant increases in IL-6 immediately post-exercise. Furthermore, the 120 min treadmill run produced a higher increase of IL-6 levels that the 60 min treadmill run and consequently a noticeably higher hepcidin production, indicating a concentration dependent response of hepcidin to IL-6.

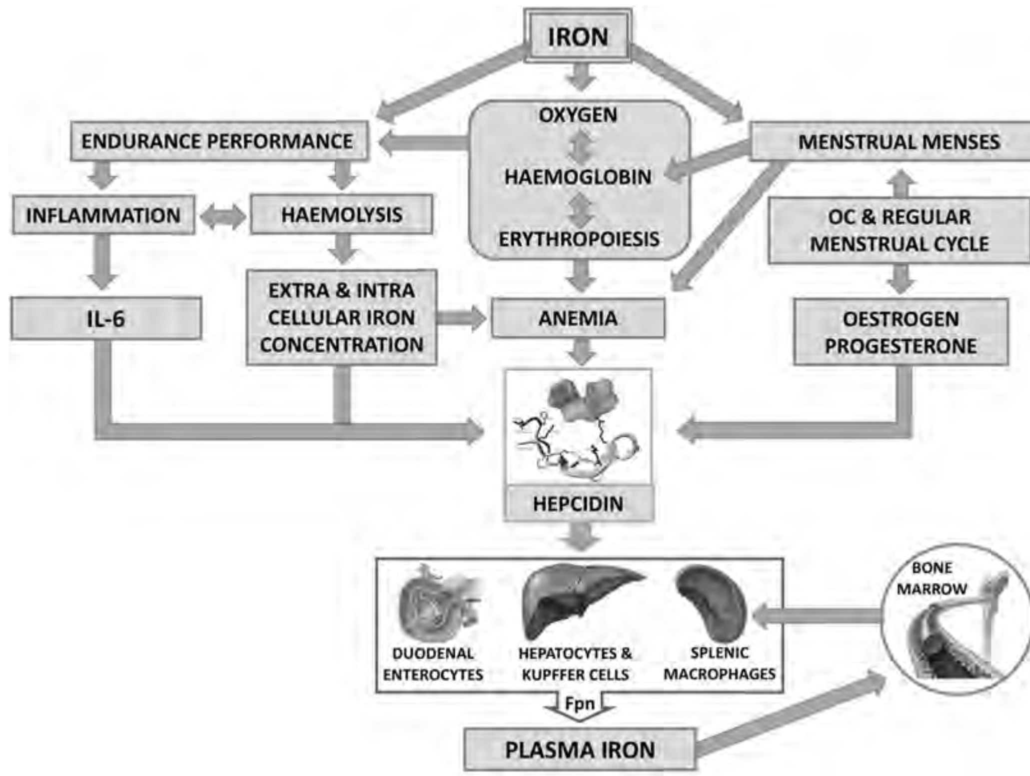
Consequently, a significant decrease in serum iron was produced 9 hours post-exercise. Several studies have reported similar results[42,44–46].

Only a few studies examined IL-6 and hepcidin response after exercise in female athletes, but none of these studies analysed these variables under the influence of sexual hormones[16,43,47] (Figure 1).

Special considerations for physically active women

Iron deficiency, with and without anaemia, has been mainly demonstrated in premenopausal females at a greater prevalence than men, because of the additional Fe demands of menstruation and preg-

Figure 1. Iron importance on oxygen metabolism and the different stimulus affecting hepcidin regulation under sexual hormones influence. Inadequate iron status may reduce haemoglobin, red blood cell production (erythropoiesis) and subsequent endurance performance. Endurance exercise is known to produce haemolysis and increase inflammatory markers as IL-6, as well as modify extra and intra cellular iron concentration. Both signals are positive regulators of hepcidin expression and may explain exercise-induced iron deficiency or anaemia in athletes. The menstrual bleeding experienced by women may have a significant impact on haemoglobin and consequently affect endurance performance. The oestrogen and progesterone variations found during a regular menstrual cycle and with OC may influence on hepcidin levels, and subsequently on iron absorption. Hepcidin regulates plasma iron concentrations by controlling ferroportin (fpn) concentrations on iron-exporting cells including duodenal enterocytes, recycling macrophages of the spleen and liver, and hepatocytes. The iron concentrations in plasma are mainly destined to the bone marrow in order to develop the erythropoiesis.



nancy[48,49]. Not only inadequate iron intake and losses of iron through menstruation could affect this population, but also regular exercise performance.

Menstrual blood loss may alter iron related parameters, as lower ferritin, haemoglobin or serum iron levels during menses[50], and therefore to reduce physical performance. Consequently, the iron homeostasis in female athletes tends to be unbalanced due to menstrual losses, which are difficult to quantify.

These variations have been found in both women, eumenorrheic and those taking OC, although fluctuations are much smaller in women under OC effects[51].

Moreover, sex hormones like estradiol and progesterone have been related with iron metabolism parameters in the literature. These hormones may have an important role over IL-6 and hepcidin, regulating iron absorption[52–54].

Endogenous hormones influence on iron metabolism

Oestrogen and progesterone may have an important effect over iron metabolism, modifying hepcidin and IL-6 activity. Despite one study reported that oestrogen was found to increase hepcidin gene expression[55], most studies have shown an inhibition of hepcidin expression by 17-β estradiol or oestrogen treatment[55–58].

Some authors reported a downregulated hepcidin expression by estradiol in fish[56]. Hepcidin regulation in this study could have been mediated by IL-6, which has also been reported to be inhibited by oestrogen or 17-β estradiol. However, IL-6 levels were not measured in these studies[56]. Shortly thereafter, a similar response in mice[53,58], and in human liver cells was reported[58]. Similarly, a mouse model of oestrogen deficiency by ovariectomy was established, demonstrating that oestrogen is implicated in modulating iron homeostasis by governing hepatic hepcidin expression[53]. In the ovariectomized mice group, hepcidin expression was more elevated under oestrogen deficiency compared to the control group, following of a decrease in serum iron.

Other authors found that transcription of hepcidin was suppressed by estradiol treatment in human liver cells and that estradiol reduced hepcidin mRNA in wild-type mice[58]. These authors suggested that hepcidin inhibition by estradiol may increase iron uptake in order to compensate iron loss during menstruation, helping as well to increase iron stores in OC users.

A recent study of in vitro fertilization reported a reduction of serum hepcidin-25 by almost 40% when oestrogen stimulation occurred compared to the castration state[57].

The results of these studies agree with an older study[59], which demonstrated that serum hepcidin is higher in postmenopausal than premenopausal women. This novel mechanism by which estradiol could inhibit hepcidin levels, may help to increase iron absorption, iron release and recycling from storage cells[58].

Furthermote, progesterone may up-regulate serum hepcidin in mice and humans. Progesterone concentrations were reported to increase hepcidin gene expression in zebrafish[60]. The same study investigated 20 women who were given progesterone as part of a standard in vitro fertilization protocol, reporting an increase in serum hepcidin levels after day 20 of a treatment with daily 50 mg of intramuscular progesterone supplementation.

The suggested regulation of estradiol and progesterone over hepcidin may also occur via IL-6 as an intermediate step. Some authors, reported in premenopausal women higher IL-6 levels during the follicular phase when progesterone levels were low, and a significant reduction in IL-6 after ovulation when progesterone levels increased[54]. Contrary, other authors reported that IL-6 concentration were significantly higher in the luteal phase[61].

Despite more scientific evidence is needed, as a result of these findings, it seems that sexual hormones may have potential effects on hepcidin and IL-6 regulation and consequently to impact iron homeostasis.

Exogenous hormones influence on iron metabolism

One of the most notable effect of OC would be the lesser quantity and shorter duration of menstrual blood loss[7]. This event may affect considerably to haemoglobin concentration and iron parameters. A previous study examined that menstrual blood loss decreased by approximately 50% in OC users[6]. In addition, these findings could be related to the improve of iron parameters in the OC group, since ferritin levels were significantly enhanced in 10%, regarding those women who did not use OC[6]. Previously to these findings, other studies reported in OC users significantly higher serum ferritin[5,7], iron and total iron binding capacity[7] in relation to nonusers.

Therefore, OC may improve iron stores by the reduction of menstrual blood loss or through the suppression of hepcidin via estradiol, and consequently optimizing exercise performance.

The steady loads of estradiol and progestin during the OC cycle have led a few authors to investigate the possible associations between exogenous sexual hormones and iron metabolism through hepcidin and IL-6 response. However, most of the studies reported that plasma IL-6 concentrations did not change between OC phases[62,63].

Similarly, the first study investigating the post-exercise IL-6 and hepcidin response, reported similar results for serum IL-6 and hepcidin for both hormone-deplete and hormone-replete phases of a monophasic OC cycle[64]. These results indicated that exercise performed during the different phases of a monophasic OC cycle does not alter exercise induced IL-6 or hepcidin production and it may be linked to the lower dosage of estradiol delivered from the OC[65].

Conclusion

Most studies presenting changes have been carried out in animals where estradiol or progesterone concentrations were infused with greater hormonal doses than those obtained from a normal menstrual cycle or with OC. Therefore, these findings cannot be applicable when premenopausal women are studied. Although literature is very reduced, for those females taking monophasic OC pills, hepcidin levels seem to be unaffected by exogenous hormones concentrations and therefore it seems it is not necessary to adjust the trainning session to the OC cycle. Regarding eumenorrheic women, more research studies are needed considering the effects of endogenous hormones concentrations on hepcidin production, since progesterone may have an opposite effect to oestrogen or even inhibiting oestrogens activity.

Hence, figure it out hepcidin behavior along menstrual cycle may provide information about when iron absorption could be more efficient. This might benefit those females suffering ID due to their demanding trainings and/or menstrual blood loss, resulting in a performance detriment or health impaired.

Conflict of interest

The authors declare no conflict of interest.

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The ARCHIVES OF SPORTS MEDICINE Journal (Arch Med Deporte) with ISSN 0212-8799 is the official publication of the Spanish Federation of Sports Medicine. This journal publishes original works about all the features related to Medicine and Sports Sciences from 1984. This title has been working uninterruptedly with a frequency of three months until 1995 and two months after this date. Arch Med Deporte works fundamentally with the system of external review carried out by two experts (peer review). It includes regularly articles about clinical or basic research, reviews, articles or publishing commentaries, brief communications and letters to the publisher. The articles may be published in both SPANISH and ENGLISH. The submission of papers in English writing will be particularly valued.

Occasionally oral communications accepted for presentation in the Federation's Congresses will be published.

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