ISSN: 0212-8799

173

Volumen 33(3)
Mayo - Junio 2016

Archivos de medicina del deporte

Órgano de expresión de la Sociedad Española de Medicina del Deporte

ORIGINALES

REVISIONES

Sociedad Española de Medicina del Deporte

AEPSAD
AGENCIA ESPAÑOLA DE PROTECCIÓN DE LA SALUD EN EL DEPORTE

CSD Consejo Superior de Deportes

Archivos de medicina del deporte

Revista de la Sociedad Española de Medicina del Deporte

Afiliada a la Federación Internacional de Medicina del Deporte, Sociedad Europea de Medicina del Deporte y Grupo Latino y Mediterráneo de Medicina del Deporte

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Publicidad
ESMON PUBLICIDAD
Tel. 93 2159034

Publicación bimestral
Un volumen por año

Depósito Legal
Pamplona. NA 123. 1984

ISSN
0212-8799

Soporte válido
Ref. SVR 389

Indexada en: EMBASE/Excerpta Medica, Índice Médico Español, Sport Information Resource Centre (SIRC), Índice Bibliográfico Español de Ciencias de la Salud (IBECS), y Índice SJR (SCImago Journal Rank).

Archivos
de medicina del deporte

Volume 33(3) - Núm 173. May - June 2016 / *Mayo - Junio 2016*

Summary / Sumario

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Training muscles and brains: being physically and cognitively active at early ages

Entrenando músculos y cerebros: ser físicamente activo para ser cognitivamente activo a edades tempranas

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Introduction

Public health institutions recommend that children and adolescents should carry out at least 60 minutes of moderate-vigorous physical activity each day to achieve substantial health benefits[1]. There are numerous benefits associated to physical health; however, the potential effects of physical activity on cognition should be examined more extensively[2]. We work under the slogan of "training muscles and brains: be physically active to be cognitively active". Physical activity stimulates some factors involved in cerebral plasticity, such as the brain-derived neurotrophic factor (BDNF)[3]. Therefore, being physically active may have beneficial effects on brain development, which in turn may play a key role in the cognition of children and adolescents.

Cognición

Cognition, understood as the mental function involved in the in¬teriorisation of knowledge and comprehension, alludes both to the cognitive and academic aspect. In fact, the cognitive function (also known as the executive function or cognitive control), plays a key role in the academic performance of students. Specifically, the cognitive function includes 3 important aspects: cognitive inhibition, work memory and cognitive flexibility. Previous studies have demonstrated that, in particular, cognitive inhibition and work memory provide the foundations for developing academic abilities in children and adolescents[4]. The stages of childhood and adolescence are characterised by periods of significant brain plasticity, providing great opportunities for stimulating the cognitive function; furthermore, despite practically all the neurons we have over our entire lives being formed in the first 3 years of life, it is from 6-7 years, at the start of childhood, when the number of neuronal connections begins to increase considerably and progressively up to the age of 15-16 years, an age at which adolescents reveal greater rates of reduced physical activity[5-6]. Paradoxically, the moment the number of neuronal connections begins to fall corresponds to the mo¬ment in which adolescents reduce their levels of physical activity. Therefore, inactive adolescents may be losing an important stimulus in improving their cognitive performance.

Physical activity and cognition during childhood and adolescence

Various systematic reviews have brought to light the influence of physical activity in the cognition of children and adolescents[7-8]. However, there is currently controversy regarding the type, frequency, duration and intensity of physical activity required to achieve the greatest cognitive benefits. Some studies affirm that short periods of vigorous physical activity should be carried out, whilst others claim that at least 60 minutes of moderate-vigorous activity should be performed each day. Others focus on the type of physical activity (aerobic exercise *vs.* strength work), however, some studies indicate that it may have no cognitive benefit, or even that high levels of physical activity could lead to unexpected effects[7-9].

Just as when following a medical prospectus, a specific dose is administered to achieve an expected response; the same would occur

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with physical activity like a "pill" in relation to cognition. It is possible that intermediate levels of physical activity, in terms of minutes of daily activity, may entail greater cognitive benefits. Along this same line, a recent study indicates that maintaining a regularly active lifestyle may have greater cognitive-associated benefits compared to acquiring changing physical activity behaviour. In other words, being "too under active" at some ages and "too active" at other ages seems to be less effective (and may even have, to a certain extent, contrary effects), than maintaining a moderately active behaviour over the whole childhood and adolescence in achieving cognitive benefits[10]. Therefore, despite physical activity maybe being beneficial in all stages of life, early intervention could be important in improving and/or maintaining cognitive functions, as the earlier the start of physical activity practice, the greater the cognitive benefits may be.

Physical activity and cognition in the school context

The school context provides a unique opportunity to promote physical activity with Physical Education, break times, active rest times or active movements at school, which may contribute substantially to the increase in physical activity levels, in turn implying benefits on a cognitive level. It is important to call to action all educational institutions, given that, in an effort to increase the academic performance of student, there have been proposed solutions to dedicate more time to instrumental subjects, reducing or eliminating the time that students have to be active (see Physical Education). However, it is worth noting that there is no scientific evidence to prove that eliminating non-instrumental subjects such as Physical Education is related to enhanced academic performance[8]. In fact, empirical evidence suggests that peak levels of physical condition may have a positive effect on academic performance, whilst obesity may have negative repercussions on academic performance[11].

Mechanisms

Various mechanisms are involved in the effects of physical activity on the brain function from early ages up to adulthood. Physical activity may increase the formation of new neurones and concentrations of BDNF, increase the blood flow to the brain and the oxygen supply to it, as well as increasing synaptic plasticity[3,12]. This collection of physiological changes are related to: 1) attention; 2) processing information, storing and recovering information; as well as 3) mental concentration[4]. Therefore, these changes may lead to improvements in the cognitive and academic performance of young people.

Future orientations

Despite the abundance of knowledge about the effects of physical activity on the brain and on cognition, there are still a multitude of issues to resolve. From a practical point of view, there is little knowledge about how to design interventions based on physical exercise that optimise the effects of cognition and brain health. Issues regarding the context, mode, frequency, duration and intensity of the physical activity to achieve the greatest cognitive results should also be resolved in future research studies. From the ActiveBrains study[13] (http://profith.ugr.es/activebrains), a random trial with the main aim of examining the effects of an exercise programme on cognition, and the structure and function of the brain in overweight/obese children, we aim to contribute towards responding to these issues.

Funding

EThis study is funded by the Ministry of Economy and Competitiveness (REF: DEP2013-47540-R; and RYC-2011-09011). IEC has a contract at the University of Granada funded by the Ministry of Economy and Competitiveness (REF: FJCI-2014-19563).

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Original article

Stretching exercises accompanied or not with music, reduce the stress level of pre-college student

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Received: 05.06.2015
Accepted: 01.09.2015

Summary

In Brazil, many students take preparatory courses for entrance exams at university. However, these tests can be a stressful event, because their realization usually coincides with a phase of students' lives when they are still finding out and structuring their identity. Considering that stress is a prevalent syndrome in young people, especially those in the admission phase in college, there is a need to evaluate the influence of strategies aiming to reduce the stress in this population. Thus, we investigate the effects of a single bout muscle stretching exercise, listening to music and stretching exercise accompanied by music sessions on the number of stress symptoms in students from preparatory schools for the university admission exams (UAE). Two hundred fifty students were evaluated using Lipp´s Inventory of Stress Symptoms for adults (ISSL). This instrument was standardized for the Brazilian population, and allows to evaluate the level of stress and determine the number of stress symptoms of an individual. Volunteers responded the ISSL before and after one of the experimental situations (30 minutes): SA (active and static stretching exercise), SM (listening to a New Age music), SAM (SA and SM combined) and control situation (SC). It was verified that 55.2% of the volunteers were stressed. The Kruskal-Wallis test observed significant differences ($H=13.63$; $p< 0.01$) between SA and SAM situations compared to SC in stressed students ($n=138$). It was observed significant reduction for physical and psychological symptoms in SA and SAM. It was concluded that static and active stretching exercises can be used before exams to reduce stress symptoms.

Key words:
Muscle stretching exercise. Music. Stress. University admission exams. Young people.

Los ejercicios de estiramiento, acompañado o no con la música, reduce el nivel de estrés de los estudiantes pre-universitarios

Resumen

En Brasil, muchos estudiantes toman cursos de preparación para los exámenes de ingreso a la universidad. Estas pruebas pueden ser un evento estresante, ya que por lo general, su realización coincide con una fase de vida de los estudiantes en la que aún están descubriendo y estructurando su identidad. Teniendo en cuenta que el estrés es un síndrome frecuente en los jóvenes que están en la fase de admisión a la universidad, se hace necesario evaluar las estrategias dirigidas a reducir la tensión en esta población. El objetivo de esta investigación fue verificar los efectos del estiramiento muscular, la audición musical y ejercicios de estiramiento con música en el número de síntomas de estrés en estudiantes de los cursos preparatorios para pruebas de admisión universitarias. Doscientos cincuenta estudiantes contestaron el Inventario de Síntomas de Estrés para adultos de Lipp (ISSL). Este instrumento permite evaluar el nivel de estrés y determinar el número de síntomas de estrés de un individuo. Los voluntarios realizaron el ISSL antes y después de una de las situaciones experimentales (duración de 30 minutos): SA (ejercicio de estiramiento estático y activo), SM (música del estilo New Age), SAM (SA y SM combinadas) y situación control (SC). Verificando que el 55,2% de los voluntarios presentaban estrés. El test de Kruskal-Wallis mostró diferencias significativas ($H=13,63$; $p< 0,01$) entre las situaciones SA y SAM comparadas a la SC en los estudiantes con estrés ($n=138$). También se observó reducción significativa en los síntomas físicos y psicológicos en SA y SAM. Permitiendo concluir que el ejercicio de estiramiento estático y activo puede ser utilizado antes de las pruebas de admisión a la universidad como estrategia para la reducción de síntomas de estrés.

Palabras clave:
Ejercicios de estiramiento muscular. Música. Estrés. Pruebas para admisión en la universidad. Joven.

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Introduction

In Brazil, many students take preparatory courses for entrance exams at university[1]. However, these tests can be a stressful event, because their realization usually coincides with a phase of students' lives when they are still finding out and structuring their identity.

The term stress has been often related to the response of the organism before challenging situations, which can harm or weaken it somehow. When exposed to external stressors, the response of the body can be identified by three stages: alert, resistance and exhaustion[2-4]. Additionally, a new phase was included by Lipp[5] during the development and validation of an instrument for assessing the symptoms of stress. It was the stage of near-exhaustion, inserted between the resistance and exhaustion phase.

Among the most susceptible populations to stress, it highlighted the teenagers and young adults[6]. In the academic context, it was observed that during the period before the entrance exams at university (UAE), young people showed an increase in the consumption of alcohol and cigarettes, as well as decrease in physical activity which aggravated even more stress caused by this situation[7]. Additionally, stress, both physical and psychological, can influence the immune system[8].

In this sense, different interventions can be effective in reducing stress levels, emphasizing relaxation techniques involving muscle stretching and music listening[9,10]. Thus, considering some physical symptoms of stress as "feeling of constant physical stress", "muscle strain", "hyperventilation" and "fatigue"[11], some body movements alternating contraction and relaxation could alleviate certain symptoms for some time or prepare the body for adverse events. With regard to music, a suitable style could help stretching programs in order to eliminate or reduce stress points in certain areas of the body[12]. In this way, the correct manner to integrate these components can affect physiological and/or psychological responses of the individual generating different reactions to reduces stress[13,14].

Considering that stress is a prevalent syndrome in young people, especially those in the admission phase in college, there is a need to evaluate the influence of strategies involving stretching and music aiming to reduce the stress in this population. Thus, the aim of this study was to investigate the effects of an acute bout of stretching muscle, listening to music sessions and the association between stretching and music on the number of stress symptoms on from pre-university students.

Material and methods

This study was approved by the Research Ethics Committee of the Institute of Biosciences of Universidade Estadual Paulista (UNESP), Campus of Rio Claro – SP, under protocol 000052/01.

Participants

Two hundred and fifty young men and women volunteered to participate in the study. The inclusion criteria in the study were that all subjects had completed high school and were regular students of all preparatory courses for UAE in the city of Rio Claro, São Paulo, Brazil. In addition to these criteria were only selected individuals who were classified in at least one of the phases of stress according to the Lipp's Inventory of Stress Symptom for Adults (ISSL). In this way, 138 individuals (45 men and 93 women) effectively participated of the study. The average age of the men was 20.3 ± 5.7 years, while the age of the women was 19.5 ± 4.7 years. The participants signed a free and informed consent form allowing their participation in the research. Later they were randomly divided into four groups: SA - participation in a stretching session; SM - participation in a Music listening session; SAM – participation in a stretching session accompanied by music listening; and SC - continuation of the activities of the preparatory course in class (control situation). Each experimental situation lasted 30 minutes. Thus, all the experimental conditions were performed on the same day during the classroom activities.

The songs used were taken from the New Age style album "Secret Garden - White Stones."[15]. This style mix classical music with elements of folk melodies, making a reinterpretation of classical music, with the addition of elements from Celtic and Norwegian folklore. This selection focuses in both music segments stimulative and sedative, the same procedure adopted by Scheufele[16]. This author was based on previous studies that suggested that such variation in musical progress is more effective for relaxation responses compared to only sedative music selections. This type of music is usually used in stretching classes, and contains only instrumental arrangements, being chosen so that there were not additional factors (vocals, lyrics) that could influence the research. The musical selection was used both in SM and SAM experimental situations. Regarding to stretching, the group SA and SAM underwent the same protocol described by Valim[17]. During these sessions were conducted 18 exercises based on the postures of *Hatha Yoga,* combining static and dynamic variations emphasizing breathing in every movement. The sequence of exercises was started with the individuals in dorsal decubitus and finalized in standing position[17]. In this protocol, every movement was verbally explained and physically demonstrated (slowly, clearly and objectively) to participants.

The ISSL was applied before and after each experimental situation. This instrument was standardized for the Brazilian population by Lipp and Guevara[18], and allows to evaluate the level of stress and determine the number of stress symptoms of an individual[4]. The ISSL consists of three tables, corresponding to three-phase Selye´s stress model[2], including the fourth phase (near-exhaustion) found at the time of standardization of the ISSL[18]. The first chart of the instrument is composed of the symptoms presented by the individual in the last 24 hours and contains 15 items referring to the symptoms (12 physical symptoms and three psychological), corresponding to the alert phase. The second table also provides 15 symptoms (10 physical and five psychological), divided into two frames corresponding to the stages of resistance and near-exhaustion, graded according to a certain minimum amount of symptoms presented during the last week. The third chart contains 23 items, 12 physical and 11 psychological, corresponding to the symptoms presented in the last month, featuring thus the exhaustion phase. The definition of stress phase is given by the sum of the symptoms presented on each frame. There is a minimum value in each of the stages, which can characterize the individual as stressed or not.

Statistical Analysis

To evaluate the homogeneity of the groups and possible differences in stress responses among groups after the experimental situation Kruskal-Wallis test was used. Multiple comparisons test was used in order to identify significant differences between treatment pairs. To separately analyze the physical and psychological symptoms and verify possible changes between before and after each experimental situation, we used Wilcoxon's test. The significance level of 95% ($p <0.05$) for all tests was used.

Results

It was observed that from the 250 subjects, 138 (55.2%) were stressed and 99 (39.6%) showed no stress. Thirteen people (5.2%) were excluded for not answering the entire quiz or incorrectly performing the research procedures (eg leave the room before the end of the experimental situation). Thus, data analysis was applied to a sample of 138 young students, so only those with stress.

Most of the stressed patients were female (67.4%) and 63.8% were enrolled in daytime, 55.8% practiced some physical activity regularly and 73.9% were not working.

All stressed volunteers were found in resistance or near-exhaustion phase, as shown in Table 1.

The Kruskal-Wallis test showed that the group of individual of SA, SM, SC and SAM were homogenous before each treatment. There were no significant differences in average levels of stress among the four groups evaluated before the execution of their respective experimental situations ($H = 6$; $p > 0.05$). After 30 minutes from baseline time differences were observed between the experimental conditions ($H = 13.63$, $p < 0.01$). The SA and SAM situations differed from the control situation ($p < 0.001$ and $p < 0.01$, respectively) and SM and SA situations differed each other ($p < 0.05$ - Figure 1).

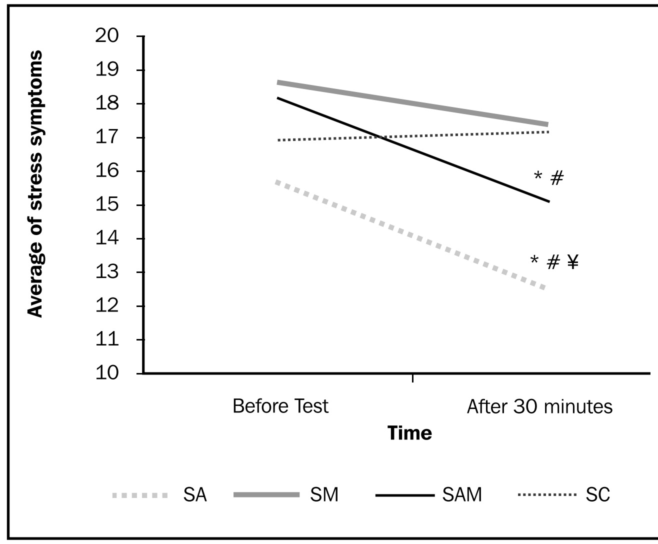
For a more detailed analysis of the reduction of stress, physical and psychological symptoms were analyzed separately within each experimental situation (Table 2). Using Wilcoxon's test it was examined whether there was superiority of one treatment over the other as to the nature of the data collected. The level of significance for differences between situations before and after the SA and SAM was high ($p \leq 0.01$) for both the physical and psychological symptoms therefore, these conditions were effective in reducing both types of symptoms of stress.

Table 1. Number of individuals situated in each stress phase.

	Stress phase			
Groups (n=138)	Alert	Resistance	Near - exhaustion	Exhaustion
SA	0	30	1	0
SM	0	30	6	0
SAM	0	38	4	0
SC	0	29	0	0
Total	0	127	11	0

SA: participation in the stretching session; SM: participation in a music listening session; SAM: participation in a stretching session accompanied by music listening; and SC: continuation of the activities of the preparatory course in class (control situation).

Figure 1. Mean number of stress symptoms of pre-college students before and after 30 minutes of their respective experimental conditions.



*: Indicates difference between before test and after 30 minutes; #: Indicates difference from SM and SC groups; ¥: Indicates difference from SAM group.

Discussion

In present study, it was expected better results for the SAM situation considering the potency of music to the effects of stretching. The music alone did not bring significant results, at least 30 minutes, and the enhancement of benefits did not happen because there were no significant differences between the stretch with music and without music stretching. It is evident the difficulty to interpret and relate the results of the research addressing the utilization of different physical activities or different types of music in stress responses. This difficulty is due to the various forms of assessment employed (physical, physiological, psychological) and the discrepancy found between the results of

Table 2. Wilcoxon test values for the physical and psychological symptoms in each group.

Groups	Physical symptoms (z)	Psychological symptoms (z)
SA	-3.91**	-2.74*
SM	-1.55	-1.01
SAM	-3.45*	-2.54*
SC	-0.29	-0.20

*$p \leq 0.01$. **$p \leq 0.0001$. SA: participation in a stretching session; SM: participation in a Music listening session; SAM: participation in a stretching session accompanied by music listening; and SC: continuation of the activities of the preparatory course in class (control situation).

a further evaluation even in just one study. An example is the study of Steptoe *et al*.[7] in which the physiological and psychological responses were compared during the performance of aerobic exercise. These researchers concluded that, through physiological measurements, aerobic training is not associated with stress reduction. However, through psychological measurements they observed responses associated with reduced stress as a decrease in tension-anxiety, depression, mental confusion and improved ability to cope with stress[7]. Similarly, it was observed that music therapy decreased anxiety and pain in patients who underwent cardiac surgery, whereas no differences were observed in blood pressure and heart rate of individuals[19]. However, Möckel *et al.*[20] observed beneficial responses related to stress only in physiological aspects when compared different types of music.

The Stretching exercises used in the present study are similar to Progressive Relaxation (RP), which is the type most often cited in the literature. RP is characterized by relaxing each muscle or muscle group so that the individual recognizes the contrast between tension and relaxation[16,21]. Thus, stretching can be considered as a sort of relaxation which promotes reduction of muscle stress levels, respiration rate and blood pressure, as well as subjective and objective states of excitation of the individual[21]. The relaxation exercise has been considered effective for stress management, being compared to hearing music sessions[16]. In this sense, Scheufele[16] compared two experimental situations by checking the effects of RP and classical music in measurements of attention, relaxation and stress responses. It has been found that the RP condition showed high scores as relaxation, despite the music have caused decreased heart rate and provided distraction from stressors.

In the present study, in addition to stretching and music listening conditions, it was also included the condition stretching accompanied by music, so that to the research could reproduce the actual situation of stretching classes and not just in a controlled experimental condition. Robb[22] compared four experimental conditions: Progressive Relaxation associated with music (RP + M), Progressive Relaxation (RP), music Hearing (M), and control situation of silence (S) on anxiety and perceived relaxation and found that each treatment might be equally effective, producing significant changes in anxiety and perceived relaxation.

Möckel *et al.*[20] stated that music with faster tempo increases heart rate whereas classical and New Age decreases and stimulates physical and mental relaxation. These researchers observed significant reductions in the concentration of plasma cortisol – the hormone related to stress - in meditative slow pace music hearing.

Regarding to the use of sedative music (slow), excitatory (quick), classical music or music preference of listeners, categorical statements about their suitability for stretching activity still cannot be made due to the few studies in this area or the contradictory results already submitted. In the Iwanaga & Moroki study[13], listening to classical exciting music brought feelings of vigor and tension to listeners while sedative classical music brought relief from tension. However, the authors also found that musical preference did not affect physiological responses as heartbeat, breathing and blood pressure, caused by hearing both songs. Thus, these authors concluded that the dominant factor on the emotional response was the type of music rather than musical preference. However, the classical style is the least heard by young people, being less present in the daily lives of these individuals[23].

In this sense, it was reported that the styles of music listened by more young people of both sexes aged 18 to 30 were the Rock (93%), Pop (66%) and the New Wave (57%), which are styles described as preferred for the practice of aerobics[24]. Thus, there are differences in the effects of the listening session according to the type and characteristics of music applied. The New Age music used in present study is similar to classical music; it is derived from the union with Folk, characterized by spatial sounds, sharp melodies and soft harmonies. The New Age style evidently differs from songs with faster tempo and stronger rhythms such as Rock or Techno-music. Listening to Techno-music, for example, may cause increase in heart rate and blood pressure, as well as, in adrenocorticotropic hormone (ACTH) and cortisol levels. Therefore this type of music is not suitable for the reduction of stress[25]. Furthermore, softer songs may promote therapeutic implications on stress treatment[26].

Regarding the physical and psychological symptoms analyzed separately, it is considered that relaxation techniques may be more effective for certain symptoms of stress[27]. The Yoga, a somatic relaxation technic, focuses directly on the body and is considered most effective in the treatment of physical stress symptoms (eg muscle tension , tachycardia, loss of energy and physical fatigue) while the cognitive techniques are more effective in changing mental processes that result in cognitive symptoms such as anxiety, worry and insomnia[27]. However, the results of the present study show that a "physical" intervention may also decrease psychological symptoms of stress.

Alter Jr[28] reports through physiological mechanisms, why and how the stretching exercise can be used to facilitate the relaxation. Such mechanisms involve the adjustment of components such as the Golgi tendon organs and muscle stretching receptors (muscle spindle) which promotes reduction of muscle tension and increase in relaxation. Additionally, proper breathing also assists in reducing tension[28].

Conclusion

Based on the results obtained in this study we can conclude that 30 minutes of stretching, accompanied or not by New Age music, significantly reduced the stress symptoms (both physical and psychological) in pre-college students. Although it cannot be statistically affirmed that New Age music style reduces stress, it can be affirmed that music listening during stretching exercise did not negatively interfere on exercise efficacy for stress symptoms reduction. Future studies should be conducted using another musical style to verify the influence on stress reduction.

The stretching, static and active, can be prescribed for young people during the period of evaluation tests as a strategy to decrease stress symptoms and can then be adopted as a coping strategy for this type of population.

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PREMIOS FEMEDE A LA INVESTIGACION 2015

Los trabajos que han logrado los premios FEMEDE a la investigación en el año 2015, consistentes en la **publicación en la revista Archivos de Medicina del Deporte, junto con una dotación de 600 euros y el certificado acreditativo** son los que se relacionan a continuación con sus correspondientes autores:

- **María Perales**, por el trabajo titulado *"Fetal and maternal heart rate responses to exercise in pregnant women. A randomized Control Trial"*, con coautoría de Silvia Mateos, Marina Vargas, Isabel Sanz, Alejandro Lucia y Ruben Barakat.
- **Oriol Abellán-Aynés**, por el trabajo titulado *"Anthropometric profile, physical fitness and differences between performance level of Parkour practitioners"*, con coautoría de Fernando Alacid.
- **Eliane Aparecida de Castro**, por el trabajo titulado *"Peak oxygen uptake prediction in overweight and obese adults"* con coautoría de Rocio Cupeiro, Pedro J. Benito, Javier Calderón, Isabel R. Fernández y Ana B. Peinado.

Original article

Heart rate deflection point determined by D_{max} method is reliable in recreationally-trained runners

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Received: 12.12.2014
Accepted: 07.09.2015

Summary

Objectives: This study examined the test-retest reliability of the speed at the heart rate deflection point (sHRDP) determined by the maximal-deviation method (D_{max}) method developed by Cheng *et al.*[10] during incremental treadmill tests. It was also aimed to verify if the regression model (i.e, exponential-plus-constant and third-order polynomial regression models) and the initial HR point used to determine the sHRDP by the D_{max} method (i.e., model considering HR values above 140 b•min^{-1} versus the model considering all the HR points) influence on the sHRDP reliability.
Methods: Twenty-eight male recreationally-trained runners performed on test-retest design two continuous incremental exercise tests on a motorized treadmill with initial speed of 8 km•h^{-1} and 1 km•h^{-1} increments each 3 min to determine the sHRDP by D_{max} method and according to exponential-plus-constant and third-order polynomial regressions models ($sHRDP_{exp}$ and $sHRDP_{pol}$). Furthermore, the sHRDP was also calculated considering HR values above 140 b•min^{-1} ($sHRDP_{exp>140}$ and $sHRDP_{pol>140}$).
Results: The sHRDP values obtained from exponential-plus-constant regression model showed higher reliability than the sHRDP values derived from third-order polynomial regression model (ICC ≥0.83; SEM ≤0.37 km•h^{-1}; CV ≤3.09%). The $sHRDP_{exp}$ was the most reliable variable with ICC of 0.87, the lowest values of SEM (0.17 km•h^{-1}) and CV (1.46%), bias near zero and narrow limits of agreement. On the other hand, the sHRDP values derived from third-order polynomial regression model were less reliable (ICC ≤0.70; SEM ≥0.67 km•h^{-1}; CV ≥5.77%). Additionally, HR values at the sHRDPexp and at $sHRDP_{exp>140}$ presented the highest reliability (SEM ≤3.74 and CV ≤2.30).
Conclusions: The $sHRDP_{exp}$ is a highly reliable variable; however, because in some participants the HR-curve demonstrated a linear behavior and the $sHRDP_{exp}$ occurred around the midpoint between initial and final speeds during incremental test, the exponential-plus-constant regression model should be used with caution.

Key words:
Reproducibility of results. Exercise test. Anaerobic threshold. Running.

Punto de deflexión de la frecuencia cardíaca determinado por el método D_{max} es reproducible en corredores de nivel recreacional

Resumen

Objetivos: Este estudio analizó la reproducibilidad test-retest de la velocidad en el punto de deflexión de la frecuencia cardíaca (vPDFC) determinado por el método de máximo desvío (D_{max}) desarrollado por Cheng, *et al.*[10], durante pruebas incrementales en tapiz rodante. Un segundo objetivo fue comprobar si el modelo de regresión (i.e., modelos de regresión exponencial-más-constante y polinómica de tercer-orden) y el punto inicial de la FC utilizado para determinar la vPDFC por el método D_{max} (i.e., modelo considerando los valores de FC superiores a 140 lat•min^{-1} versus el modelo teniendo en cuenta todos los puntos de FC) tienen influencia en la reproductibilidad de la vPDFC.
Métodos: Veintiocho corredores recreacionales entrenados ejecutaron en un diseño test-retest mediante dos pruebas incrementales continuas en la cinta rodante con la velocidad inicial de 8 km•h^{-1} y con incrementos de 1 km•h^{-1} cada 3 min para determinar la vPDFC por el método D_{max} y de acuerdo con los modelos de regresión exponencial-más-constante y polinómica de tercer-orden ($vPDFC_{exp}$ y $vPDFC_{pol}$). Además, la vPDFC también fue calculada teniendo en cuenta los valores de FC superiores a 140 lat•min^{-1} ($vPDFC_{exp}$>140 y $vPDFC_{pol}$>140).
Resultados: Los valores obtenidos de vPDFC por medio del modelo de regresión exponencial-más-constante mostró una mayor reproductibilidad en comparación a los valores de vPDFC derivados desde el modelo de regresión polinómico de tercer-orden (ICC ≥0,83; SEM ≤0,37 km•h^{-1}; CV≤ 3,09%). La $vPDFC_{exp}$ fue la variable más reproducible con ICC de 0,92, los valores más bajos de SEM (0,17 km•h^{-1}) y CV (1,46%), el sesgo cerca de cero y con estrechos límites de acuerdo. Por otro lado, los valores de vPDFC derivados del modelo de regresión polinómico de tercer-orden fueron menos reproducibles (ICC ≤0,70; SEM ≥0,67 km•h^{-1}; CV ≥5,77%). Además, valores de FC con la $vPDFC_{exp}$ y con la $vPDFC_{exp>140}$ presentarón mayor reproductibilidad (SEM ≤3,74 y CV ≤2,30).
Conclusiones: La $vPDFC_{exp}$ es una variable muy reproducible; no obstante, debido a que en algunos participantes la curva de FC demostró comportamiento lineal y la $vPDFC_{exp}$ ocurrió alrededor del punto medio entre las velocidades inicial y final durante el test incremental, el modelo de regresión exponencial-más-constante debe ser utilizado con precaución.

Palabras clave:
Reproducibilidad de resultados. Prueba de esfuerzo. Umbral anaeróbio. Carrera.

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Introduction

Variables determined during incremental exercise tests, such as lactate threshold and heart rate deflection point (HRDP) are predictors of endurance performance and are used as parameters for prescription and monitoring training intensities[1-3]. The reliability of these variables is defined by the replication of the same result in one or more repeated trials by the same participant under similar conditions[4].

The HRDP, as an intensity related to the anaerobic threshold (AT)[1,5,6] has demonstrated high correlations with endurance running performance[1,7,8], Conconi, *et al.*[7] proposed a visual determination of the HRDP that has been used in many studies[5,8,9].

However, other studies preferred to determine the HRDP by the maximal-deviation method (D_{max}) method, that was developed by Cheng, *et al.*[10] and consider as AT the point on an intensity regression curve that is furthest away from a straight line which connects the first and last points of that curve, mainly because it is possible to determine this point in most subjects, different of the Conconi, *et al.*[7] method which may not be identifiable due to a linear behavior[1,11,12]. Furthermore, da Silva, *et al.*[1] showed that the speed associated with deflection point (sHRDP) determined by D_{max} method was highly correlated with lactate threshold and with 10-km running performance in endurance recreationally-trained female runners.

Moreover, other factors could influence the determination of the HRDP by D_{max} method, such as the regression model for fitting data (i.e. exponential-plus-constant model vs third-order polynomial model) and the number of heart rate (HR) points used (all points or those above 140 b•min^{-1})[1,11]. Recently, da Silva, *et al.*[1] showed that the sHRDP obtained from exponential-plus-constant regression model resulted in a better estimation of lactate threshold which was better correlated with running performance than the one derived from the third-order polynomial regression model, independently of the HR values used (all points or those above 140 b•min^{-1}), demonstrating that the regression model could influence the HRDP values obtained from D_{max} method. However, the authors state that because the deflection point often occurred around the midpoint between initial and final speeds during the incremental test suggesting that the exponential-plus-constant may not be an appropriate regression curve.

Some studies have examined the reliability of the HRDP based on Conconi, *et al.*[7] method[13,14]. These studies reinforced the difficult to analyze reliability of the Conconi, *et al.*[7] method because it is not possible to determine HRDP in all subjects. This lack of identification of the HRDP by Conconi, *et al.*[1] method is explained by the behavior of the HR curve during incremental tests shows large inter-subject variability, which may reflect in a convex, concave or linear curve behavior, and influences the identification of a visual deflection point[15]. For instance, Jones and Doust[14] only observed HRDP in 6 out of 15 participants in both test and retest.

Thus, the application of D_{max} method could contribute to identifying HRDP in all subjects as showed in previous studies[1,11,12]. However, the reliability of the HRDP determined by D_{max} method is unknown. We hypothesized that the regression model and the number of HR points would influence the reliability of the sHDRP determined by D_{max} method. Therefore, the aim of this study was to examine the test-retest reliability sHRDP determined by D_{max} method during incremental treadmill test-retest reliability of sHRDP. It was also aimed to verify if the regression model (i.e, exponential-plus-constant and third-order polynomial regression models) and the initial HR point used to determine the sHRDP by the D_{max} method (i.e., model considering HR values above 140 b•min^{-1} *versus* the model considering all the HR points obtained) influence on the sHRDP reliability.

Material and methods

Participants

Twenty-eight male recreationally-trained runners with experience in 10-km running races and involved in systematic training were recruited. Characteristics of the participants (mean ± SD) were: age 26.1 ± 3.9 years, stature 177.1 ± 7.0 cm, body mass 75.6 ± 9.0 kg, body mass index (BMI) 24.1 ± 2.6 kg•m^{-2} and body fat 14.1 ± 4.2%. Body density (BD) was determined using the seven skinfolds protocol of Jackson and Pollock[16] and subsequently, body fat percentage was calculated from BD using Siri's equation[17]. The training characteristics were experience 4.2 ± 4.8 years, frequency 3.2 ± 1.4 days•wk^{-1} and distance 25.8 ± 16.9 km•wk^{-1}. The 10-km running times of the participants were between 40 and 60 min (i.e. a pace between 10 and 15 km•h^{-1}; ≅ 44–66% of the world record). We used the following inclusion criteria: age between 18 and 35 years; be apparently healthy (without chronic medical complications such as diabetes, hypertension, asthma and/or cardiovascular diseases); practice running for at least six months; be able to complete 10-km between 40 and 60 minutes (recreational level). The exclusion criteria were the following: be a smoker; present health problems such as diabetes, hypertension, asthma and/or cardiovascular disease according to anamnesis screening. Before testing, written informed consent was obtained from all participants. The researchers responsible for the study were committed to perform the tests within the safety standards, being knowledgeable of procedures to be performed. Thus, there are no risks for the participants, only that they can felt possible discomfort after the tests such as tiredness, muscle pain, sweating that will be similar to the symptoms felt during the routine of physical exercise. The experimental protocol was approved by Human Research Ethics Committee of the State University of Maringá (# 719/2010) and is in accordance with the Declaration of Helsinki in 2008.

General Procedure

The anthropometric measures (e.g., body mass, height and skinfolds to predict body fat) were obtained in laboratory conditions during the first visit. The participants who were habituated to running tests performed two continuous incremental exercise tests on a motorized treadmill (Super ATL; Inbrasport, Porto Alegre, Brazil) set at a gradient of 1%. The tests were performed separated by one week. Participants were instructed to report for testing well-rested, well-nourished, and well-hydrated, wearing lightweight comfortable clothing and were also instructed to avoid eating two hours before the tests, to abstain from caffeine and alcohol, and to refrain from the training routines and competitions during testing. Additionally, the participants not performed training or competition for at least 72 hours prior to the first test.

Incremental exercise tests

After a warm-up that comprised walking at 6 km•h^{-1} for three minutes, the continuous tests started with a speed of 8 km•h^{-1}, followed by an increase of 1 km•h^{-1} among each successive stage of three minutes, following the recommendation of Conconi, *et al*.[18] and Pokan, *et al*.[13] of small increments in speed and fixed stage duration. Furthermore, submaximal HR values obtained during protocols with three minutes stage duration are highly reproducible[19]. Each participant was encouraged to give maximum effort until volitional exhaustion. To minimize circadian variations in performance, the tests were performed at the same time of the day in the morning, under stable laboratory conditions (temperature = 20 –22 ºC and relative humidity = 50–60%). No feedback of the results was given to participants. The reliability of sHRDP was assessed by means of a test-retest design.

Heart rate (HR) was measured throughout the incremental test by a HR monitor (Polar RS800, Kempele - Finland) and rating of perceived exertion (RPE) was assessed by the Borg scale (6-20)[20]. At the end of each stage (i.e., exactly during the last 15 s of the stage) of the incremental test, the HR values were registered. The maximal HR (HR_{max}) was defined as the highest HR value recorded during the tests and the highest RPE was adopted as the maximal RPE (RPE_{max}). Steady HR points at the end of each stage were included in the analysis. Earlobe capillary blood samples (25 µL) were collected into a capillary tube after the end of each test at the fifth minute of passive recovery during which participants sat in a comfortable chair, for the determination of post-exercise peak blood lactate concentration. From these samples, blood lactate concentration was subsequently determined by electroenzymatic methods using an automated blood lactate analyzer (YSI 2300 STAT, Ohio, USA) that was calibrated according to manufacturer's instructions. The peak treadmill speed (V_{peak}) was considered as the speed of the last complete stage added to the product of the speed increment and the completed fraction of the incomplete stage[21] ($V_{peak\text{-}P}$), calculated according to the equation $V_{peak}\text{-}P = V_{complete} + (Inc^* t/T)$, in which $V_{complete}$ is the running speed of the last complete stage, Inc the speed increment (i.e., 1 km•h^{-1}), t the time in seconds sustained during the incomplete stage, and T the time in seconds required to complete a stage (i.e., 180 s).

Maximal effort was deemed to have been achieved if the incremental test produced two of the following criteria: 1) peak blood lactate concentration ≥ 8 mmol•L^{-1} [1, 2]) HR_{max} ≥ 95% of endurance-trained age-predicted HR_{max} (APMHR) using the age-based equation [206 – (0.7 × age)][22] and 3) RPE ≥ 19 in the 6–20 Borg scale[23].

Determination of the speed and heart rate values at the heart rate deflection points by the D_{max} (sHRDP and HR at sHRDP)

Data were fitted by two different models: 1) the exponential-plus-constant regression curve[24] and 2) third-order polynomial regression curve10 based on all points of HR and HR points above 140 b•min^{-1} (Figure 1). The calculations of both models were based on a previous study.[1]

The determination of HR values at sHRDP determined by D_{max} method were analyzed by linear interpolation considering the HR values and the speed above and below $sHRDP_{exp}$, $sHRDP_{exp>140}$, $sHRDP_{pol}$ and $sHRDP_{pol>140}$.

Statistical analyses

Data are presented as mean ± SD and were analyzed using the Statistical Package for the Social Sciences 17.0 software (SPSS Inc., USA) and spreadsheets of Hopkins[25]. Normality of data distribution was tested according to the Shapiro-Wilk test. Considering that data distribution was normal we used parametric analysis. Variables were compared using Student's t-test for dependent samples to identify systematic differences. Residual analysis (plotting the absolute differences between test and retest against the individual means) was applied to examine heteroscedasticity[26]. Relative reliability was examined using the intraclass correlation coefficient (ICC; two-way mixed model, consistency, single measures)[27,28]. The reliability was considered high for ICC values, moderate for values between 0.80 and 0.89 and questionable for values below 0.80[29]. The absolute reliability was determined based on SEM and coefficient of variation (CV). The SEM was calculated by dividing the SD of the differences between the variables of the test and retest by the square root of two (√2)[4,30]. The CV was determined by obtaining the SEM of the natural logarithm of the variables (SEM_{ln}). Thereafter, the CV was calculated using the formula CV (%) = 100 × [exp(SEM_{ln})$^{-1}$], where exp is the natural exponential function[4]. The magnitude of differences (effect size) estimated from the ratio of the mean difference to the pooled standard deviation was calculated to assess meaningfulness of differences and was interpreted as trivial (≤ 0.2), small (0.21 to 0.5), moderate (0.51 to 0.8) and large (>0.8)[31]. Bland Altman plots were used to check agreement. Statistical significance was set at $p < 0.05$.

Results

The variables obtained during the maximal incremental tests (mean ± SD) were: V_{peak} = 15.2 ± 0.8 km•h^{-1} (test) and 15.2 ± 0.8 km•h^{-1} (retest); HR_{max} = 192 ± 7.8 b•min^{-1} (test) and 190 ± 8.3 b•min^{-1} (retest); percentage of age-predicted maximal heart rate (%APMHR) = 102.1 ± 4.2% (test) and ¨101.1 ± 4.2 (retest); RPE_{peak} = 20 ± 0.5 (test) and 20 ± 0.3 (retest); LA_{peak} = 7.5 ± 2.0 mmol•L^{-1} (test) and 7.6 ± 1.9 mmol•L^{-1} (retest). These variables did not differ significantly between the two tests (test and retest) ($p > 0.05$).

The comparisons between test and retest for the variables related to the sHRDP and the HR at the sHRDP obtained during the incremental tests are presented in Table 1. The sHRDP determined by D_{max} from the exponential-plus-constant regression model using HR values above 140 b•min^{-1} (i.e., $sHRDP_{exp>140}$) and the HR at the sHRDP determined by D_{max} from the exponential-plus-constant regression model using all HR values (i.e. $sHRDP_{exp}$), were significantly different between the test and retest ($p < 0.05$). Furthermore, the percentage in which $sHRDP_{exp>140}$ corresponds to V_{peak} and the percentage in which HR values at $sHRDP_{exp}$ corresponds to HR_{max} were different between test and retest.

The measures of test-retest reliability (i.e., ICC, SEM, CV and ES) of the speeds at the heart rate deflection point are given in Table 2. The sHRDP

Figure 1. Determination of the sHRDP by D_{max} method from exponential-plus-constant regression model considering all HR values (A) and values above 140 b•min⁻¹ (B) and from third-order polynomial regression model considering all HR values (C) and values above 140 b•min⁻¹ (D).

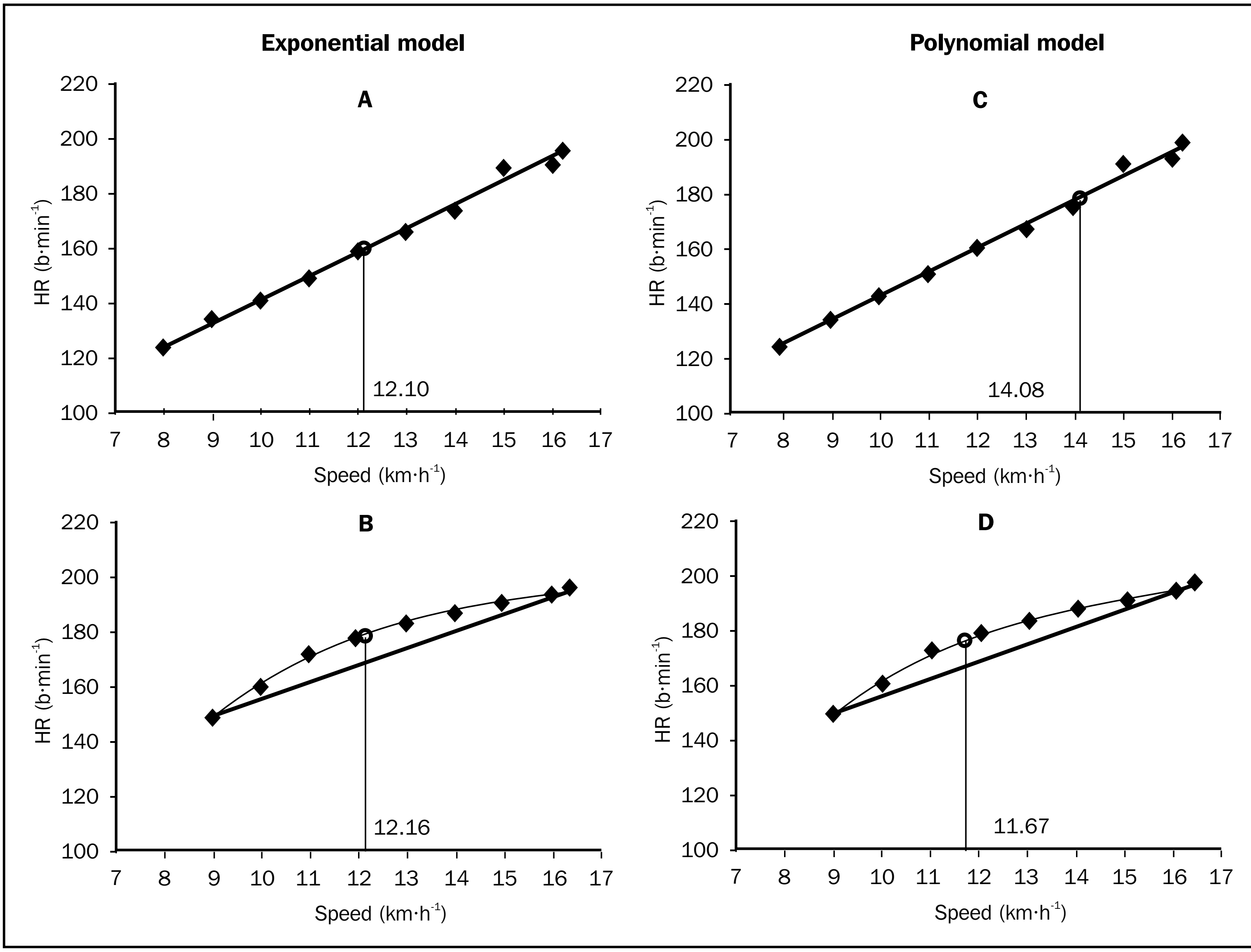


Table 1. Variables obtained during incremental treadmill tests (test-retest).

Variables	Test	Retest	% from V_{peak} (km•h⁻¹) or HR_{max} (b•min⁻¹) (test)	% from V_{peak} (km•h⁻¹) or HR_{max} (b•min⁻¹) (retest)
$sHRDP_{exp}$ (km•h⁻¹)	11.5 ± 0.5	11.4 ± 0.5	75.6 ± 1.3	75.5 ± 1.8
$sHRDP_{exp>140}$ (km•h⁻¹)	12.1 ± 0.8	12.4 ± 1.0[a]	79.8 ± 3.8	81.7 ± 4.1[a]
$sHRDP_{pol}$ (km•h⁻¹)	11.4 ± 1.1	11.5 ± 1.1	75.1 ± 7.3	75.6 ± 8.7
$sHRDP_{pol>140}$ (km•h⁻¹)	11.4 ± 1.1	11.8 ± 1.2	75.4 ± 7.4	77.5 ± 6.7
HR at $sHRDP_{exp}$ (b•min⁻¹)	164 ± 9.3	160 ± 10.5[a]	85.5 ± 3.0	84.5 ± 3.6[a]
HR at $sHRDP_{exp>140}$ (b•min⁻¹)	170 ± 5.5	168 ± 6.6	88.5 ± 2.1	88.8 ± 1.9
HR at $sHRDP_{pol}$ (b•min⁻¹)	164 ± 11.2	160 ± 13.0	85.4 ± 5.1	84.6 ± 6.2
HR at $sHRDP_{pol>140}$ (b•min⁻¹)	164 ± 9.7	163 ± 11.9	85.7 ± 5.2	86.1 ± 4.3

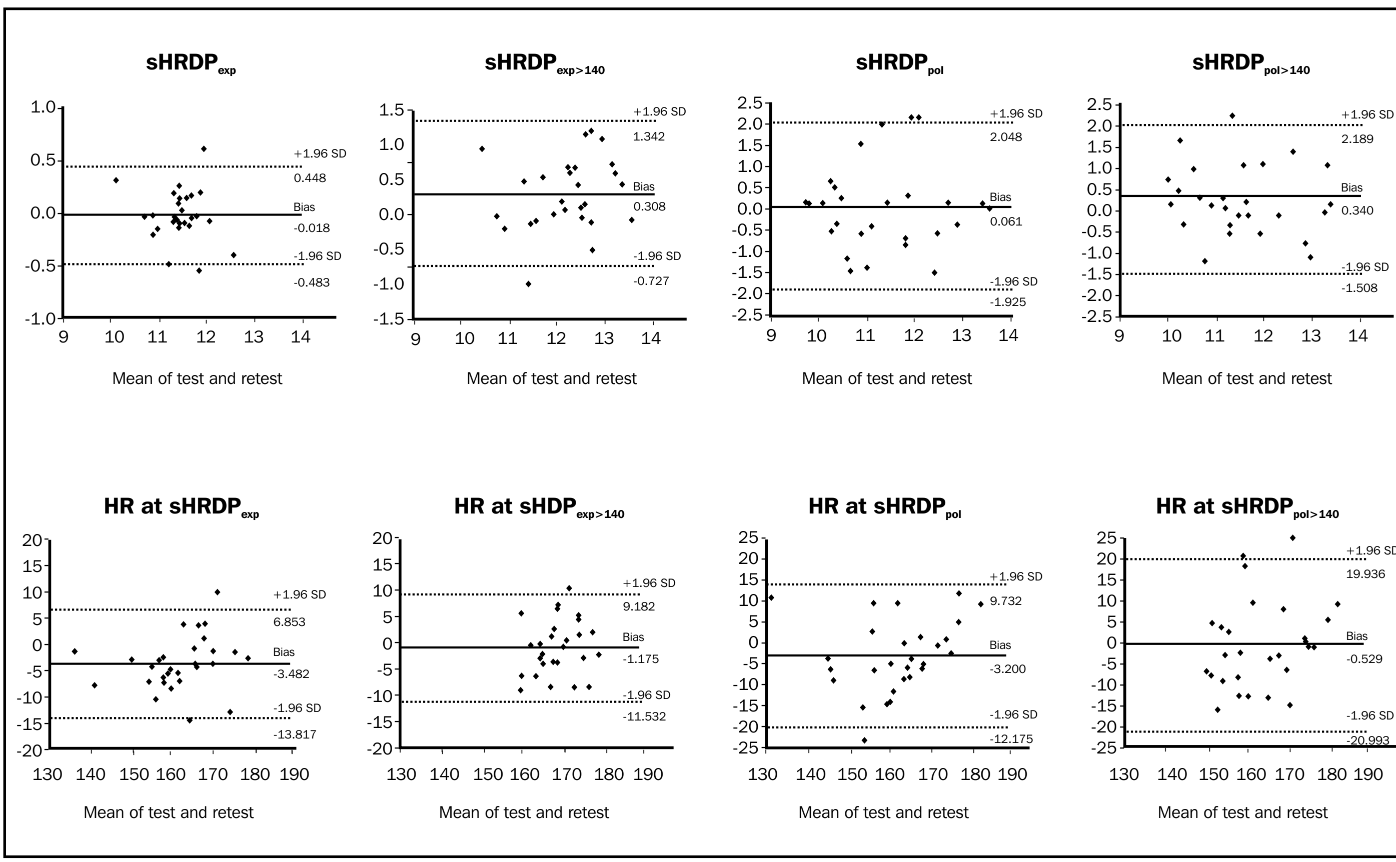
Values are mean ± SD, n=28. $sHRDP_{exp}$, speed at heart rate deflection point determined by D_{max} from the exponential-plus-constant regression model using all HR values; $sHRDP_{exp>140}$, speed at heart rate deflection point determined by D_{max} from the exponential-plus-constant regression model using HR values above 140 b•min⁻¹; $sHRDP_{pol}$, speed at heart rate deflection point determined by D_{max} from the third-order polynomial regression model using all HR values; $sHRDP_{pol>140}$, speed at heart rate deflection point determined by D_{max} from the third-order polynomial regression model using HR values above 140 b•min⁻¹; HR_{max}, maximal heart rate, V_{peak}, peak speed at incremental test. [a]p <0.05 compared with test.

Table 2. Reliability of the speeds and heart rate values at heart rate deflection point determined during incremental treadmill tests.

Variables	ICC (CI 95%)	SEM (CI 95%)	CV (%) (CI 95%)	ES
$sHRDP_{exp}$ (km•h^{-1})	0.87 (0.75-0.94)	0.17 (0.13-0.23)	1.46 (1.15-1.99)	0.04
$sHRDP_{exp>140}$ (km•h^{-1})	0.83 (0.66-0.92)	0.37 (0.30-0.51)	3.09 (2.44-4.23)	0.34
$sHRDP_{pol}$ (km•h^{-1})	0.70 (0.45-0.85)	0.72 (0.57-0.98)	6.46 (5.07-8.89)	0.05
$sHRDP_{pol>140}$ (km•h^{-1})	0.68 (0.41-0.84)	0.67 (0.53-0.91)	5.77 (4.54-7.94)	0.29
HR at $sHRDP_{exp}$ (b•min^{-1})	0.86 (0.72-0.93)	3.73 (2.95-5.08)	2.30 (1.81-3.14)	-0.35
HR at $sHRDP_{exp>140}$ (b•min^{-1})	0.62 (0.33-0.80)	3.74 (2.95-5.09)	2.24 (1.77-3.07)	-0.19
HR at $sHRDP_{pol}$ (b•min^{-1})	0.75 (0.52-0.88)	6.12 (4.84-8.33)	3.96 (3.12-5.43)	-0.26
HR at $sHRDP_{pol>140}$ (b•min^{-1})	0.54 (0.21-0.76)	7.38 (5.84-10.05)	4.64 (3.65-6.37)	-0.05

n=28; CI, confidence interval; CV, coefficient of variation; ES, effect size; ICC, intraclass correlation coefficient; SEM, standard error of measurement; $sHRDP_{exp}$, speed at heart rate deflection point determined by D_{max} from the exponential-plus-constant regression model using all HR values; $sHRDP_{exp>140}$, speed at heart rate deflection point determined by D_{max} from the exponential-plus-constant regression model using HR values above 140 b•min^{-1}; $sHRDP_{pol}$, speed at heart rate deflection point determined by D_{max} from the third-order polynomial regression model using all HR values; $sHRDP_{pol>140}$, speed at heart rate deflection point determined by D_{max} from the third-order polynomial regression model using HR values above 140 b•min^{-1}.

Figure 2. Bland-Altman statistics relating to the comparison of variables between test and retest.



All the vertical axes of the figures corresponds to: Difference (retest-test).

values obtained from exponential-plus-constant regression model (i.e., $sHRDP_{exp}$ and $sHRDP_{exp>140}$) showed higher reliability than the sHRDP values derived from third-order polynomial regression model (i.e., $sHRDP_{pol}$ and $sHRDP_{pol>140}$). Furthermore, the $sHRDP_{exp}$ was the most reliable variable, presenting the lowest values of SEM, CV and ES. On the other hand, the sHRDP values derived from third-order polynomial regression model were less reliable, mainly because the ICC value below 0.80 and the higher CV values. Effect size (ES) interpretations were that $sHRDP_{exp}$ and $sHRDP_{pol}$ were trivial, and the $sHRDP_{exp>140}$ and $sHRDP_{pol>140}$ were small.

For the HR values at the different sHRDP, it was demonstrated a similar response to the reliability of the speeds related to the HRDP, in which the HR values at $sHRDP_{exp}$ and at $sHRDP_{exp>140}$ were more reliable (i.e., SEM ≤ 3.74 b•min^{-1} and CV ≤ 2.30%) than the HR values at $sHRDP_{pol}$

and at $sHRDP_{pol>140}$ (i.e., SEM ≥ 6.12 b•min^{-1} and CV ≥ 3.96%). The ES values were considered trivial for HR at $sHRDP_{exp>140}$ and at $sHRDP_{pol>140}$, and small for the HR at $sHRDP_{exp}$ and at $sHRDP_{pol}$.

Systematic bias and the random variation as 95% limits of agreement are shown in Figure 2 for sHRDP and HR at HRDP. The Bland-Altman analyses indicated a high reliability for the $sHRDP_{exp}$, in which the systematic bias was near zero and the range in the limits of agreement was narrow. For the HR values at the sHRDP, the best agreement between the test and retest were demonstrated by HR at $sHRDP_{exp>140}$, given the lower bias associated with lower limits of agreement. Despite HR at $sHRDP_{pol>140}$ presented lower bias, its limit of agreement were higher.

Discussion

The aim of this study was to examine the test-retest reliability of sHRDP determined by D_{max} method during incremental treadmill tests. It was also aimed to verify if the regression model (i.e, exponential-plus-constant and third-order polynomial regression models) and the initial HR points used to determine the sHRDP by D_{max} method (i.e., model considering HR values above 140 b•min^{-1} *versus* the model considering all the HR points obtained) influence the sHRDP reliability.

The main finding was that the sHRDP presented high reliability when derived from exponential-plus-constant regression model, in which the $sHRDP_{exp}$ was the most reliable variable (ICC = 0.87; SEM = 0.17 km•h^{-1}; CV =1.46%; ES = 0.04; Bias =-0.018). Furthermore, HR values at the $sHRDP_{exp}$ (ICC = 0.86; SEM = 3.73 b•min^{-1}; CV = 2.30%) and at $sHRDP_{exp>140}$ (ICC=0.62; SEM = 3.74 b•min^{-1}; CV = 2.30%) presented the highest reliability. It seems that the regression model influenced the reliability of the sHRDP values and HR values at sHRDP. Moreover, the number of HR points slightly influenced the reliability estimates.

Since HRDP was proposed by Conconi, *et al.*[7], some studies observed high correlations among this variable and other AT methods, and for this reason the HRDP determined by Conconi, *et al.*[7] was consider an accurate predictor of the AT (i.e., ventilation and lactate thresholds)[5,6,8]; however other works found that HRDP overestimated the AT[9,14,32]. For example, while Vucetic, *et al.*[5] observed a good relationship, demonstrated by coefficient of determination (R2), between the ventilation threshold and HRDP in forty-eight trained runners who performed two treadmill test protocols (R2=0.72 and 0.74), Jones and Doust[14] and Vachon, *et al*[9]. found that HRDP was significantly higher than AT. Regarding the D_{max} method, few studies investigated the relationship between the HRDP determined by D_{max} and AT or maximal lactate steady state (MLSS)[1,11,33]. Da Silva, *et al.*[1] and Siahkouhian and Meamarbashi[11] showed high correlations among AT determined by lactate concentrations and HRDP obtained by D_{max} (0.82 ≤ r ≤ 0.95). On the other hand Silveira, *et al.*[33] assessed 13 runners and found that the HRDP was not significantly different from the MLSS, but it was not found correlation between these variables (r = 0.42).

However, only few studies previously examined the reliability of HRDP[7,13,14], but none of them were based on D_{max} method. Despite Conconi, *et al.*[7] and Jones and Doust14 have found a high correlation between test-retest (r = 0.99 and 0.89, respectively), there are other statistical approaches to be applied when reliability was analyzed (e.g., relative and absolute reliability)[4,30]. Moreover, the HRDP obtained by Conconi, *et al.*[7] and Jones and Doust[14] did not occur in the entire sample and there are significant differences both within observers[7,14]. This weak point of the method proposed by Conconi *et al.*[7] was highlighted on other studies[6,9,12,14]. For example, Vachon *et al.*[9] observed only in four of the eight subjects no signs of HR deflection on a treadmill incremental test, and Hoffman *et al.*[6] in 14% of their sample showed no deflection or inverse deflection of the HR curve. Jones and Doust[14] investigated 15 well-trained runners in test and retest and only in six subjects the HR deflection point was determined; in four subjects no deflection point from HR linearity could be discerned in either test.

In the Conconi *et al.*[7] method the linear behavior of the HR-curve not allow the identification of the visual deflection point. The same weak point was observed for the $HRDPD_{max}$ determination in ten participants of our study. It is important to emphasize that in this cases (i.e.,linear HR-curve) the $HRDPD_{max}$ often occurred at the midpoint between initial and final speeds during the incremental test. Additionally, it seems that $HRDPD_{max}$ don´t occurs between midpoint and final speed in a concave downward exponential-plus-constant model because the D_{max} is a mathematical model highly dependent on the shape of the curve[34].

Previous studies used the D_{max} method to obtain the HRDP.[1,11,12] Siahkouhian and Meamarbashi[11] determined the HR value at the HRDP in 15 active male during incremental cycle ergometer test using all HR points ($L.D_{max}$) and with points above 140 b•min^{-1} ($S.D_{max}$). The authors showed significant correlation between the $S.D_{max}$ and the criterion method (i.e., lactate threshold) (r = 0.94) and no significant correlation between $L.D_{max}$ and the criterion (r=0.16), concluding that the $S.D_{max}$ method is an accurate alternative to substitute the lactate method. Moreover, Kara, *et al.*[12] reinforce the use of the D_{max} method mainly because this point can be easily and objectively found in all subjects, differently from the Conconi, *et al.*[7] method.

Recently, da Silva, *et al.*[1] examined the relationship between sHRDP values calculated by D_{max} (i.e., $sHRDPD_{max}$) method and 10-km endurance running performance in female recreational runners, and found that only the $sHRDPD_{max}$ determined by the exponential-plus-constant regression model correlated with s10km ($sHRDP_{exp}$, r = 0.96; $sHRDP_{exp>140}$, r = 0.79). Correlations with lactate threshold showed similar results, in which the $sHRDPD_{max}$ derived from exponential-plus-constant regression model showed higher correlations than the $sHRDPD_{max}$ derived from third-order polynomial regression model. However, the authors concluded that despite the high correlations with performance, the exponential-plus-constant regression model seems not be an appropriate regression curve because this regression model very often occurred around the midpoint between initial and final speeds during the incremental test.

It is important to emphasize that a variable must be highly reliable for its application in training prescription[4,30]. One measure to demonstrate reliability is the coefficient of variation. In the present study, this value was 1.46% for $sHRDP_{exp}$. Despite we cannot compare it to other values of HRDP reliability, the reliability of the lactate and ventilatory thresholds determined during incremental exercise tests are well reported in previous studies[30,35,36] , in which CV values between 1.6 and 3.3% were found. Hence, reliability of the $sHRDP_{exp}$ can be considered very high and recommendable for practical and scientific purposes.

In conclusion, the sHRDP determined by D_{max} method from exponential-plus-constant regression model considering all the HR values and those above 140 b•min^{-1} (i.e., $sHRDP_{exp}$) is a highly reliable variable. Additionally, the HR values at the $sHRDP_{exp}$ and at $sHRDP_{exp>140}$ were highly reliable. However, in some participants the HR-curve demonstrated a linear behavior and the $sHRDP_{exp}$ occurred around the midpoint between initial and final speeds during incremental test. Thus, despite the high reliability, the exponential-plus-constant regression model should be used with caution and when the HR-curve is linear this regression curve seems not be appropriate. In contrast, $sHRDPD_{max}$ determined by the third-order polynomial regression model presented a moderate reliability. Future studies are required to analyze the practical application of sHRDP to prescribe endurance training and monitor adaptations.

Acknowledgements

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, Brazil.

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Original article

Influence of ladder climbing exercise on bone of rats induced to osteoporosis and immobilization

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Received: 15.05.2015
Accepted: 25.09.2015

Summary

Introduction: The oophorectomy surgery produces menopause, which in turn predisposes to many problems such as osteoporosis, with consequent deterioration of microarchitecture and decreasing mass bone, predisposing to the risk of fractures, which are often treated with immobilization, which negatively affects the muscle, cartilage and bone tissues. Despite the rich literature on exercise as a way of remobilization, both in humans and in animals, there is a gap with respect to some types of exercise, such as that performed with climb ladders. The aim of this study was to analyze the effects of ladder climbing exercise on rats bone histomorphometry induced osteoporosis and subjected to immobilization.
Methods: 36 female Wistar rats were separated into six groups: G1, G2 and G3 subjected to pseudo-oophorectomy; and G4, G5 and G6 to oophorectomy. After 60 days rest, G2, G3, G5 and G6 had immobilized the right hind limb for 15 days, followed by remobilization for the same period, being free in the box to G2 and G5, and ladder climbing exercise to G3 and G6. At the end of the experiment, the rats were euthanized, their tibias removed bilaterally and submitted to histological routine.
Results: There was a significant decrease in cortical bone (area and thickness) and osteocytes numbers, and increased medullary canal, in immobilized limbs of ovariectomized rats. However, the exercise of climbing a ladder was able to reverse these losses due to oophorectomy associated with immobilization. There was also a significant decrease in the area and trabecular thickness in members subjected to immobilization, being reversed with the free remobilization and in ladder.
Conclusions: the ladder climbing exercise was effective in the recovery process of bone tissue damaged by immobilization on osteoporosis model by ovariectomy in rats.

Key words:
Ovariectomy
Immobilization.
Exercise therapy.

Influencia del ejercicio en escalera sobre el hueso de ratas inducidas a la osteoporosis e inmovilización

Resumen

Introducción: La cirugía ooforectomía produce menopausia, que a su vez predispone a muchos problemas tales como la osteoporosis, con el consiguiente deterioro de la microarquitectura ósea, lo que aumenta el riesgo de fracturas, que a menudo son tratadas con la inmovilización, que afecta negativamente el tejido muscular, cartilaginoso y óseo. A pesar de la abundante literatura sobre el ejercicio físico como medio de recuperación, tanto en humanos como en animales, existe una brecha con respecto a algunos tipos de ejercicios, como los realizados con escaleras de ascenso. El objetivo de este estudio fue analizar los efectos del ejercicio de subir una escalera sobre el hueso de ratas con osteoporosis inducida y sometidos a inmovilización.
Métodos: Se dividieron 36 ratas Wistar en seis grupos: G1, G2 y G3 sometidos a pseudo-ooforectomía; y G4, G5 y G6 a ooforectomía. Después de 60 días de descanso, G2, G3, G5 y G6 habían inmovilizado la extremidad posterior derecha durante 15 días, seguido de removilización durante el mismo tiempo, realizando ejercicio libre en la jaula los grupos G2 y G5, o ejercicio subir escaleras para los grupos G3 y G6. Al final del experimento, las ratas fueron sacrificadas, sus tibias fueron retiradas bilateralmente y sometidas a un análisis histológico.
Resultados: Se observó una disminución significativa en el hueso cortical (área y espesor) y del número de osteocitos, y el aumento del canal medular, en las extremidades inmovilizadas de ratas ovariectomizadas. Sin embargo, el ejercicio de subir una escalera fue capaz de revertir estas pérdidas debidas a ooforectomía asociada con la inmovilización. También hubo una disminución significativa en el espesor de la área trabecular de los miembros sometidos a inmovilización, siendo revertido con la removilización libre y en escalera.
Conclusiones: El ejercicio de subida en una escalera fue eficaz en el proceso de recuperación del hueso dañado por inmovilización en el modelo de osteoporosis por la ovariectomía en ratas.

Palabras clave:
Ovariectomía.
Inmovilización.
Terapia por ejercicio.

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Introduction

Menopause in humans is characterized by last menstrual period and may occur spontaneously at about 51 years of age or by surgical induction in cases of oophorectomy and hysterectomy[1]. This alteration in the female body causes a slow and gradual decrease in estrogen and progesterone hormones, predisposing the onset and aggravation of some disorders and diseases such as osteoporosis. Consequently, this metabolic bone disease causes deterioration of the tissue microarchitecture and decrease of bone mass, leading to bone fragility and predisposing to fractures, even for minor traumas[1-3]. Around a third of postmenopausal women are affected by osteoporosis, with higher prevalence between 65 and 75 years of age and it may reach 70% in women over 80 years old. It is related to high rates of morbimortality, mainly due to fractures caused by bone fragility[2,4-7]. In the United States, from the total fractures treatment expenditure, about 50-67% are specifically of osteoporotic fractures, which increases the health care costs[8].

The studies involving the effects of immobilization in osteoporotic bones, especially when it comes to women's life situation, such as postmenopausal are still few. Immobilization is a conservative therapeutic resource used in post-operative, post-fractures, sprains and muscle injuries, in order to maintain a body segment at rest; aims to reduce the pain symptoms and protect the affected musculoskeletal structures affected by injuries[9]. However, the immobilization also leads to problems for tissues, affects negatively the muscle, bone and cartilaginous tissues, causing mass and joint range of motion decreasing, besides functional deficits[10-12]. In addition to these effects, is still observed decrease in strength and in the cross sectional area of the muscle; loss and reduction of the synthesis of proteoglycans of the cartilage matrix; necrosis and ulceration of cartilage; mass reduction and total cartilage volume; significant loss of cortical and cancellous bone[9,13-19].

Several studies emphasize that remobilization exercise has been shown to be effective in the recovery of patients and animals that were subjected to a body segment immobilization, promoting muscle hypertrophy, improving and maintaining bone mass[20-23]. Kemmler *et al.*[24] reinforces that even a single session of exercise can positively influence the hormones that affect bone metabolism, improving the remodeling process. However, up to the present, were not found in literature studies about morphological changes that the exercise of stair climbing can cause on the bone tissue of animals, osteoporotic females due to hormonal deprivation, in mimetization of postmenopausal period. The increase of life expectancy of the population, with consequent increase in the arising of chronic diseases, osteoporosis as a major public health problem nowadays, the disabilities and deleterious effects on tissues resulting from a restraint, and also from the need of making scientific evidence therapeutic resources used in clinical practice justify this study, which aimed to analyze the effects of ladder climbing exercise on bone histomorphometry in female rats induced to osteoporosis and submitted to immobilization.

Materials and methods

Experimental Groups

It were used 36 adult female Wistar rats, (10 ± 2 weeks), nulliparous, with a mean weight of 317.2 ± 22.1 g, kept in polypropylene cages, with free access to water and food, photoperiod light/dark of 12 hours, controlled room temperature (25 ± 1° C). The study was conducted according to the international ethical standards of animal experimentation and was approved by the Ethics Committee on Animal Experimentation of the State University of West of Paraná (Unioeste), under the number 4112.

The rats were divided randomly into six groups:

- G1 (n=6): submitted to simulated oophorectomy surgery (pseudo-oophorectomy) and remained 60 days without intervention;
- G2 (n=6): submitted to pseudo-oophorectomy and remained 60 days without intervention. After that, an immobilization of the right hind limb was performed (RHL) for two weeks. Posteriorly, they remained in free remobilization for the same period, being just put in contact with a ladder, in the last 10 centimeters (cm);
- G3 (n=6): submitted to pseudo-oophorectomy and remained 60 days without intervention. After that, an immobilization of the RHL was performed for two weeks and subsequently subjected to the exercise of stairs climbing for 10 days, with an interval of two days after the fifth session;
- G4 (n=6): bilateral oophorectomy surgery and remained 60 days without intervention;
- G5 (n=6): bilateral oophorectomy and remained 60 days without intervention. After that, it was performed immobilization and remobilization similar to G2;
- G6 (n=6): bilateral oophorectomy and remained 60 days without intervention. After that, it was performed immobilization procedure and remobilization similar to G3.

Pseudo and Oophorectomy Protocol

To carry out oophorectomy, pseudo-oophorectomy, immobilization and euthanasia, the rats were weighed and subjected to a protocol of anesthesia, which consisted of intraperitoneal injection of xylazine (12 mg/kg) and ketamine (80 mg/kg). To do the oophorectomy a trichotomy and antisepsis with iodine alcohol was performed in the region of the lower abdomen, followed by a longitudinal incision with a scalpel blade nº 11. After accessing the peritoneal cavity, the adipose tissue was removed until the oviduct and ovaries could be identified. Then, a suture with simple wire catgut 4.0 was done on the area of the uterine horns, promoting ovarian resection bilaterally. At the end of the procedure, internal sutures with resorbable simple wire catgut 4.0, and external with nylon 4.0 were performed. The pseudo-oophorectomy consisted of all surgical steps similar to ovariectomy, except for the ovaries removal. Subsequent to surgery, the rats remained for 60 days without any intervention, free in the cage[25].

Immobilization Protocol

Prior to immobilization, the anesthetic procedure was performed, and then the RHL, from the hip to the ankle, wrapped in a tubular mesh with cotton bandages. Then the static orthosis was shaped, of approximately 50 grams, using quick drying plaster bandages, with the RHL in full extension of the knee and ankle in maximum plantar flexion, in rats of G2, G3, G5 and G6 as model proposed by Booth and Kelso[26], being reconfigured to only one of the members according to a study by Matheus *et al*.[27].

Ladder Climbing Protocol

After immobilization removal, the rats of G3 and G6 were submitted to the exercise of ladder climbing, with 10 repetitions per day in the first week (five days) and 20 repetitions per day in the second week (five days), with an interval of one minute between the climbs and two days between the weeks. The rats of G2 and G5 performed remobilization free of the cage, being placed in contact with a ladder at 10 cm from the end of it, only once, in the same period in which the exercise was performed on the ladder to G3 and G6. The equipment used to perform the exercise consisted of a ladder with 67 steps, spaced 1.5 cm between the steps of the grid, 20.5 cm wide, 118 cm height and vertical with 80° inclination angle. At the upper area there was a 28.5 cm long, 18.5 cm high and 15 cm wide darkroom, used for the rats to rest between the climbing sets, as well as to make them feel attracted to a shelter and encouraged to perform the exercise[20,22,28,29].

Histomorphometric Analysis

At the end of the experiment, all the rats were weighed and underwent anesthetic protocol being subsequently euthanized by decapitation in guillotine. After these procedures, the right and left tibia (left hind limb – LHL) were dissected and placed in flasks containing formalin at 10%. Then, after the fixation, the tibiae were washed with distilled water and immersed in trichloroacetic acid (TCA) at 5%, during five days, for decalcification. The next steps were dehydration, diafanization, measurement of the parts in its length with the help of a digital pachymeter to perform in the medial region of the bone a cut in the transversal plane.

In the proximal part, a cut in the frontal plane was performed, being reserved the anterior portions. After that, the bone pieces were impregnated and embedded in paraffin, being arranged in the blocks for subsequent microtomization as follows: the proximal portion, in frontal cut; and the distal portion, with transversal cut, positioned with the upper region for visualization. Cuts of 7 µm were performed in microtome, and confectioned blades with hematoxylin and eosin (HE) and photomicrographed with Olympus DP71® microscope.

The photomicrographs were submitted to analysis using the Image-Pro Plus 6.0® program, in the transversal plane performed with magnification of 40 times, for measurements of medullary canal area and cortical bone area, being the cortical thickness measured at three points (M1 , M2 and M3)[30] (Figure 1A); and magnification 400 times for counting the osteocytes, measured in three regions (between the points M1 and M2, M1 and M3, and M2 and M3) by means of a rectangle of 200 µm base by 100 µm height, overlaid on the image. There were excluded from the count, the cells that were in contact with the upper edge and right side (Figure 1B). In the frontal plane, photomicrographs were taken in three regions, designated P1, P2, and P3, which correspond respectively to the lateral, intermediate and medial portions of the proximal region of the tibia, with a magnification of 40 times to measure the length (vertical tracing) and area (circular tracing) trabecular bone (region between epiphyseal plate the and upper articular cartilage)[31,32] (Figure 1C). After collecting the data, the means were made and the statistical analyzes were performed.

Statistical Analysis

The survey data were evaluated by comparing the results obtained on the left hind limb (control) and right (immobilized), between the rats of the same group and between the experimental groups. For this, the BioEstat 5.0® program was used, with values presented as mean and standard deviation. The Student t paired test was applied for comparison between the right and left side, within the same group, and one-way ANOVA with Bonferroni post hoc test, for comparison between experimental groups. The level of significance was $p \leq 0.05$.

Figure 1. Photomicrographs with statements of histomorphometric analyzes performed on the ovariectomized rat right tibia subjected to immobilization and remobilization ladder. In A, measuring the thickness of cortical bone in three points (M1, M2 and M3) and medullar canal area, cross-section and 40X magnification; B overlaid base of 200 µm by 100 µm high for counting osteocytes in the area between the points M1 and M2, 400X magnification (obs .: rectangle asterisks exclusion demarcate the edges (top and right side); and C, measurement of area (circular route) and thickness (vertical layout) of trabecular bone (upper region of the plate and articular cartilage) in medial (P3), 40X magnification. HE staining.

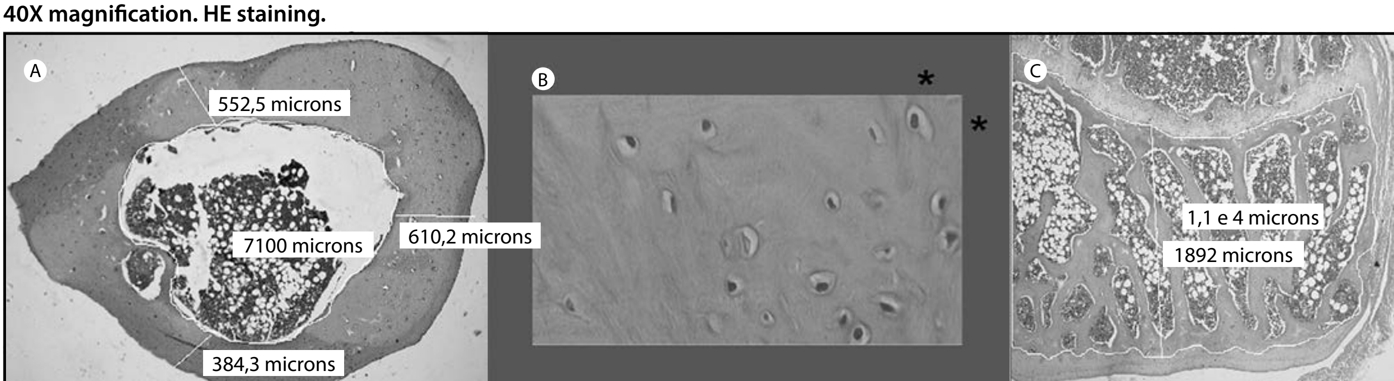

Table 1. Area and cortical thickness data, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Cortical Area (µm²) Note.: values elevated to the 6th Power RHL (mean ± sd)	LHL (mean ± sd)
G1	4.20 ± 5.47 (a)	4.90 ± 8.91 (cd)
G2	4.65 ± 11.71 (ab)	4.78 ± 9.97 (c)
G3	5.60 ± 18.36 (ab)	5.80 ± 12.58 (cd)
G4	3.80 ± 6.78 (a)	3.73 ± 4.77 (d)
G5*	4.00 ± 5.57 (a)	5.40 ± 7.91 (cd)
G6	6.66 ± 9.22 (b)	6.22 ± 12.32 (c)

Groups	Cortical Thickness (µm) RHL (mean ± sd)	LHL (mean ± sd)
G1	600.38 ± 64.44 (ab)	661.46 ± 122.49 (c)
G2	617.68 ± 69.30 (ab)	690.43 ± 89.07 (c)
G3	683.07 ± 69.83 (ab)	702.05 ± 39.53 (c)
G4	394.68 ± 35.83 (a)	427.55 ± 103.78 (d)
G5*	374.96 ± 28.40 (a)	624.19 ± 90.31 (c)
G6	646.12 ± 112.36 (b)	711.63 ± 78.82 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Results

Cortical Bone Area and Thickness

In the comparison between rats of the same group, it could be noted a significant decrease in cortical bone of the RHL regarding the LHL in G5, as evidenced by data from both the area (p=0.0178) and thickness (p=0.0024). The other groups (G1, G2, G3, G4 and G6) showed no significant differences between their hind limbs so that remobilization (G6) was able to reverse the loss of cortical bone in the immobilized limb when compared to the contralateral non-immobilized (Table 1).

When the comparison was made between the groups, with mean values of RHL, it was observed a significant increase in G6 compared to G1, G4 and G5 (p=0.0007), to area, but also to G4 and G5 (p=0.0062), for thickness. Thus, it is evident that the exercise was able to reverse the cortical bone loss caused by ovariectomy and immobilization, with values that exceed even those found in rats only pseudo-oophorectomized (G1). It is noteworthy that the free remobilization was not able to recover the loss of cortical bone in the limb of ovariectomized rats (Table 1).

In the LHL analysis between groups, there was a significant decrease in area of G4 in relation to G2 and G6 (p=0.0031), and in thickness of G4 in relation to the other groups (p=0.0001). This finding shows that ovariectomy was able to promote cortical bone loss and that both, free remobilization, and in ladder were able to recover from this loss (Table 1).

Table 2. Data of the medullary canal area, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Medullary Canal Area (µm²) Note.: values elevated to the 6th Power RHL (mean ± sd)	LHL (mean ± sd)
G1	1.90 ± 2.53 (a)	2.13 ± 4.89 (c)
G2	2.48 ± 6.61 (ab)	2.00 ± 4.29 (c)
G3	1.77 ± 4.32 (a)	2.08 ± 7.57 (c)
G4	2.00 ± 5.21 (a)	2.25 ± 4.93 (c)
G5*	3.42 ± 1.07 (b)	2.22 ± 3.34 (c)
G6	2.00 ± 8.74 (a)	2.40 ± 5.73 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Table 3. Data of osteocytes number, comparing the rats distributed between the study groups (G1 - G6), right (RHL - target) and left hind limbs (LHL - control).

Groups	Osteocytes Number (un) RHL (mean ± sd)	LHL (mean ± sd)
G1	22.67 ± 5.32 (a)	20.06 ± 3.32 (c)
G2	18.83 ± 3.48 (a)	23.33 ± 6.36 (c)
G3	20.44 ± 1.76 (a)	21.33 ± 4.73 (c)
G4	20.33 ± 1.63 (a)	19.67 ± 1.71 (c)
G5*	10.07 ± 0.83 (b)	20.07 ± 1.93 (c)
G6	23.94 ± 1.97 (a)	24.94 ± 2.89 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side. * Significant difference between the sides of the same group.

Medullary Canal Area

As to the medullar canal area, G5 rats showed a significant increase of RHL in relation to LHL (p=0.0384), also for the G5 RHL showed higher values than G1, G3, G4 and G6 (p=0.0043). In LHL there was no significant difference between the groups, ie, the area of the medullary canal was maintained even after the interventions (Table 2).

Thus, it was found that oophorectomy associated with immobilization and free remobilization (G5), produced a significant increase in the medullary canal area of the immobilized limb, when compared to the RHL from the other groups and that the ladder climbing exercise was able to reverse this increase. This was evident in G3 and G6, which had similar means between themselves and to the non-immobilized groups (G1 and G4) (Table 2).

Osteocytes Number

Regarding the osteocytes number, it was observed a statistically significant difference between RHL and LHL of G5 (p<0.0001), revealing that the free remobilization is not able to reverse the cell loss caused by immobilization in ovariectomized rats. However, as there was no

Table 4. Data referring to the trabecular bone area and thickness, comparing the rats distributed between the study groups (G1 - G6), right (target) and left hind limbs (control).

Groups	Trabecular Area (μm²) Note: values elevated to the 6th power RHL (mean ± sd)	LHL (mean ± sd)
G1	2.67 ± 9.22 (a)	2.15 ± 6.74 (b)
G2	3.55 ± 7.73 (a)	3.55 ± 5.56 (b)
G3	3.34 ± 5.07 (a)	3.45 ± 7.85 (b)
G4	3.52 ± 1.24 (a)	3.84 ± 1.02 (b)
G5	3.03 ± 8.43 (a)	3.02 ± 3.72 (b)
G6	3.70 ± 6.83 (a)	4.69 ± 2.40 (c)
	Trabecular Thickness (μm)	
Groups	**RHL (mean ± sd)**	**LHL (mean ± sd)**
G1	1587.6 ± 255.82 (a)	1353.87 ± 118.44 (b)
G2	1774.83 ± 215.35 (a)	1795.06 ± 81.48 (c)
G3	1927 ± 156.10 (a)	1928.89 ± 257.36 (c)
G4	1655.11 ± 175.77 (a)	1716.03 ± 206.04 (bc)
G5	1728.07 ± 227.24 (a)	1645.6 ± 232.20 (bc)
G6	1880.72 ± 307.6 (a)	1928.5 ± 354.04 (c)

Obs.: same letters mean similarities, and the different letters mean significant differences between the experimental groups to the same side.

difference between the hind limbs of rats belonging to the G6; it is believed that the ladder exercise was able to reverse this loss (Table 3).

In the comparison between the groups, it was observed lower values only in RHL at G5 comparing to the other groups (p=<0.0001). Thus, immobilization with free remobilization caused severe loss of osteocytes in ovariectomized rats and the ladder climbing exercise proved to be effective in recovering the number of osteocytes (Table 3).

Trabecular Bone Area and Thickness

In the trabecular bone area and thickness analysis of the same group rats, there was no significant difference between RHL and LHL. In the intergroup analysis there were significant differences in comparison of G1 LHL with G6 (p=0.0265) for the area and thickness, and G1 to G2 and G3, only for thickness (p=0.0015) (Table 4).

Discussion

According to the results, it was observed a significant loss of cortical bone and the osteocytes number in immobilized limbs in ovariectomized rats subjected to the free remobilization, which was reversed by remobilization climbing ladder exercise. It was also observed that the osteoporotic rats, not immobilized, had significant loss of cortical bone, however, there was reversibility with the free remobilization and ladder climbing exercise. It was also observed an increase in trabecular bone in the not immobilized limbs of rats submitted to exercise ladder. Thus, the results obtained are consistent and affirm the hypothesis of this study that the ladder climbing exercise is effective in reduction or reversibility of bone loss caused by osteoporosis and immobilization.

In the present study, it was opted for the use of ovariectomized rats that mimetize some osteoporotic features found in postmenopausal women and for immobilization in plaster static orthoses, using the histomorphometry method for evaluation. These were chosen for been effective, and for best representing the daily clinic routine and with lower operational costs. There are several experimental models for inducing osteoporosis, either by hormonal interventions (surgical or pharmacological), dietary, genetic alterations, or immobilization (conservative or surgical)[30,31]. The oophorectomy consists on the removal of the ovaries, the main source of estrogens, thus inducing the restriction of this hormone circulating in the body. After surgery already begins the process of bone remodeling, with reabsorption exceeding neoformation, causing bone loss[29,30].

In the evaluation by means of histomorphometry is possible to analyze bone mass and architecture with accuracy as well as indexes of bone fragility, number of osteoblasts, osteoclasts, osteocytes and active osteoblasts in relation to the bone perimeter. Some authors have emphasized that the histological analysis technique is considered to be more effective, however it shows some limitations, such as difficulty in assessing various areas in different structures and at the same time. It is usually possible to analyze only a small area of the tissue, at a particular bone, which does not represent changes throughout the skeleton. However, it presents a great reliability when the sampling area is comparable in all groups[30,32]. Thus, it was opted for the bone histomorphometry in the proximal and medial region of the tibia, this bone was chosen due to its anatomical and biomechanical importance.

In some studies on oophorectomy significant bone losses were observed after 14, 72 and 74 days at the proximal tibia metaphysis, after 60 and 73 days in the lumbar vertebral body and after 30 days in the femoral neck[30,33]. Other authors report that the first changes in the width of the cortical bone and the medullar cavity of the femur and tibia, are noted in periods around 90 and 120 days after ovariectomy[34-36]. In the present study, was observed significant bone loss at 60 days after ovariectomy and 90 days after the association of oophorectomy, immobilization and free remobilization in the proximal region (trabecular) and tibia medial (cortical). However, the loss was more pronounced in 90-day period in the oophorectomized group, immobilized and free remobilization, and may be associated with longer duration of hormone estrogen deprivation, but also by association with immobilization, as it accentuated the decrease in bone mass.

Strong evidences show that the combination of oophorectomy and immobilization can reduce the time of significant bone loss, especially the cortical, when compared to the techniques isolated[30,34-36]. In view of the findings of this study, such association may also accentuate the effects of the significant loss of bone mass. These findings corroborate with previous studies showing the accuracy of measurements taken in the medial inferior areas of the cortical axis, which are very reliable, because most bone losses occur in this location[30,33,37].

The reduction in bone mass was clearly demonstrated in this study, because it is well seen in the cortical bone by the enlargement of the medullar cavity. This occurs due to the increasing in endosteal reabsorption and periosteal bone apposition[30,33,37]. With area measurements and cortical thickness as well as the medullar canal area, only G5 RHL showed significant decrease compared to the LHL, which may be related to the effects of estrogen hormone deprivation associated with immobilisation of the RHL. The free remobilization was not effective to recover this loss, as in G2, G3 and G6 there was no difference. Thus, it is believed that both the free exercise and in stairs were effective to recover the bone loss associated with immobilisation in the pseudo-ovariectomized groups (G2 and G3) and that only the ladder exercise was effective in this reversibility in immobilized osteoporotic rats.

In intergroup comparison to the RHL, it was found that the exercise on ladder was effective in the recovery of cortical bone loss caused by ovariectomy alone or in combination with immobilization and free remobilization. It was also found that G6 was significantly higher than G1 regarding the cortical area, ie, showing that the exercise on ladder produces increasing in the upper bone mass even when compared with animals that did not undergo significant interventions, only the surgical procedure stress without removal of the ovaries.

In intergroup comparison for LHL, there was a significant decrease in cortical bone area and cortical bone thickness of G4 compared to G2 and G6. Regarding G2 can be linked to the fact that there was an overload on the LHL in relation to the immobilized limb (RHL), leading to a growth of cortical bone in higher values than those from the group which was subjected only to hormone deprivation (G4) with significant loss of cortical bone. However, in G6, it is evident the outcomes of therapeutic intervention, ie, that the ladder exercise was a clinical resource very well employed in remobilization for the recovery of bone loss caused due to hormonal estrogen deprivation and immobilization. As there was no difference compared to G5, it is believed that osteoporosis may have been determinative in the reduction of bone mass, as in G4, as well as in the free activity in the box was not enough to reverse the decrease in cortical bone mass in osteoporotic rats.

Regarding the trabecular bone, there was a significant LHL loss of G1 compared to G2, G3 and G6. This may be related to overload exerted on LHL due to immobilization of RHL associated with the effectiveness of exercise of climbing stairs, in ovariectomized and pseudo-oophorectomized rats being even able to promote an increase in this type of tissue. For rats not subjected to hormone deprivation, even the free exercises associated to the overload in LHL were able to promote growth of the trabecular bone. It was not observed diminution in consequence of ovariectomy and immobilization, which may be related to post-surgical period and immobilization. Maeda *et al.*[18] found a significant loss of cancellous bone in rats subjected to six weeks of immobilization.

Even with therapeutic interventions applied correctly, the complete reversibility of the damage caused by immobilization will not always occur[38,39]. Nevertheless, the exercise has been a therapeutic resource widely used for both the treatment of osteoporosis and in remobilization periods. However, the benefits of physical exercise on the skeleton depend on the intensity and bone characteristics, such as age, type and region involved[40,41]. In this study it was found that both the free exercise and the stairs climbing were effective in recovering the bone loss in rats that have undergone hormonal deprivation, through the protocol used. However, when these ovariectomized rats underwent immobilization, it was found that the free exercise was not able to recover bone loss, unlike the ladder exercise.

Several authors studied the therapeutic action of various resources and remobilization techniques in animal models, such as muscle stretching[42], free remobilization1[5,21,42], swimming[15], jump[21] and treadmill running[40,43]. It were found improvements in gait, cartilage conditions, subchondral bone, biomechanical and articular capsule[15]; restoration of the trabecular architectural integrity[21]; increase in bone mass and mineral density[21,40,43]; decrease in the number of osteocytes, even after remobilization[42]. This study verified that the osteocytes number of immobilized limbs was reestablished in both free remobilization and climbing stairs, in pseudo-ovariectomized rats. In osteoporotic rats, only the exercise of stairs climbing was able to recover the number of osteocytes, with values that even resemble the groups not exposed to hormone deprivation.

Study performed with rats subjected to tail suspension for 21 days and climbing stairs exercise for the same period, doing eight sets, with overload equivalent to 80% of their maximum strength, five times a week, observed that this type of exercise was able to restore the BMD and bone stiffness values[44]. In this study, the exercise of climbing stairs was held without load. Nevertheless, the protocol used for this exercise was effective in the recovery of bone mass and cell losses due to ovariectomy and immobilisation verified by the histomorphometry method. Cassilhas *et al.*[20] add that the ladder exercise promotes hypertrophy of the gastrocnemius muscle, the flexor digitorum longus and plantaris, which can help on the protection of the ankle joint and the bone matrix. Nascimento *et al.*[22] showed that this climbing exercise also promotes hypertrophy of the brachial triceps muscle, ie, achieving, with positive results with inferences even for the forelimbs.

Thus, it is apparent that the stair climbing exercise promotes the adaptation of muscle tissue, cartilage and bone, with an improvement in BMD, bone stiffness, hypertrophy of gastrocnemius muscles, flexor digitorum longus, plantaris and triceps proprioceptive stimulation and resistance, thus proving to be an important and effective kinesiotherapeutic feature, mainly because of its global action throughout the body, not only in a focus or member[20,22,45]. It is believed that exercise stimulates ladder climbing action osteoprotegerin, neutralizing the effects of osteoclastic bone resorption by the interaction of receptor activator kB ligand (RANKL) and receptor activator kB (RANK) due to hypoestrogenism. This possibly leads to an increased formation of vitamin D and serum calcium uptake in the bone matrix, providing increased mass and bone strength, which was evidenced by area and cortical thickness, and number of osteocytes, in the groups submitted to exercise climb staircase[46].

Thus, it might be noted that the ladder climbing exercise appears to be a good resource for clinical practice, taking in account many benefits that it provides to the treatment of osteoporosis in periods of remobilization and the combination of both situations. This causes it to expand the clinical reasoning of professionals and to increase the possibilities of therapeutic methods to be applied with greater accuracy and resolution in the proposed treatments. One limitation of the present study was the absence of a control group subjected only to immobilization, without remobilization and hormonal intervention. It is reiterated regarding the difficulty of other comparisons with the exercise protocol used, using times, series and different intensities. However, following the 3Rs ethical rule (replacement, reduction and refinement) for the use of animals in research, it was opted to follow this model due to the number of rats to be used.

In summary, upon the climbing stairs exercise protocol used in this study and with its limitations, it is concluded that this therapeutic approach was effective in the recovery of bone loss in rats subjected to a post-menopausal osteoporosis experimental model and immobilization, with recovery of thickness, and cortical and trabecular area, as well as the number of osteocytes. It is emphasized that future studies could be conducted with greater times of hormonal deprivation and immobilization, implementing other exercise protocols, in association with other therapeutic resources, as well as evaluating the effects on other systems and potential impact on functional activities.

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Semi-longitudinal analysis of physical status in madrilenian adolescents

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Received: 12.05.2015
Accepted: 30.09.2015

Summary

Physical fitness is very important for health, but is also essential to adjust the training load and adapt it to the age of individuals. The Physical Education teachers can assess the fitness of their students, from seven tests that are part of the curricular contents of that subject. The aim of the present work was to determine the physical aptitude of Madrilenian students of both sexes aged 13 to 18, and to develop an updated reference patterns that could serving to assess the physical fitness in High Schools students.
Longitudinal study was performed, since each individual was evaluated among two and eight times. The sample consisted of 4271 records (2333 boys and 1938 girls) from more than 500 individuals between 13 and 18 years old. The tested exercises determine the physical capabilities of students, strength, endurance, speed and flexibility. These tests are the following: Sit-Ups in 30 seconds, 4 x 9 meter Shuttle Run test, 50 m shuttle-run test, Standing Broad Jump, medicine ball explosive power test and trunk flexion test. Results allow characterizing the physical fitness of the Madrilenian students. Ontogenic and sexual variability were examined and the corresponding tables are provided with mean, standard deviation and percentile distribution for each test, according to sex and age. The statistical analysis, made using the SPSS v.20.0, shows a significant sexual dimorphism ($p < 0.001$) for all the studied tests.
The obtained values in this research also prove that boys outperform girls in all the physical test, except in those that measure flexibility.

Key words:
Physical fitness. Physical activity. Adolescents. Spain. Standards.

Análisis semilongitudinal de la condición física en adolescentes madrileños

Resumen

La condición física tiene gran importancia sobre la salud de los sujetos, pero también es primordial ajustar la carga del entrenamiento y adecuarla a la edad de los individuos. El profesorado de Educación Física puede evaluar la condición física de su alumnado a partir de siete pruebas que forman parte de los contenidos curriculares de la mencionada asignatura. Los objetivos de este trabajo son, por un lado, conocer la condición física de los estudiantes madrileños de ambos sexos de 13 a 18 años y, por otro, elaborar unos patrones actualizados que sirvan de referencia al profesorado de Educación Física para su alumnado de ESO y Bachillerato. Se ha efectuado un análisis semilongitudinal, ya que cada sujeto ha sido evaluado entre dos y ocho ocasiones. La muestra se compone de 4.271 registros (2.333 de chicos y 1.938 de chicas) de más de 500 escolares de 13 a 18 años. Los ejercicios examinados determinan las capacidades físicas de los estudiantes, fuerza, resistencia, velocidad y flexibilidad. Estas pruebas son: abdominales en 30 segundos, carrera de 9 m x 4, salto horizontal, carrera de 50 m lisos, lanzamiento del balón medicinal de 3 kg, flexión del tronco y carrera de 1.000 m. Los resultados presentados permiten caracterizar la aptitud física de los escolares madrileños. Se ha examinado la variabilidad ontogénica y sexual y se aportan las correspondientes tablas con la media, desviación estándar y la distribución percentilar para cada una de las pruebas, según sexo y edad en años cumplidos. En el análisis efectuado con el paquete estadístico SPSS (versión 20.0) se manifiesta un dimorfismo sexual significativo ($p < 0,001$) para todos los ejercicios estudiados. Asimismo los valores aportados por esta investigación muestran que los varones obtienen mejores resultados que las mujeres en todas las pruebas físicas, excepto en aquellas que miden la flexibilidad.

Palabras clave:
Aptitud física. Actividad física. Adolescentes. España. Estándares.

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Introduction

Physical capabilities are unique to each individual, are genetically influenced, and develop with exercise. There is controversy among authors regarding their title; as such, Álvarez Villar[1] calls them basic physical qualities and describes them as the factors that determine the physical condition of the subject, guiding them to performing a specific activity and enabling them to develop their physical potential via training. The majority of specialists in this field establish that these capacities are strength, resistance, speed and flexibility.

Meanwhile, physical activity is defined by the World Health Organisation (WHO) as any bodily movement produced by the skeletal muscles that requires an expenditure of energy[2]. This should not be confused with exercise, which is a variety of the aforementioned, but that requires planning, structure and repetition. In other words, physical activity encompasses exercise and other activities that imply movement of the body. If we bring together and assess the physical capacities of an individual with activity or exercise, we reach the idea of physical condition.

The definition of this concept has evolved and has significantly transformed over time, but the importance of physical condition in personal well-being and health[3] was established in the early 21st century. A sedentary lifestyle is a risk associated with the propagation of excess weight and other metabolopathies in childhood and adolescence[4-6]. The AVENA study (Feeding and Assessment of the Nutritional Status of Spanish Adolescents)[7] estimates that 1 out of every 5 Spanish young people face future cardiovascular risks, due to their low level of physical shape, but as well as this it has been proven that exercise should form part of the treatment for diverse pathologies, such as type 1 diabetes[8]. There are also very recent studies, such as the MOCA study[9], which reveal a significant relationship between practising sports, self-esteem and adequate perception of their Body Mass Index (BMI).

Exercise presents additional advantages, as it also constitutes a practice that socialises the individual, allowing participants to interact with their surroundings and other people[10]. Unfortunately, the majority of western populations do not perform the recommended amount of physical exercise to lead a healthy life[11]. As highlighted in the research carried out by the WHO[12] on 11,230 students aged between 11 and 18 years, the percentage of Spanish students that perform 1 hour of exercise each day is very low. Furthermore, this proportion is lower in girls, and gets lower as the age increases. Therefore, at 11 years the figures stand at 21% for females, and 41% for males, whilst by 15 years they stand at 8% for females and 25% in males. Similar results were obtained in a study performed on Catalan students from 5 to 17 years, in which the recommendation was to increase the number of Physical Education teaching hours at school[13], the advantage of which is proven in the EDUFIT intervention programme (Physical Education and Education for Fitness)[14].

In short, it is essential to raise awareness among families, teaching staff, school centres and governments, that promoting physical exercise ensures a healthier future for young people[15]. In this respect, educational institutions could play an important role, for example, by providing students with sporting materials during break times, or by designating sporting spaces on playgrounds in schools[16]. The guidelines of the PERSEO Programme (Pilot Programme in Schools for Health, Physical Exercise and against Obesity) assign an important role to Physical Education teaching staff[17]. These teachers must strive to increase the time spent on motor activities in classes, and to encourage exercise habits. But first, to increase the quality of their subject, they should update their knowledge regarding the importance of physical condition on health and its age appropriateness for students. Regarding this point, the majority of teachers consider that it is key to resort to aptitude tests to assess the physical condition of students and in turn adjust the training load[18].

The aims of this work are, firstly, to discover the physical condition of Madrilenian students in the second cycle of Compulsory Secondary Education (ESO) and Baccalaureate. Secondly, to check if there is sexual dimorphism in the results of the different tests analysed, as signalled by multiple research studies. Finally, to create some current reference values that can be used by Physical Education teaching staff to assess the capacity and physical activity of Madrilenian students at the aforementioned educational stages.

Material and methods

The population studied includes 4,271 records (2,333 from males and 1,938 from females) from over 500 students aged between 13 and 18 years from the Santa Eugenia de Madrid Secondary Education Institute (IES). The data was obtained between the 2000/01 and 2011/12 academic years, and was collected by two Physical Education teachers from the cited centre, adhering to the Helsinki regulations dictated by the *World Medical Association* (WMA)[19].

The teaching staff involved did not carry out a specific course to ensure the reliability of the measurements, nor did they design a pilot sample because the tests analysed here had to be performed by all ESO and Baccalaureate students, and each of the tests followed the corresponding protocol in the programmes established by the Ministry of Education of the Autonomous Community of Madrid, in Physical Education.

As the methodology for a semi-longitudinal study dictates, each subject was assessed on at least two separate occasions, i.e. at the start and finish of the course and on a maximum of eight occasions, as the students repeated the same tests on each of the four academic years (3rd and 4th of ESO and 1st and 2nd of Baccalaureate) that stayed on at the IES.

The sample is heterogeneous because it covers all the IES students from previously mentioned educational stages during the cited time period, apart from those students that were excluded from the Physical Education subject by means of an official medical certificate. The series was classified by sex and age in completed years. To assess the physical condition, a series of tests was selected to establish the basic physical capacities of the student: strength, resistance, speed and flexibility. A warm-up is recommended before each of the exercises. The protocols followed have already been described and standardised[20-22].

Abdominals for 30 seconds are used to determine the strength and resistance of the abdominal muscles. To perform this test, subjects had to adopt the supine position, with legs flexed at 90º, feet supported, hip distance apart and with hands interlocked behind the neck. At the same time, another person held their feet to the floor. Students must touch both knees with their elbows and count the number of times this movement is performed in 30 seconds. Students must touch the mat with their backs, their knees with their elbows and the floor with their forearms. Only one attempt is made for this exercise.

The 9 m x 4 run measures agility and speed of movement. To perform this exercise, a flat, non-slip floor is required, upon which two parallel lines are marked 9 m apart. Subjects are positioned behind one of the lines and on the starting signal, must run and touch the other line with their hand. Next, they must return to the first line and bend down to touch it. Again, the same pattern is repeated returning to the opposite point and returning to the starting point. The time taken is measured in seconds and split seconds. Two attempts are made, with the best result noted down.

The horizontal jump with feet together is used to assess the explosive strength of the leg muscles. Students stand behind the line with feet in line, slightly separated with the knees lightly flexed. Students must jump forward as far as possible over the starting line and fall without touching the ground with their hands. The distance is measured in m and cm from the starting line and the back heel mark. Two attempts are made with the best result noted down.

The 50 m flat run checks movement and reaction speed. Students stand behind the starting line. Upon hearing the signal, they must cover the distance as quickly as possible. The time is measured in seconds and split seconds, from the moment the back foot is lifted to the moment the finishing line is crossed. Two attempts are made and the best result is noted.

Throwing the medicine ball measures the explosive strength of the arm and shoulder. Subjects stand with legs hip distance apart next to the starting line, and must throw a 3 kg ball as far as possible. The ball's trajectory should be as perpendicular as possible to the starting line and upwards with an angle of around 45º. The distance is measured in m and cm from the starting point to the nearest landing point. It is worth practising the throw, indicating the most suitable launch angle. Two attempts are made, with the best result noted down.

The sitting trunk flex assess the flexibility of the thorax, hip and legs. A bench is needed upon which a 55-cm long millimetre ruler is positioned with a sliding bar. Students sit on the floor with no shoes on, with legs parallel and stretched out, so that the feet rest completely on the front wall of the bench. With arms completely extended, they flex their trunk forward, push the bar as far down as possible with the fingertips and hold the position for at least 2 seconds. The bar movement along the scale is measured in cm but if the student does not reach 0, the numeration is negative and if it is passed it is positive. Knees should not bend, nor should the sliding bar be pushed forward hard. Two attempts are made, with the best length noted down.

The 1,000 m run determines their mid-distance aerobic resistance. Students must cover the set distance in as short a time as possible. Students stand behind the starting line and start running upon hearing the signal. The time is measured in minutes and seconds, from the moment the back foot is lifted to the moment the finishing line is crossed. Only one attempt is made for this exercise.

Analysis has been carried out using the SPSS statistics package (20.0 version). The normality of the distributions has been verified and the average, standard deviation and percentile value have been calculated for each of the trials, by sex and age in completed years. The Student T test has been used to analyse the sexual dimorphism and the ANOVA test to check the variation experienced with age in each of the exercises.

Results

Tables 1 to 7 display the averages, standard deviations and percentiles 3, 10, 25, 50, 75, 90 and 97 by age (in completed years) and sex, which correspond to each of the physical tests analysed. Likewise, in all the figures (Figures 1 to 7), sexual dimorphism is represented based on the average values obtained in each of the tests.

Table 1 presents the results of the abdominals in 30 seconds; in both sexes it can be seen that the number of repetitions varies significantly with age (males $F = 15.36$ $p < 0.001$, females $F = 7.26$ $p < 0.001$). There is a first stage (from 13 to 16 years) in which performance increases, then later there is a relative stabilisation, with a slight fall in values in the 17 year-old female series. Likewise, Figure 1 displays the sexual differences, which are significant ($p < 0.001$) across all age intervals analysed.

Table 2, corresponding to the 9 m x 4 run, clearly shows that in the male series the average time is reduced as age increases ($F = 33.84$ $p < 0.001$). However, in the female tests this reduction is not as clear, though it is significant ($F = 2.05$ $p < 0.05$). For this reason, the difference between the age extremes is 0.839 s for males and 0.076 for females. Equally, Figure 2 reveals that there is significant sexual dimorphism ($p < 0.001$) from 13 to 18 years. Effectively, the time taken in the test is higher for females than for males and this divergence increases with age.

Table 3 reflects the results obtained in the horizontal jump test. It seems obvious that for males, the average distance increases with age ($F = 47.81$ $p < 0.05$). For females, despite the variation being less, it is still significant ($F = 2.45$ $p < 0.05$). The average differences between the age extremes is greater for males (171.41 cm at 13 years and 219.61 cm at 18 years) than for females, with the respective figures of 155.84 and 157.13 cm. Figure 3 reveals the disparity between both sexes ($p < 0.001$), which is easily explainable because the power of the jump is directly related to muscle strength.

In the 50 m run, the strength of the lower limb muscles comes into play, which is why the results are better in males than in females (Table 4). It can be observed that for females, time increases with age ($F = 8.19$ $p < 0.001$) and varies by 2.92 seconds from 13 to 18 years. For males, this increase is less, though the variation of just 0.5 seconds between the age extremes is significant ($F = 4.38$ $p < 0.05$). Figure 4 reveals the marked sexual differences across all ages ($p < 0.001$) except for in the 13-year old set which reveals no significant differences ($p < 0.140$).

Table 1. Abdominals in 30 seconds (number of repetitions).

Males									
Age (years)	Average	S.T.	P3	P10	P25	P50	P75	P90	P97
13 (N=104)	26.66	3.70	19.00	22.00	24.00	27.00	29.00	32.00	33.00
14 (N=454)	28.33	4.73	20.00	22.00	25.00	28.00	31.00	34.00	38.00
15 (N=700)	29.64	4.26	22.00	25.00	27.00	30.00	32.00	35.00	38.00
16 (N=677)	30.32	4.31	22.00	25.00	28.00	30.00	33.00	36.00	38.00
17 (N=304)	30.81	4.39	22.00	25.00	28.00	31.00	34.00	36.00	38.00
18 (N=70)	30.59	4.61	22.13	24.00	27.00	31.00	34.00	37.00	40.74
Females									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=108)	23.47	3.77	16.27	18.00	21.00	24.00	26.00	29.00	30.00
14 (N=423)	23.67	3.65	17.00	19.00	21.00	24.00	26.00	28.00	31.00
15 (N=438)	24.37	4.14	16.00	19.00	22.00	24.00	27.00	29.00	32.83
16 (N=615)	25.34	4.46	16.00	19.60	23.00	26.00	28.00	31.00	33.00
17 (N=228)	24.68	4.64	14.74	19.00	22.00	25.00	28.00	30.00	33.00
18 (N=38)	25.71	4.55	16.17	19.70	22.75	26.00	29.00	31.00	34.49

S.T.: Standard deviation.

Table 2. Running 9 m x 4 (seconds and split seconds).

Males									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=104)	9.862	0.705	8.800	9.000	9.400	9.700	10.300	10.900	11.485
14 (N=455)	9.482	0.649	8.500	8.760	9.000	9.400	9.800	10.400	11.000
15 (N=696)	9.235	0.545	8.500	8.700	8.900	9.100	9.500	9.900	10.500
16 (N=672)	9.130	0.499	8.400	8.600	8.800	9.000	9.400	9.800	10.281
17 (N=305)	9.026	0.474	8.218	8.500	8.700	9.000	9.300	9.600	10.182
18 (N=70)	9.023	0.476	8.300	8.400	8.700	9.000	9.300	9.500	10.074
Females									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=109)	10.457	0.595	9.430	9.800	10.000	10.400	10.900	11.300	11.600
14 (N=439)	10.551	0.626	9.520	9.800	10.200	10.500	10.900	11.300	11.900
15 (N=437)	10.396	0.635	9.200	9.700	10.000	10.300	10.800	11.200	11.800
16 (N=615)	10.409	0.708	9.200	9.700	10.000	10.300	10.800	11.200	11.800
17 (N=230)	10.435	0.645	9.300	9.700	10.100	10.400	10.700	11.190	11.900
18 (N=37)	10.381	0.559	9.142	9.660	10.000	10.400	10.700	10.960	11.700

S.T.: Standard deviation.

In the medicine ball throw, it can be observed (Table 5) that the averages are significantly higher for males than for females ($p < 0.001$). Males improve appreciably with age ($F = 81.70$ $p < 0.001$) with the distance increasing by 2.82 m in the age range analysed. However, there was barely any variation among females. Figure 5 displays the discrepancies between males and females ($p < 0.001$).

Females had better physical performance than their male classmates in terms of flexibility (Table 6). This is the only test of those described

Table 3. Horizontal jump (centimetres).

Male									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=104)	171.41	0.23	123.20	141.50	154.25	170.00	188.00	204.50	214.55
14 (N=457)	195.59	0.26	140.00	160.00	180.00	198.00	213.00	227.20	242.00
15 (N=697)	205.91	0.24	152.94	174.80	192.00	208.00	222.00	237.00	245.00
16 (N=677)	211.77	0.23	161.02	181.60	200.00	213.00	228.00	240.00	252.00
17 (N=304)	216.16	0.22	170.00	182.50	203.50	220.00	232.00	243.00	255.00
18 (N=70)	219.61	0.21	170.00	190.00	210.00	220.00	235.00	246.60	261.96
Female									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=109)	155.84	0.21	110.10	123.00	141.50	156.00	173.00	182.00	196.70
14 (N=442)	153.06	0.21	110.00	127.00	140.00	152.00	167.00	180.00	195.00
15 (N=443)	158.11	0.26	107.00	123.00	143.00	160.00	175.00	187.60	209.76
16 (N=618)	158.55	0.23	115.00	130.00	143.00	160.00	173.00	185.00	210.00
17 (N=226)	157.04	0.24	120.00	129.40	141.50	154.50	173.00	182.30	203.38
18 (N=38)	157.13	0.31	55.34	129.00	145.00	162.00	176.00	181.40	209.96

S.D.: Standard deviation

Table 4. Running 50 m (seconds and split seconds).

Male									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=46)	4.32	0.34	3.70	3.80	4.10	4.30	4.62	4.80	4.90
14 (N=146)	4.72	1.41	3.40	3.60	3.80	4.10	4.90	7.23	8.04
15 (N=239)	5.19	1.65	3.50	3.60	3.80	4.20	6.90	7.30	8.18
16 (N=216)	5.34	1.81	3.40	3.50	3.70	4.20	7.00	7.80	8.49
17 (N=108)	4.71	1.54	3.30	3.40	3.60	3.80	6.60	7.00	7.50
18 (N=34)	4.85	1.60	3.40	3.50	3.67	3.85	6.82	7.05	7.59
Female									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=60)	4.41	0.27	3.87	4.10	4.30	4.40	4.60	4.70	5.00
14 (N=144)	6.10	2.05	3.90	4.20	4.40	4.80	8.37	8.95	9.56
15 (N=126)	6.16	2.12	3.88	4.00	4.30	4.70	8.20	8.73	9.91
16 (N=165)	6.48	2.05	3.99	4.20	4.50	7.40	8.30	8.90	9.70
17 (N=56)	6.31	2.02	3.57	4.27	4.60	4.90	8.20	8.83	9.73
18 (N=20)	7.33	1.73	4.20	4.34	5.45	8.10	8.60	8.98	9.70

S.D.: Standard deviation

in which they achieved better results ($p < 0.001$). It can be observed that the males increased trunk flex over the period analysed ($F = 8.25$ $p < 0.001$) yet their female classmates maintained more stable average values, which even reduced after 17 years. Figure 6 reveals the disparity between both sexes, which is significant in all ages ($p < 0.001$) except for in the 18-year old set ($p = 0.805$).

Table 7 reveals how the time taken for males in the 1,000 run reduces with age ($F = 8.49$ $p < 0.001$), whilst in the female series the values are

Table 5. Throwing medicine ball (metres).

Male									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=103)	5.26	1.07	3.41	3.74	4.50	5.30	6.00	6.70	7.39
14 (N=465)	6.31	1.17	4.20	4.80	5.45	6.20	7.20	7.80	8.50
15 (N=702)	7.19	1.17	5.20	.70	6.30	7.20	8.00	8.77	9.50
16 (N=682)	7.62	1.29	5.25	6.10	6.70	7.60	8.50	9.40	10.10
17 (N=312)	7.95	1.43	5.20	6.30	7.10	7.90	9.00	9.80	10.60
18 (N=69)	8.08	1.47	5.14	6.40	7.00	8.20	9.15	10.00	10.80
Female									
Age (years)	**Average**	**S.D.**	**P3**	**P10**	**P25**	**P50**	**P75**	**P90**	**P97**
13 (N=109)	4.71	0.77	3.70	3.80	4.20	4.60	5.00	6.00	6.60
14 (N=440)	4.65	0.79	3.40	3.70	4.20	4.50	5.00	5.80	6.50
15 (N=448)	4.93	0.89	3.60	4.00	4.30	4.80	5.50	6.00	6.80
16 (N=637)	4.95	0.85	3.60	4.00	4.40	4.80	5.40	6.00	6.80
17 (N=240)	5.06	0.96	3.70	4.10	4.30	4.95	5.50	6.00	7.38
18 (N=38)	4.81	0.72	3.28	4.09	4.27	4.65	5.40	6.00	6.00

S.D.: Standard deviation

Table 6. Trunk flex (centimetres).

Male									
Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
13 (N=104)	5.327	7.213	-11.550	-1.500	1.000	4.500	9.000	15.500	21.850
14 (N=462)	5.619	7.580	-9.000	-4.000	1.000	6.000	10.000	15.000	20.000
15 (N=700)	7.579	6.853	-7.000	-2.000	3.000	8.000	12.000	15.900	21.000
16 (N=674)	8.798	7.578	-7.000	-1.000	4.000	9.000	14.000	18.000	21.750
17 (N=304)	7.954	8.095	-9.850	-3.000	2.000	9.000	14.000	18.000	21.000
18 (N=69)	9.377	8.048	-6.900	-4.000	5.000	11.000	15.000	17.000	23.000
Female									
Age (years)	**Average**	**S.D.**	**P3**	**P10**	**P25**	**P50**	**P75**	**P90**	**P97**
13 (N=110)	12.005	6.928	-2.670	4.100	7.000	11.500	17.000	20.000	24.670
14 (N=444)	12.689	6.369	0.000	4.000	9.000	13.000	17.000	21.000	24.000
15 (N=455)	12.545	7.229	-2.320	4.000	8.000	12.000	17.000	22.000	26.000
16 (N=652)	12.948	7.441	-1.410	4.000	8.000	13.000	18.000	23.000	26.000
17 (N=238)	11.483	7.137	-5.490	3.000	7.000	11.500	16.000	21.000	25.000
18 (N=39)	9.795	8.600	-13.800	0.000	5.000	9.000	16.000	22.000	26.400

S.D.: Standard deviation

more stable. This is confirmed upon checking that the result between the age extremes is four times less for females (0.35 min), than for males (1.57 min). There are significant discrepancies (Figure 7) between the markers of both sexes ($p < 0.001$ in all ages apart from 13 years in which $p < 0.05$), again higher for males than for females.

Discussion

The *Eurydice network*, adopted by 40 European countries including Spain and coordinated by the European Union Education, Audiovisual and Culture Executive Agency, has produced a report entitled *Physical*

Table 7. Running 1,000 m (minutes and tenths of a minute).

Age (years)	Average	S.D.	P3	P10	P25	P50	P75	P90	P97
Male									
13 (N=25)	5.66	1.84	3.44	3.57	4.36	5.12	6.27	8.79	10.02
14 (N=285)	4.46	0.97	3.33	3.41	3.58	4.28	5.04	5.52	7.07
15 (N=491)	4.34	0.99	3.25	3.37	3.55	4.20	4.52	5.37	6.45
16 (N=454)	4.24	0.99	3.20	3.35	3.49	4.15	4.45	5.28	6.66
17 (N=188)	4.22	0.90	3.23	3.38	3.53	4.14	4.40	5.29	6.43
18 (N=40)	4.09	0.61	3.29	3.40	3.54	4.10	4.34	4.49	6.18
Female									
Age (years)	**Average**	**S.D.**	**P3**	**P10**	**P25**	**P50**	**P75**	**P90**	**P97**
13 (N=29)	6.63	1.39	4.25	5.19	5.58	6.36	7.43	9.42	10.02
14 (N=299)	6.13	1.18	4.35	5.05	5.33	6.03	6.55	7.50	9.04
15 (N=315)	5.96	1.14	4.26	5.00	5.26	5.58	6.45	7.31	8.42
16 (N=418)	5.98	1.22	4.15	4.52	5.20	5.57	6.50	7.49	8.92
17 (N=153)	6.24	1.35	4.38	5.09	5.35	6.12	6.57	8.36	9.79
18 (N=26)	6.28	1.05	4.27	5.04	5.39	6.32	7.08	8.15	8.33

S.D.: Standard deviation

Figure 1. Comparison between sexes in abdominals in 30 seconds.

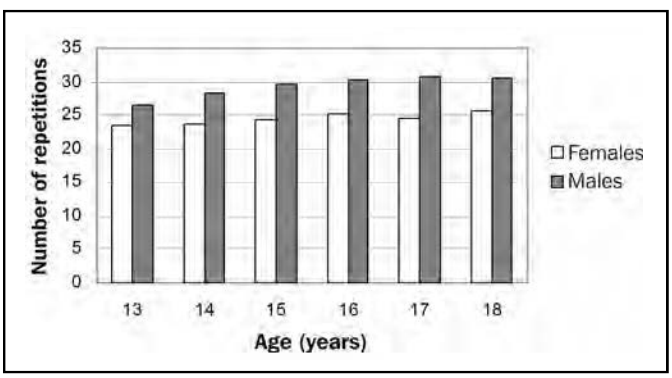


Figure 2. Comparison between sexes in running 9 m x 4.

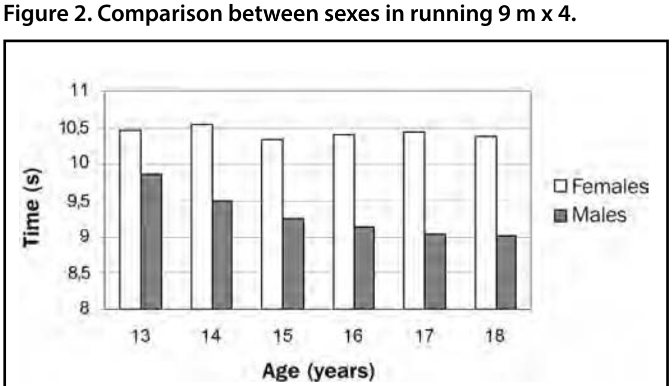


Figure 3. Comparison between sexes in horizontal jump.

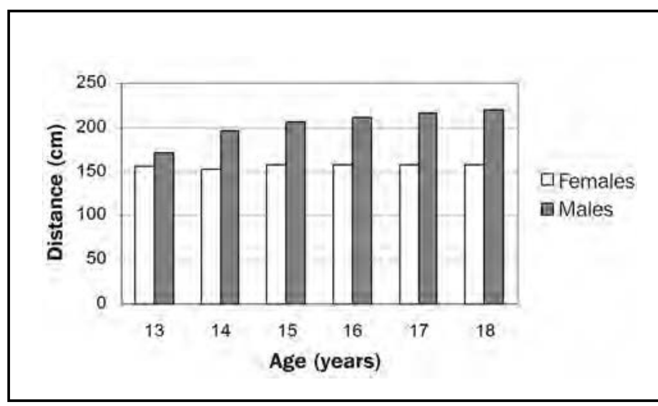


Figure 4. Comparison between sexes in running 50 m.

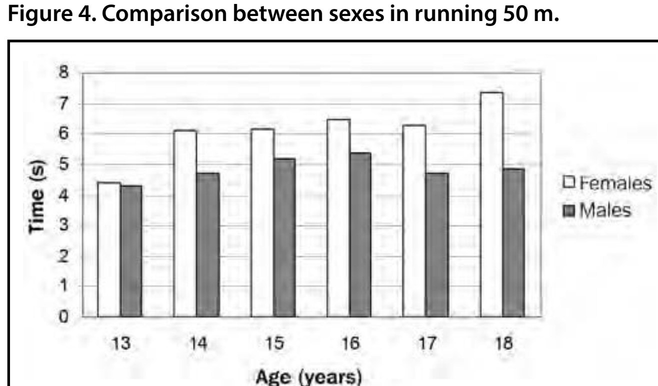


Figure 5. Comparison between sexes in throwing medicine ball.

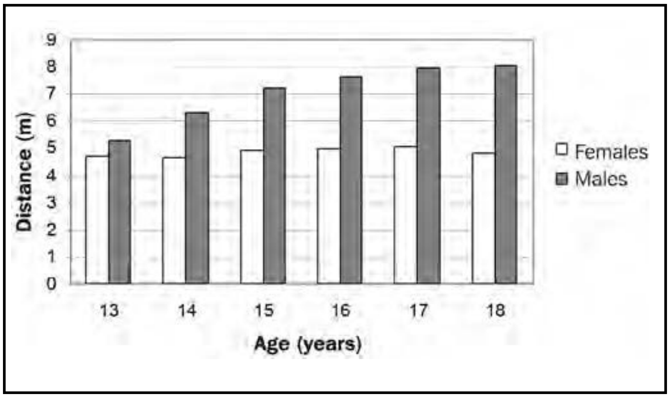


Figure 6. Comparison between sexes in trunk flex.

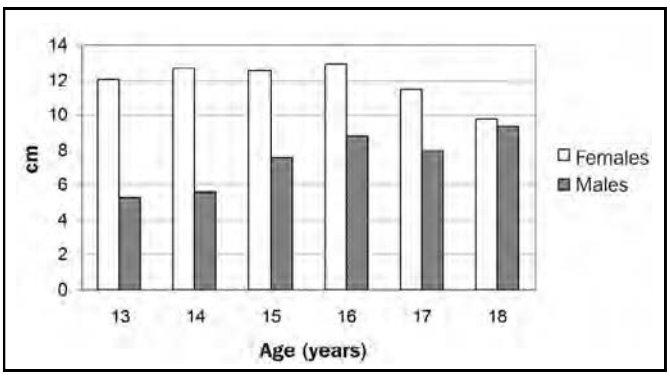
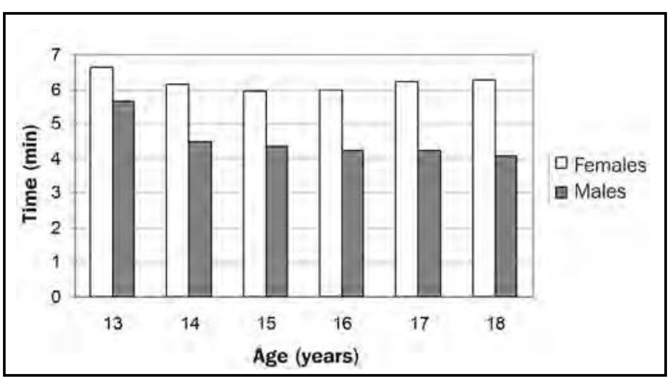


Figure 7. Comparison between sexes in running 1,000 m



Education and sports at school in Europe[23]. This report examines the current state of Physical Education and sporting activity in the schools of participating countries. For both primary and secondary schools, aspects such as the position of the subject, number of recommended teaching hours per stage, teacher qualification, etc. are analysed. It is important not to reduce the time dedicated to this regulated activity in the school curriculum, as for a high percentage of children, particularly girls, this is the only exercise they do[24].

In the cited *Eurydice* dossier, there is also an analysis of the way assessment processes are carried out, and it highlights the need to include the assessment of the physical condition of students in curricular contents. Some authors even indicate that it would be recommendable to do this for each educational stage in the different itineraries corresponding to a course[25]. To perform this assessment, updated reference models are needed, adapted to the demographic to be analysed, given that, as in many anthropometric values, physical condition also reveals secular and demographic variations.

In this respect, in research carried out on school children from North America, Australia, Denmark, the Baltic States and Venezuela[26-30] significant generational changes that affect the average marks obtained in specific aerobic-type tests, or included in the *Eurofit* test battery have been detected. Equally, a meta-analysis[31] that covers 77 studies from 23 European countries with a sample size of over a million students aged between 7 and 18 years notes the considerable variability between the performance of subjects from different nationalities, which could be a reflection of socio-cultural type issues, or the importance that each society grants the teaching and practice of exercise.

The need for suitable reference models has driven the development of different research studies in Latin America and Europe. As such, in Montería (Colombia), the physical capacities of aerobic resistance, strength, speed and flexibility were assessed among 612 students aged between 12 and 18 years, and the percentile ranges were established by age and by sex[32] for each of these functional capacities. Using similar methodology, but on a larger scale, a transverse study was carried out on 48,738 students from Bogota aged 7 to 18 years[33], with the aim of classifying the physical aptitudes of Colombian adolescents. Along this same line is another study[34], which examined 7,843 students of both sexes from the central region of Peru, from 6 to 17 years.

In terms of Europe, in Latvia, Sauka *et al.*[35], based on a transverse study on 10,464 students aged 6 to 17 years, have established *Eurofit* test battery reference values to be used, on the one hand, to evaluate the school population, and on the other hand, to eventually establish comparisons with other Nordic countries. In the same continent, the HELENA study has been undertaken *(Healthy Lifestyle in Europe by Nutrition in Adolescence)*[36] on a sample group of 3,428 adolescents aged between 12.5 and 17.5 years, recruited between 2006 and 2008 from 10 cities in Austria, Germany, Belgium, Spain, France, Greece, Hungary, Italy and Sweden.

However, all the aforementioned studies are based on transverse-type research, in which the subject is measured just once at a specific time. Furthermore, the majority include *Eurofit* test batteries that do not completely coincide with the normal protocol applied to Physical Education curricula in the different educational stages in Spain. Instead, the tests presented in this work are those habitually carried out in Spanish schools, they meet the methodology applied by Physical Education teaching staff and the results adhere to a semi-longitudinal study. As such, the exercises analysed are easily applied and do not require a large infrastructure or technology, meaning they could be performed in any educational institution. Consequently, the values reflected here can be used as a reference to assess the nutritional condition of students. To some extent, these models complement those already published by researchers in our group, regarding the dynamometric hand strength of Spanish students[37].

Likewise, in all the tests analysed there is a clear sexual dimorphism, which coincides with the majority of the studies that assess these or other physical activities, both in adolescents and adults. In the field of young people, the values contributed by this research demonstrate that males obtain better results in all the tests that measure functional capacity, except for those that determine flexibility. In the male series, the physical condition assessed in different tests clearly increases with age, whilst for females, capacity levels tend to be more stable over the ontogenetic period studied; this fact coincides with that observed in preceding research studies38-41 that also analyse some of the tests shown here.

Conclusions

This investigation firstly enables the establishment of the physical aptitudes of Madrilenian students in the final two years of the ESO (3rd and 4th) and the two educational levels of the Baccalaureate (1st and 2nd). Likewise, all the values presented here can be used by the Physical Education teaching staff at the Madrid teaching centres, to assess the physical condition of their students.

At the same time, as the measurements are expressed in the form of averages, standard deviations and percentile value by age and sex, they can be used as reference model to characterise the physical condition (using strength, resistance, speed and flexibility tests) of young Madrilenians of both sexes, aged between 13 and 18 years.

Finally, it should be highlighted that in all the exercises analysed there is a clear difference between both sexes; males obtained better markers in all the tests, apart from the trunk flex in which females were better. Furthermore, for males there was a noticeable improvement in performance with age, whilst for females the values remained more similar over the period studied.

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PREMIOS FEMEDE A LA INVESTIGACION 2015

Los trabajos que han logrado los premios FEMEDE a la investigación en el año 2015, consistentes en la **publicación en la revista Archivos de Medicina del Deporte, junto con una dotación de 600 euros y el certificado acreditativo** son los que se relacionan a continuación con sus correspondientes autores:

- **María Perales**, por el trabajo titulado *"Fetal and maternal heart rate responses to exercise in pregnant women. A randomized Control Trial"*, con coautoría de Silvia Mateos, Marina Vargas, Isabel Sanz, Alejandro Lucia y Ruben Barakat.
- **Oriol Abellán-Aynés**, por el trabajo titulado *"Anthropometric profile, physical fitness and differences between performance level of Parkour practitioners"*, con coautoría de Fernando Alacid.
- **Eliane Aparecida de Castro**, por el trabajo titulado *"Peak oxygen uptake prediction in overweight and obese adults"* con coautoría de Rocio Cupeiro, Pedro J. Benito, Javier Calderón, Isabel R. Fernández y Ana B. Peinado.

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