ISSN: 0212-8799

181
Volumen 34
September - October 2017

Archivos de medicina del deporte

Órgano de expresión de la Sociedad Española de Medicina del Deporte

ORIGINAL ARTICLES

REVIEWS

ARCHIVOS DE MEDICINA DEL DEPORTE

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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

Edita
Sociedad Española de Medicina del Deporte
Iturrama, 43 bis.
31007 Pamplona. (España)
Tel. 948 267 706 - Fax: 948 171 431
femede@femede.es
www.femede.es

Correspondencia:
Ap. de correos 1207
31080 Pamplona (España)

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).

La Revista Archivos de Medicina del Deporte ha obtenido el Sello de Calidad en la V Convocatoria de evaluación de la calidad editorial y científica de las revistas científicas españolas, de la Fundación Española para la Ciencia y la Tecnología (FECYT).


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Volumen 34(5) - Núm 181. September - October 2017 / *Septiembre - Octubre 2017*

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Gene doping. Are we willing to risk it?

Dopaje genético. ¿Estamos dispuestos a arriesgar?

Raquel Blasco Redondo

Unidad de Medicina Interna. Centro Regional de Medicina Deportiva de la Junta de Castilla y León. Dirección General de Asistencia Sanitaria. Valladolid. Departamento de Nutrición y Bromatología. Facultad de Medicina. Universidad de Valladolid.

Gene doping is defined as the non-therapeutic use of genes, genetic elements and/or cells that have the capacity to improve sporting performance. This can be achieved by introducing and subsequently expressing a transgene, or by modulating the activity of an existing gene to achieve an additional physiological advantage[1].

Since 2001, when the improvement of the abilities of athletes that used the principles of gene therapy was discussed for the first time in the prestigious Nature[2] magazine, gene doping has been the focus of many debates, some controversial in terms of its banning.

The decoding of the human genome has opened the door to a wide set of possibilities for genetic treatments, as well as for the technologies that will develop them. This has not just been in the field of therapy, but also in improvements in sporting performance. Considering that gene therapy is a new form of medicine, and until recently it had only been tested on patients with terminal illnesses, its long-term consequences are as of yet unknown. Therefore, the key questions continue to be unanswered regarding the possible use of transgenes in the sporting sphere.

Perhaps the fundamental question refers to the theoretical possibility that the transgenics used in gene doping could inadvertently affect germinal cells, and produce permanent alterations that could be transferred to future generations. There are currently no solid answers to this question.

The basic principles of gene doping: Much simpler than it seems

The non-therapeutic use of genes can modify genetic expression in that proteins are produced in the organism that give muscles more growth, faster recovery and greater strength. The proteins produced this way will be the same as those generated normally by the body.

The idea is simple: alter our genetic composition, the building bricks of which we are made, to make us stronger or faster. However, the practical aspects are highly complex[3-5].

Genetic therapists add a synthetic gene to the patient's genome, and reintroduce it into the body via a de-activated virus. The new gene is extracted by the patient's stem cells, and acts as a treatment, becoming permanently incorporated into the body.

It is still a rare and highly specialised treatment, but the principle is used for the research of any variety of illnesses, including those in which there is muscle deterioration, a point that makes it easier to imagine how athletes could benefit from this mechanism.

Types of genetic interventions: Are they all equally dangerous?

Generally speaking there are two types of genetic interventions:

Somatic

SThis involves the intervention into cells to modify the genome of already existing beings in order to make them more resistant to certain illnesses or to improve their physical capacities. In theory they are not variations that can be transmitted genetically from people to their descendants. In principle these would be those that are (currently) applied in gene doping.

On this level there are currently two possibilities of using somatic gene therapy to improve sporting performance:

Ex vivo: For this, a cell line must be extracted from the athlete (using a biopsy). The gene is transferred to these cells, which are then re-introduced into the body. This is an invasive method (as it requires a biopsy) but it has the great advantage that it allows for the exclusive treatment of specific cell lines (usually muscle lines).

In vivo: The gene is transferred directly to the patient using a drug. The gene would be transported in a vector such as a virus or plasmid, and the modified DNA would be injected into the athlete's cells.

Genetic modifications to the germ line

- *In vitro fertilization:* development of the embryo before its implantation and the genetic modification of the embryonic stem cells of the

Correspondencia: Raquel Blasco Redondo
E-mail: rblasco@saludcastillayleon.es

foetus, by which the genetic data of the future individual is changed.
- *Cloning:* an adult somatic cell is genetically modified. Next, the nucleus of this modified cell is introduced into an egg that has no nucleus, and fertilization is simulated, with which an embryo is created that contains the same genetic data as the first adult, plus the added genetic modification. This has not yet been applied to human beings… Are we considering applying it to be stronger, faster and more resistant?

Which genes are candidates for use in gene doping?

There are many, but perhaps the most well known are:

Erythropoietin: Achieving an increase in energy production by the aerobic metabolism. The high-profile Repoxygen

Repoxygen leapt into the media during the trial that took place in 2006: A genetic therapy developed by the British laboratories Oxford Biomedica, in 2002 found it to be a very effective treatment for severe anaemia in neoplastic processes and in kidney failure.

Treatment with Repoxygen is based on the direct intramuscular administration of an inactivated virus that carries the erythropoietin gene. The drug attaches to a specialised gene in the DNA of its host, in this case, the gene that is responsible for the synthesis of EPO. In the right conditions, the gene directs the cells to start producing extra erythropoietin (EPO).

The majority of Repoxygen studies carried out reached the conclusion that the EPO gene injected produces higher levels of circulating EPO and has a much more pronounced biological effect that the endogenous gene in all the species studied, thus revealing its high potential in gene therapy strategies for EPO[6-7].

Vascular endothelial growth factor (VEGF): Increase of the oxygen supply

Oxygen is vital for the synthesis of ATP for aerobic breathing. Oxygen, being a small molecule, is able to spread through the plasmatic membrane of endothelial cells. Therefore, an increase in vascular branching promotes quicker and more effective oxygen diffusion to tissues, and a greater availability of it to produce energy. VEGF promotes the branching of the pre-existing vessels, thus increasing capillarisation. In gene doping, various copies of the gene that codifies the VEGF are inserted into the muscle, probably using viral vectors. Therefore, if it is successful in athletes, muscle microcirculation will be stimulated and the oxygen supply to muscles will be increased[8].

Type 1 insulin-like growth factor IGF1: Increase in the growth and differentiation of the muscle

In 2007, whilst Lee Sweeney - a professor at the Pennsylvania University – researched the possible ways of restoring muscle growth in patients with muscular dystrophy, mice were created that continued to have enormous muscles and that preserved a significant percentage of their strength into old age.

These super-mice were created by injecting normal mice with a virus that held the gene for type 1 insulin-like growth factor, which has receivers in the surface of muscle cells and stimulates their growth. These mice were nicknamed "Schwarzenegger mice" after the North American body-builder.

In gene doping, multiple copies of the gene that codifies for IGF-1 could be inserted into the skeletal muscle and would produce an increase in muscle mass due to the hypertrophy of the muscle cells. This somatic gene insertion, according to experts, could be achieved by using two alternative vectors: plasmid or virus, and would always be performed using the ex vivo technique, i.e. via muscle biopsies, strengthening the muscle groups required.

It is essential to recall at this point that IGF-1 also has activities that act beyond muscular effects, including the capacity to boost tumour development and progression, that is, it is potentially pre-neoplastic[9].

The antagonists of myostatin, the gene PPAR-Delta and its agonist the gene GW501516: The increase of hypertrophy and hyperplasia of the muscle and also the perpetual movement machine

Myostatin, a member of the growth factor family, could be useful in gene doping with the aim of improving muscle percentage and sporting performance. It is closely linked to the gene PPAR-Delta. In 2008, Evans developed a strain of "marathon mice" by stimulating the gene called PPAR-Delta. The genetically modified mice were able to run twice as far as normal mice, and were capable of possessing high muscle definition, even when they were fed with a high-fat diet10. It is key to remember and reiterate that the activity of these genes goes further than the target organ, and they therefore have the capacity to proliferate tumours, as in they are potentially pre-neoplastic.

The list would be endless, as there are currently at least 181 active clinical trial treatments in the USA alone, and over 2000 internationally. The majority of them focus on treatment for severe anaemia and muscle weakness, which could undoubtedly be used in athletes. Mention should be made of growth hormone modulating genes, the hypoxia induced factor (HIF), peroxisome proliferator activated receptors (PPARα), etc.

Where does the problem lie?

Permanently changing the genome could be complex, using a de-activated virus to take medicine genetically to cells. Nevertheless, there is a shortcut that offers temporary results: injecting the purified gene directly into the muscle. In fact, this is simple, and now, thanks to the Internet, it is possible to access Repoxygen.

We could suggest that a tempting aspect for those aspiring to use doping is that this temporary improvement, after a couple of days, could be difficult to detect by the authorities.

If this is the case, what is the World Anti-Doping Agency (WADA) doing about this?

WADA: Detection or prevention strategies?

Unfortunately, science is a step behind those that turn to cheating, and when a new substance is detected, there is already another one in the market.

In 2003 the WADA banned gene doping, as from the agency's scientific management perspective, not only would the carrying out of this practice be unfair, it could also be lethal[11].

It is highly unlikely that anyone is benefitting from gene doping, and undoubtedly it is much more effective to focus attention on more standardised doping systems such as anabolic steroids and different blood doping methods. However, the WADA upholds that it is investing significant amounts of money and research resources into finding an effective diagnostic method to detect the intervention of genetic material in athletes. In fact, the latest challenge in the fight against doping is to be able to detect gene doping, for which molecular biology techniques have been used. Work is currently being undertaken from a new perspective: instead of tracking substances, as in a standard examination, changes are looked for in genetic expression and in the production of protein. Another very curious idea that is being contemplated is the shaping of images, using a similar process to magnetic resonance, to explore the body in the search for less common gene expression locations. In light of the studies that are being carried out, experts from WADA feel that it is only a matter of time before a detection test is created.

In all honesty, to date, no one can be sure of whether or not genetically modified Olympic athletes are already in the pools or on the tracks, as the temptation of winning the gold medal can lead athletes to take the hugely dangerous genetic step towards the unknown.

Some say that it may not be long before we see the first genetically modified athlete. Others, including myself, consider that the use of gene therapy to improve sporting performance is already a reality. However, given that the diagnostic methodology still lacks the sophistication needed to contest "gene doping", its status continues to be unclear.

A certainty is that the use of genetic modification to improve sporting performance is technically feasible, at least in animals, and that some athletes are prepared to risk their lives in the quest for guaranteed gold medals[12].

Yet there is an even greater question mark. Even if there were already an effective test to detect gene doping, what would happen if gene therapy became a widespread, or even routine practice? What would happen if we could all purchase genetic medicine to reduce muscle deterioration? Should we – or could we – prevent athletes from using this medicine to prolong their careers or to speed up their recovery after an injury?[13-15].

Where are we headed?

This is where I leave my own question: If gene therapy used to prevent muscle deterioration were safe, it would become an exclusively ethical issue, given that we could maintain normal quality in the field of sporting medicine for longer, and optimise ageing, which is where the issue returns to sport.

We know that the earlier the intervention, the better the ageing process will be. Could it be unethical to stop treating people with something that could truly enable their muscles to be much healthier now and in the future?

In any case, we are decades from having the need to cover this issue, given the slow rate at which gene therapy is advancing. When the moment arises, the Agency will have to mark the limits that it does with all drugs: Do they unfairly improve the athlete's performance?

Though with that in mind, it seems that the nature of gene doping will make it technically difficult and ethically cumbersome to mark these limits. Authorities, athletes and the sporting sphere agree upon a whole new definition of what they want sport to mean.

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Original article

Leisure time physical inactivity and self-perception of health status in colombian adults from 18 to 64 years old

Dario Mendoza Romero[1], Adriana Urbina[2]

[1]Universidad Santo Tomás de Aquino, Bogotá, Colombia. [2] Escuela de Medicina y Ciencias de la Salud, GI en Salud Pública, Universidad del Rosario, Bogotá. Colombia.

Received: 07.12.2016
Accepted: 01.02.2017

Summary

Objective: To explore the relationship between leisure time physical inactivity (PI) and self-perceived health status in the Colombian population between 18 and 64 years of age reported in the National Nutrition Situation Survey (ENSIN) and National Demography and Health Survey (ENDS) 2010.
Methods: Cross-sectional analytical study to explore the association between physical inactivity and self-perception of the health status of Colombians between 18 and 64 from the National Survey of Nutrition Situation in Colombia (ENSIN) and the National Demographic and Health Survey (ENDS) 2010.
Results: PI explains a regular and poor self-perception of health (OR= 1.37; IC95% 1.10-1.70) in women after adjusting for confounding factors. Other associated variables included: age, educational level, affiliation to the health system, marital status, living in eastern region and Bogota. PI was not associated with self-perception of health status in men (OR=1.04; IC95% 0.85 – 1.29), but age and low educational level did show association.
Conclusion: Low levels of leisure time physical activity were associated with regular and poor self-perceived health status in women, whereas in men this relationship was not observed.

Key words:
Physical activity. Health status. Subjective health.

Inactividad física en el tiempo libre y auto percepción del estado de salud de colombianos entre los 18 y 64 años

Resumen

Objetivo: Explorar la relación entre la inactividad física en el tiempo libre y la auto percepción del estado de salud en la población colombiana entre 18 y 64 años de edad reportados en la Encuesta Nacional de la Situación Nutricional (ENSIN) y Encuesta Nacional de Demografía y Salud (ENDS) año 2010.
Metodología: El diseño del estudio es analítico de corte transversal y pretende con los datos de la Encuesta Nacional de Situación Nutricional en Colombia (ENSIN) y la Encuesta Nacional de Demografía y Salud (ENDS) 2010, explorar la asociación entre inactividad física en el tiempo libre y auto percepción del estado de salud de los colombianos entre 18 y 64 años.
Resultados: Para el 2010, en mujeres, la inactividad física explica una regular y mala auto percepción de la salud (OR=1,37; IC95% 1,10–1,70) después de haber ajustado por factores de confusión. Variables como edad, nivel escolar, afiliación al sistema de salud, estado civil, vivir en determinadas regiones como la oriental y Bogotá en mujeres también influyen en la auto percepción de la salud. En el grupo de los hombres no se observó que la inactividad física esté asociada con auto percepción regular o mala del estado de salud (OR=1,04; IC95% 0,85 – 1,29), pero sí la edad y la baja escolaridad.
Conclusión: Niveles bajos de actividad física en el tiempo libre se asocian con regulares y pobres auto percepciones de salud en mujeres, mientras que en hombres no se observó esta relación, lo que hace necesario tener en cuenta otro tipo de variables que pueden modificar esta percepción.

Palabras clave:
Actividad física. Estado de salud. Salud subjetiva.

Correspondence: Dario Mendoza Romero
E-mail: dariomendoza@usantotomas.edu.co

Introduction

Physical inactivity is related to 21-25% of colon and breast cancer cases, 27% of diabetes cases and almost 30% of ischemic heart disease cases[1]. Physical inactivity is therefore a major risk factor for the development of non-communicable chronic diseases (NCDs), which represent 35 million deaths each year, equivalent to 60% of all deaths in the world, with 80% of them occurring in countries with low and medium income levels[2].

In Colombia, 22.4% of men and 27.3% of women perceive their own health to be average and poor3, and 80% of adults between 18 and 64 years of age are physically inactive in their leisure time4, which could entail an increase in health risks for people in that country. Leisure time physical inactivity contributes to the development of other risk factors, such as excess weight and obesity, which affect 17.5% of young Colombians aged between 5 and 17 years (13.4% are overweight and 4.1% are obese)[4]. A lack of knowledge surrounding the factors associated to physical inactivity among the Colombian population contributes to the growing threat of the development of non-communicable chronic diseases, which characteristically have long latency periods. Clearly establishing the factors that lead to this inactivity in leisure time will not only contribute to improving the population's lifestyle, but also to preventing psycho-social risk behaviour, by maintaining good mental health and overall improved physical health[5].

In turn, the self-perception of health has proven to be a mortality and morbidity predictor in study populations[6]. Having an average and poor self-perception of health has been associated to a low socio-economic level, a low level of schooling, being over 60 years of age and being a woman, among other factors[7]. There is no unanimous explanation behind this predictive power of self-perception of health status, but some authors argue that individuals are able to detect the signs and symptoms related to illness early, even before medical professionals[8]. Knowing the factors associated to a poor self-perception of health status could allow for earlier primary care interventions, before the established onset of chronic illnesses and their complications[9]. On the other hand, assessing the link between a poor self-perception of health and physical inactivity enables the earlier detection of the negative consequences of a sedentary lifestyle.

The aim of this study was to explore the link between leisure time physical inactivity and the self-perception of health status in Colombians aged between 18 and 64 years, in accordance with the National Survey on the Nutritional Situation in Colombia (ENSIN) 4 and the National Demographic and Health Survey (DHS) from 2010[3].

Material and method

Study design

A cross-sectional analytical study was carried out using information collected from the 2010 ENSIN and DHS studies. Both surveys were combined, taking into account that the ENSIN survey was a sub-sample of the DHS survey, and that they were carried out over the same period of time. The ENSIN 2010 study was applied to 50,670 homes, 4,987 segments of 258 municipalities from the 32 provinces and Bogotá[4].

Study group

The study group used for the ENSIN 2010 is probabilistic and representative of the Colombian population, which required a complex composition process and in which stratified multi-staged cluster samples were used[4]. The segments were selected systematically with an interval consistent of the segments list by provinces and municipality of the DHS to ensure coverage of the 258 Primary Sampling Units from 2010 in urban and rural areas across the entire Colombian territory, apart from the rural part of the Amazon and Orinoquia, which represents less than 1% of the population and which is scattered across 500,000 km^2[4].

Selection of variables

The subjects chosen for the ENSIN 2010 survey were aged between 18 and 64 years, to whom the international physical activity questionnaire (IPAQ)[10] was applied, and they were asked about their self-perception of their health status (in the DHS)[3]. Other variables were also included, which could be related to these. The files with the corresponding information from the two surveys were provided by the Management Assessment of the *Asociación Pro-bienestar de la Familia Colombiana (Profamilia)* for DHS[3], and by the Colombian Family Welfare Institute (ICBF) for the ENSIN[4]. Once the confidentiality agreements were signed, these entities provided the keys required to access, on a maximum of two occasions, the database download of the surveys. A final file comprised 6,241 men (43.15%) and 8,224 women (56.85%).

The physical activity variables were handled in accordance with the guidelines established in the IPAQ analysis guide[11] and the amount of leisure time was taken into consideration, in accordance with the recommendations for the Latin American context[1].

Statistical analysis

A logistic regression model was applied, recoding the variable depending on the self-perception of health in excellent, very good and good as 1, and average/poor as 0 in men and women. The model was adjusted by age group, physical activity, schooling, affiliation to the social security healthcare system, body mass index, risk of obesity, means of transport, socio-economic level, and civil status. An assessment by confidence intervals at 95% was taken into account to establish significance between the variables. The models were carried out using the IBM SPSS version 20 software.

Results

The frequency at which individuals reported to have a good self-perceived state of health is similar among men and women (53.6% and 52.7% respectively). However, upon adding the positive perceptions (good, very good and excellent state of health), the percentage obtained from men is 77.1% and 68.6% from women ($p < 0,0001$).

In terms of physical activity, the majority of both men and women do not carry out the minimum amount required to maintain a healthy lifestyle – 150 minutes a week of preferably aerobic activity[12] - with women being less active 93.7% compared to 87.8% of men. In terms of schooling, the differences begin to emerge from secondary level, with the most advantaged group in this category being men.

In the body mass index (BMI) classification, men have proportionally more excess weight, 36.8% compared to 35.1% of women, but in obesity this situation inverts, with the larger proportion being women, with 21.4% compared to 14.5% of men. In abdominal obesity the situation is similar to that of BMI, in the first levels (abdominal obesity and high risk of coronary disease) the prevalence among men is greater, but at the highest level (very high risk of coronary disease), women hold the largest proportion, with 37.3% compared to 14.4% of men.

The distribution in terms of geographical regions was similar between men and women, apart from in the central region, in which there is a larger percentage of men than women (28.3% and 26.4% respectively; $p = 0.012$) and in national territories, in which there is a larger proportion of men (14.5% and 12% respectively; $p < 0.001$) (Table 1).

Tables 2 and 3 display the raw and adjusted analysis for an average and poor self-perception of health status. In this model for the results from 2010, the amount of adjustment variables included is greater than in a previous study for results from 2005[13].

When all the variables mentioned in Table 2 are adjusted, women present a greater OR for average and poor self-perception of health status, if their leisure time physical activity is low (OR: 1.30 CI95%: 1.03 – 1.62). This trend was not observed among men in the adjusted analysis (OR: 1.04 CI95%: 0.85 – 1.29) (Table 3).

Figure 1 displays some of the variables that are significantly associated with an average and poor self-perception of health. It reveals that the most strongly associated variables are not having a level of schooling among women, and being over 50 years among men.

Discussion

This study used information from 14,465 people aged between 18 and 64 years, chosen in the 2010 Colombian ENSIN and DHS demographic studies. The results reveal that low levels of leisure time physical activity are associated with a poor and average self-perception of health among the group of women, whilst this relationship was not found among the group of men.

Leisure time physical activity is starting to become an important way of promoting people's health[14]. The health-related benefits associated with physical activity have led to the understanding that there is a positive relationship with other predictive indicators of good health, such as the self-perception of state of health[9,14,15]. However, in this study this association in men has not been found, with this result reiterated with results obtained from 2005[13].

Previously it has been demonstrated that there is a relationship between performing physical activity and the self-perception of the state of health as positive (excellent, very good and good) [9,14,19]. Particularly noteworthy is that in this study no such evidence was found in the group of men (OR = 1.04, CI95% 0.85-1.29; Table 3), despite this demographic performing proportionally more physical exercise in their leisure time than women (13% compared to 5.8% respectively). It is necessary to investigate and adjust other variables that could influence these results, such as the consumption of alcohol, smoking and dietary habits, which were not included in the analysis either because these variables were not included in the national survey or because they were not obtained for all the subjects from the analysed study group. With regard to this, a study on a demographic from the south of Brazil discovered associations between the consumption of fruit and physical activity with a positive self-perception of health status, whilst the use of tobacco and alcohol abuse did not reveal this relationship[20].

One recommendation for future demographic surveys in Colombia is to include questions regarding the consumption of alcohol in men and women, both adults and adolescents, and regarding the issue of current smoking habits, which is mainly asked of women; this should also be asked of men.

Schooling and economic income levels have revealed connections with positive or negative self-perceptions of health. Previous research has shown that a high level of schooling and better income favour positive self-perceptions of health[6,13,21]. This aspect was consistent with the results of this study in both sexes, and although inquiries were not made into economic income, the fact that individuals were asked about their social-economic level by asking if they owned a home, a bicycle, a motorbike or a car, is a way of categorising the income levels of the Colombian demographic. These variables were entered into the models with the aim of perceiving differences in the self-perception of health, if they got about by bicycle, compared to the other means of transport that did not involve physical effort, but given the specific conditions of a developing country, the fact that a person owns any of these items means that they have the necessary income required to acquire and maintain this kind of vehicle.

Both women and men that were affiliated to a healthcare system perceived their health positively. In the data from the 2005 demographic surveys, this relationship is only found in women[13], whilst a study carried out with a sample group of inhabitants of the city of Bogotá in 2014, which was adjusted by demographic variables of inequality and education, also did not find this connection[7].

Upon analysing the relationship between BMI and the self-perception of health, no association was found in either of the sexes, whilst a study carried out using data from different countries did find this relationship and describes a U-shaped behaviour[22].

In terms of civil status, the results found, particularly among the group of women, were contradictory compared to those found previously in some studies[23,24], as being married is a favourable condition for self-perceiving health as good in countries such as Sweden and Spain. For Colombian women in 2010, being married was a risk factor (OR = 1.16; CI95% 1.00-1.35) for assessing their health as average or poor, whilst no association at all was discovered for men. On the other hand, these results are similar to those found by Onadja, in Ouagadougou, the capital

Table 1. Characteristics of the study demographic of the variables selected from the ENSIN and DHS 2010.

Variables	Women N = 8.224 (56,85%)	Men N = 6.241 (43,15%)	p
Age group			
18 – 29	2679 (32.6%)	2149 (34.4%)	0.019
30 – 49	3741 (45.5%)	2790 (44.7%)	0.356
50 – 64	1804 (21.9%)	1302 (20.9%)	0.124
State of health			
Excellent	604 (7.3%)	755 (12.1%)	<0.001
Very good	709 (8.6%)	713 (11.4%)	<0.001
Good	4333 (52.7%)	3345 (53.6%)	0.284
Average	2403 (29.2%)	1346 (21.6%)	<0.001
Poor	175 (2.1%)	82 (1.3%)	<0.001
Physical activity			
Low	7702 (93.7%)	5477 (87.8%)	<0.001
Moderate	390 (4.7%)	434 (7.0%)	<0.001
Vigorous	132 (1.6%)	330 (5.3%)	<0.001
Level of Schooling			
None/Not known	272 (3.3%)	223 (3.6%)	0.409
Pre/Primary	2223 (27.0%)	1671 (26.8%)	0.745
Secondary	3734 (45.4%)	2957 (47.4%)	0.019
Tech./University	1995 (24.3%)	2390 (22.3%)	<0.001
Affiliated to a healthcare system			
Yes	7354 (89.4%)	5272(84.5%)	<0.001
No/Not known	870 (10.6%)	969 (15.5%)	<0.001
BMI classification	n = 7864	n = 5676	
Thinness	261 (3.3%)	146 (2.6%)	0.013
Normal	3164 (40.2%)	2615 (46.1%)	<0.001
Excess weight	2759 (35.1%)	2091 (36.8%)	0.037
Obesity	1680 (21.4%)	824 (14.5%)	<0.001
Abdominal obesity	n = 7614	n = 5608	
Abdominal obesity	2902 (38.1%)	3080 (54.9%)	<0.001
High risk of CD +	1875 (24.6%)	1722 (30.7%)	<0.001
Very high risk of CD +	2837 (37.3%)	806 (14.4%)	<0.001
Owns a motorbike	n = 8224	n = 6241	
Yes	2168 (26.4%)	1887 (30.2%)	<0.001
No	6056 (73.6%)	4354 (69.8%)	<0.001
Owns a car	n = 8224	n = 6241	
Yes	1000 (12.2%)	808 (12.9%)	0.163
No	7224 (87.8%)	5433 (87.1%)	0.163
Owns a bicycle	n = 8224	n = 6241	
Yes	3090 (37.6%)	2535 (40.6%)	<0.001
No	5134 (62.4%)	3706 (59.4%)	<0.001
Socio-economic level	n = 8224	n = 6241	
From 0 to 3	7842 (95.4%)	6003 (96.2%)	0.016
From 4 to 6	382 (4.6%)	238 (3.8%)	0.016
Civil status	n = 8224	n = 6241	
Married/Free union	4620 (56.2%)	3811 (61.1%)	<0.001
Separated/Widowed	1758 (21.4%)	570 (9.1%)	<0.001
Single (a)/Not known	1846 (22.4%)	1860 (29.8%)	<0.001
Area			
Municipal capital	8224 (100%)	6241 (100%)	<0.001
Rest (town)			
Rest (disperse)			
Regions			
Atlantic	1770 (21.5%)	1408 (22.6%)	0.140
East	1217 (14.8%)	893 (14.3%)	0.422
Central	2328 (28.3%)	1649 (26.4%)	0.012
Pacific	1172 (14.3%)	843 (13.5%)	0.209
Bogotá	754 (9.2%)	541 (8.7%)	0.310
National territories	983 (12%)	907 (14.5%)	<0.001

+CD: coronary disease.

Table 2. Odds ratio (OR) and its confidence interval at 95% for average and poor self-perception of health status regarding all the characteristics considered for this study on women, ENSIN 2010 and DHS 2010.

Variables	n (%)	OR (IC 95%)	
		Raw	Adjusted*
Age group			
18 – 29	520 (19.4%)	1	1
30 – 49	1242 (33.2%)	2.06 (1.83 – 2.32)	1.68 (1.46 – 1.92)
50 – 64	816 (45.2%)	3.42 (3.00 – 3.91)	2.32 (1.96 – 2.74)
Physical activity			
Low	2453 (31.8%)	1.48 (1.20 – 1.82)	1.30 (1.03 – 1.62)
Moderate to vigorous	125 (23.9%)	1	1
Level of schooling			
None/Not known	138 (50.7%)	5.47 (4.19 – 7.14)	2.77 (2.06 – 3.72)
Pre/Primary	1036 (46.6%)	4.63 (4.00 – 5.36)	2.70 (2.29 – 3.19)
Secondary	1088 (29.1%)	2.18 (1.90 – 2.51)	1.70 (1.46 – 1.98)
Tech./University	316 (15.8%)	1	1
BMI classification			
Thinness	79 (30.3%)	0.65 (0.49 – 0.86)	1.28 (0.89 – 1.82)
Normal	827 (26.1%)	0.53 (0.46 – 0.60)	0.91 (0.74 – 1.13)
Excess weight	887 (32.1%)	0.71 (0.62 – 0.80)	0.88 (0.76 - 1.03)
Obesity	672 (40.0%)	1	1
Abdominal obesity			
Abdominal obesity	715 (24.6%)	1	1
High risk of CD ++	563 (30.0%)	1.31 (1.15 – 1.49)	1.02 (0.87 – 1.21)
Very high risk of CD ++	1099 (38.7%)	1.93 (1.72 – 2.16)	1.19 (0.97 – 1.46)
Owns a motorbike			
Yes	594 (27.4%)	1	1
No	1984 (32.8%)	1.29 (1.15 – 1.43)	1.20 (1.06 – 1.36)
Owns a car			
Yes	198 (19.8%)	1	1
No	2380 (32.9%)	1.99 (1.69 – 2.34)	1.46 (1.21 – 1.76)
Owns a bicycle			
Yes	851 (27.5%)	1	1
No	1727 (33.6%)	1.33 (1.20 – 1.47)	1.21 (1.08 – 1.34)
Socio-economic level			
From 0 to 3	2520 (32.1%)	2.64 (1.99 – 3.51)	1.98 (1.42 – 2.77)
From 4 to 6	58 (15.2%)	1	1
Civil status			
Married/Free union	1524 (33.0%)	1.78 (1.57 – 2.02)	1.16 (1.00 – 1.35)
Separated/Widowed	655 (37.3%)	2.15 (1.85 – 2.49)	1.07 (0.90 – 1.28)
Single (a)/Not known	399 (21.6)	1	1
Affiliated to a healthcare system			
Yes	2297 (31.2%)	1	
No/Not known	281 (32.3%)	1.05 (0.90 – 1.22)	1.22 (1.03 – 1.44)
Regions			
Atlantic	581 (32.8%)	1.01 (0.85 – 1.19)	0.96 (0.80 – 1.15)
East	358 (29.4%)	0.86 (0.72 – 1.03)	0.81 (0.66 – 0.99)
Central	738 (31.7%)	0.96 (0.82 – 1.12)	0.90 (0.76 – 1.08)
Pacific	406 (34.6%)	1.09 (0.91 – 1.31)	1.07 (0.88 – 1.31)
Bogotá	175 (23.2%)	0.62 (0.50 – 0.77)	0.67 (0.53 – 0.85)
National territories	320 (32.6%)	1	1

*The logistics regression model is adjusted by the variables of age group, physical activity, level of schooling, affiliation to a healthcare system, area, regions, body mass index (BMI) classification, abdominal obesity, owning a motorbike, car or bicycle, socio-economic level and civil status.
++CD: Coronary disease.

of Burkina Faso[18], whereby being single did not reveal any connection, with adjustment of the other variables (OR = 0.87; CI95% 0.62-1.21).

Some limitations of this study are related to the use of self-declared information from the study subjects, such as, for example, the self-perception of health, which can lead to bias[24]. However, strong links have been mentioned between this indicator and the prediction of mortality, level of education, age and physical activity, among others[8,14-18]. Despite some of these links not appearing in the male study group, the

Table 3. Odds ratio (OR) and its confidence interval at 95% for average and poor self-perception of health status regarding all the characteristics considered for this study on men, ENSIN 2010 and DHS 2010.

Variables	n (%)	OR (IC 95%)	
		Raw	Adjusted*
Age group			
18 – 29	318 (14.8%)	1	1
30 – 49	638 (22.9%)	1.70 (1.47 – 1.98)	1.61 (1.34 – 1.95)
50 – 64	472 (36.3%)	3.27 (2.77 – 3.85)	2.83 (2.27 – 3.52)
Physical activity			
Low	1281 (23.4%)	1.28 (1.05 – 1.55)	1.04 (0.85 – 1.29)
Moderate to vigorous	147 (19.2%)	1	1
Level of schooling			
None/Not known	87 (39.0%)	4.35 (3.18 – 5.94)	2.34 (1.64 – 3.33)
Pre/Primary	551 (33.0%)	3.35 (2.77 – 4.04)	2.08 (1.68 – 2.58)
Secondary	612 (20.7%)	1.77 (1.48 – 2.12)	1.43 (1.17 – 1.74)
Tech./University	178 (12.8%)	1	1
BMI classification			
Thinness	24 (16.4%)	0.63 (0.39 – 1.01)	0.95 (0.54 – 1.65)
Normal	565 (21.6%)	0.88 (0.73 – 1.07)	1.08 (0.78 – 1.50)
Excess weight	492 (23.5%)	0.99 (0.82 – 1.20)	1.09 (0.84 – 1.43)
Obesity	195 (23.7%)	1	1
Abdominal obesity			
Abdominal obesity	630 (20.5%)	1	1
High risk of CD ++	409 (23.8%)	1.21 (1.05 – 1.39)	1.00 (0.81 – 1.23)
Very high risk of CD++	213 (26.4%)	1.39 (1.16 – 1.67)	1.20 (0.87 – 1.65)
Owns a motorbike			
Yes	354 (18.8%)	1	1
No	1074 (24.7%)	1.41 (1.23 – 1.62)	1.40 (1.20 – 1.63)
Owns a car			
Yes	127 (15.7%)	1	1
No	1301 (23.9%)	1.68 (1.38 – 2.06)	1.40 (1.11 – 1.78)
Owns a bicycle			
Yes	522 (20.6%)	1	1
No	906 (24.4%)	1.24 (1.10 – 1.41)	1.23 (1.07 – 1.40)
Socio-economic level			
From 0 to 3	1403 (23.4%)	2.59 (1.71 – 3.95)	2.16 (1.31 – 3.55)
From 4 to 6	25 (10.5%)	1	1
Civil status			
Married/Free union	961 (25.2%)	1.59 (1.38 – 1.83)	1.03 (0.86 – 1.24)
Separated/Widowed	143 (25.1%)	1.58 (1.26 – 1.98)	0.85 (0.65 – 1.11)
Single (a)/Not known	324 (17.4%)	1	1
Affiliated to a healthcare system			
Yes	1192 (22.6%)	1	1
No/Not known	236 (24.4%)	1.10 (0.93 – 1.29)	1.22 (1.02 – 1.46)
Regions			
Atlantic	345 (24.5%)	0.84 (0.69 – 1.01)	0.77 (0.62 – 0.96)
East	213 (23.9%)	0.81 (0.66 – 1.00)	0.81 (0.64 – 1.03)
Central	350 (21.2%)	0.70 (0.58 – 0.84)	0.63 (0.51 – 0.78)
Pacific	179 (21.2%)	0.70 (0.56 – 0.87)	0.76 (0.60 – 0.98)
Bogotá	89 (16.5%)	0.51 (0.39 – 0.67)	0.55 (0.41 – 0.75)
National territories	252 (27.8)	1	1

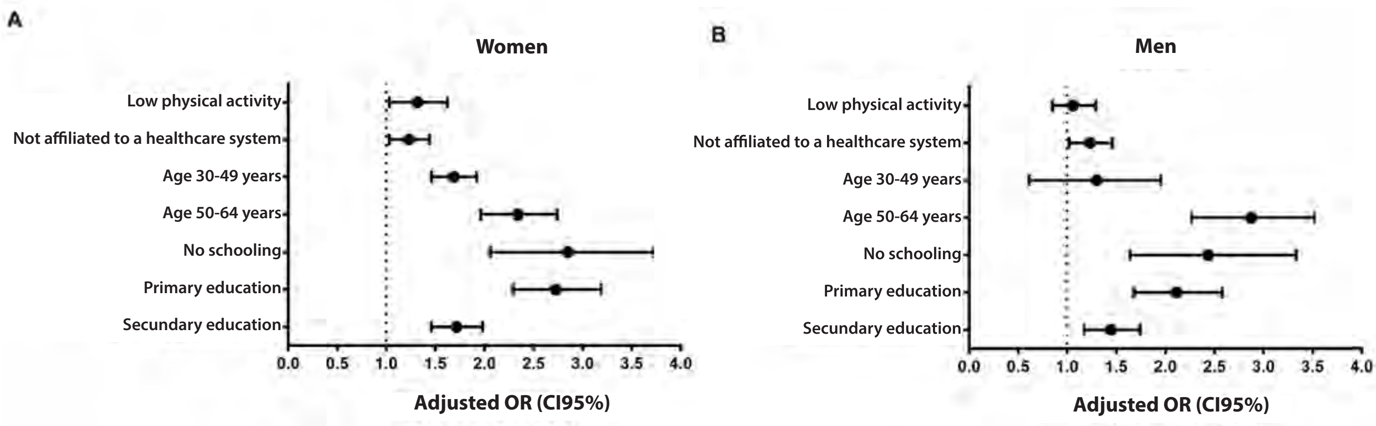
*The logistics regression model is adjusted by the variables of age group, physical activity, level of schooling, affiliation to a healthcare system, area, regions, body mass index (BMI) classification, abdominal obesity, owning a motorbike, car or bicycle, socio-economic level and civil status.
++CD: Coronary disease.

results allow us to understand the phenomena that are inherent to the Colombian population and that should be taken into considerations when implementing policies, plans and programmes related to the promotion of healthy lifestyles.

Conclusions

In accordance with the information from the 2010 Colombian national demographic and health surveys, among women physical

Figure 1. Graphic comparison of the degree of association of some variables with an average and poor self-perception of health in women (A) and in men (B).



inactivity is linked to an average and poor self-perception of health after adjustment for confounding factors. Age, the level of schooling, affiliation to a healthcare system, civil status, living in the eastern region and in Bogotá also influenced the self-perception of health among women. In contrast, among men there was no indication that physical inactivity is linked to an average or poor self-perception of health, but a link was found with age and low levels of schooling. From the aforementioned, it can be observed that the factors associated with the self-perception of health can be different between men and women, therefore health interventions aimed at improving this indicator should be different and specific.

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Original article

Changes in the soleus muscular tissue of rats with experimental periodontitis under physical exercise influences

Lidiane Ura Afonso Brandão, Bruna Martinazzo Bortolini, Pedro Henrique de Carli Rodrigues, Ana Claudia Paiva Alegre-Maller, Christian Giampietro Brandão, Lucinéia de Fátima Chasko Ribeiro, Rose Meire Costa Brancalhão, Gladson Ricardo Flor Bertolini, Danielle Shima Luize, Marcela Aparecida Leite, Carlos Augusto Nassar, Patricia Oehlmeyer Nassar

State University of West Parana (UNIOESTE), Cascavel, Parana, Brazil.

Received: 30.11.2016
Accepted: 23.03.2017

Summary

Until the moment, no study explored conjunctively the physical activity relation, using the inflammatory biomarkers, with the periodontitis. This way, the objective of this experiment was to evaluate the muscular tissue behavior of rats submitted to physical exercise in aquatic environment with experimental disease. Twenty-four male Wistar rats were divided in 4 groups: 1) control and sedentary (CS); 2) control and active (CA); 3) with the periodontal disease and sedentary (PDS); with the periodontal disease and active (PDA). On the group that the periodontitis was induced, it was for ligature and the groups with active swimming activity have practiced it for 4 weeks. At the end of 30 days the animals were euthanized and a portion of the gingival tissue and the soleus muscle were removed and underwent analysis by ELISA and morphological and morphometrical analysis of the muscle. Data drawn from the analysis was analyzed through ANOVA and Tukey. Results have shown that there is a higher expression of TNF-α in the gingival tissue and on the muscular tissue of the rats that underwent the induced periodontitis independently of the physical activity (PDS and PDA), as a meaningful decrease on the conjunctive tissue on the groups with induced periodontitis, that have or have not undergone active swimming activity which could suggest a predisposition to muscular injury or difficulty of muscular recovering on these groups. Therefore, it was possible to highlight a correlation between the periodontal disease and the muscle morphological changes, and, moreover, the physical swimming activity promoting an acceleration of the regeneration of the muscle tissue.

Key words:
Physical activity.
Periodontitis.
Soleus muscle.

Cambios en el tejido muscular del sóleo de las ratas con periodontitis experimental bajo influencia del ejercicio físico

Resumen

Hasta este momento, ningún estudio ha explorado conjuntamente la relación de la actividad física con la periodontitis utilizando biomarcadores de inflamación. Siendo así, el objetivo de este estudio fue evaluar el comportamiento del tejido muscular de ratas con periodontitis experimental al realizar ejercicio físico en medio acuático. Veinticuatro ratas Wistar machos fueron divididas en cuatro grupos: 1) control y sedentario (CS); 2) control y ejercicio (CA); 3) con enfermedad periodontal y sedentario (PDS); 4) con enfermedad periodontal y ejercicio (PDA). En los grupos con periodontitis, la enfermedad periodontal fue inducida por ligadura y los grupos con ejercicio realizaron natación durante cuatro semanas. A los treinta días, los animales fueron sacrificados y una parte del tejido de las encías y del músculo soleo se resecaron y utilizaron para análisis con ELISA y para análisis morfológicos y morfométricos. Los datos obtenidos fueron analizados y evaluados a través de los tests ANOVA y TUKEY. Los datos mostraron una mayor expresión de TNF-α tanto en el tejido de las encías como en el tejido muscular de los ratones sometidos a periodontitis inducida independiente del ejercicio físico (PDS y PDA). Se percibió también una disminución significativa en el tejido conjuntivo en los grupos con periodontitis inducida sometidos o no al ejercicio de natación, lo que podría sugerir una predisposición a lesión muscular o una dificultad en la reparación de las lesiones musculares de esos grupos. Por lo tanto, fue posible destacar una correlación entre la enfermedad periodontal y los cambios morfológicos musculares y, además, que la actividad física de natación favoreció una aceleración de la regeneración del tejido muscular.

Palabras clave:
Esfuerzo físico.
Periodontitis.
Músculo soleo.

This paper was supported by the scholarship CAPES B. S.; UNIOESTE.

Correspondence: Patricia Oehlmeyer Nassar
E-mail: ponassar@yahoo.com

Introduction

Physical activity regularly practiced leads to the physiological and morphological adaptation, relevant to the maintenance of the organism homeostasis and these changes influenced on many diseases control, especially those of cardiovascular nature and endocrine-metabolic. This way, several studies have shown that the physical activity is capable to promote changes in various functions of the human organism and of rats[1,2]. Besides, they are capable to promote the acceleration of the process of the inflammation recovery[2,3]. Under this sense, some studies pinpointed that the exercises infer on several stages of the inflammatory process, promoting migration of the leukocytes to the direction of the inflammation focus (chemiotaxis) and the increase of the capacity of phagocytosis of this cells in human beings and animals, besides increasing the antitumor macrophage activity[2,4,5].

The inflammation is an adaptive response, which is broken by stimulus and harmful conditions, such as the infection and the tissue injury. A considered process has been reached on the comprehension of the cells and molecular events, which are involved on the acute inflammatory response and, in a lower scale, the tissue aggression. Still, events that have broken the located chronical inflammation, especially the chronical infections and autoimmune diseases, are partially understood. Little is known, however, about its causes and mechanisms of systemic chronical disease, which occurs in a several variety of diseases, including the diabetes type 2 and cardiovascular diseases. These inflammatory chronical states seem not to be caused by the classical inflammation causes: Infection and aggression. Instead, they seem to be associated with the bad-function of the tissue: a lack of balance of the homeostatic of one of the physiological system, which does not have direct relation to the function of the host defense or tissue repair[6].

The inflammatory response to an infection, while aiming at recovering the homeostasis, can turn out to be harmful if excessive or not balanced. Under these conditions, inflammation plays an essential role in the pathogeneses of many chronical diseases, including periodontitis[6].

Periodontitis is a chronic inflammatory disease triggered by a pathogenic microbe organized by a biofilm that results on the selective destruction of the periodontal tissue that can cause the loss of the tooth. The destruction of the periodontal tissue, however, may not be linear. Periodontitis is actually characterized on alternate phases of the exacerbation and remission of the disease, as well as on the inactivity period. Under this context, it is possible that the cytokines that directs the cellular response during the overlapped inflammatory and healing of the tissues phase that can interact in different levels. Little is known, however, about the interaction between the cytokines and the growth factors that frame the inflammation and the tissue recovering[7].

Tumoral Necrosis Factor-α (TNF-α) is an important inflammatory cytokine expressed during the inflammatory phase of tissue healing and in chronic inflammatory conditions, such as the periodontal disease. The TNF-α inhibits the synthesis of the Extracellular Protein Matrix (EPM) and the active production of the matrix metalloproteinases. Studies have shown that the TNF-α can affect the antagonist upon several induced answers or stimulated by the transforming growth factor of β1 (TGF-β1), such as, the synthesis of the collagen type I and III, and the expression of α- de smooth muscle actin (α-SMA) in fibroblasts of skin and the production of tropo-elastin in fibroblasts on the rats lungs[7].

Epidemiological data suggests that the periodontal diseases can be associated with other systemic pathological conditions[8-10]. Besides that, it is possible to find some evidences of infections caused by odontogenesis origin that has a relation with the damage development caused by the exacerbation and immunological response, which is willing to generate destructions and changes on the expression of myosin[11-13].

Considering that the same proinflammatory biomarkers are involved in both periodontal disease and muscle metabolism, it is plausible that the systemic challenge generated by periodontal disease could also influence physical fitness[2,5].

Although it is possible that the physical activity protects the periodontal area due to the fact that it attenuates the excessive inflammatory response on the host, there are some evidences on the longitudinal studies and a prospective study showing that the adults physically active have lowered the risk of periodontitis. Until the moment, no study has explored conjunctively the relation between the physical activities, using the inflammatory biomarkers, with the periodontitis[14].

Therefore, the objective was to evaluate the muscular tissue behavior of rats submitted to physical exercise in aquatic environment with experimental periodontitis.

Material and method

Obtaining the animals

There were used 24 Wistar Rats weighting an average of 100 g, from the Unioeste central animal research area. These animals were maintained on controlled thermic conditions (23 ± 2º C) and light (cycle of 12 hours light and 12 hours dark – 07:00 A.M – 7:00 P.M) and received water and commercial food all the time. The experimental protocols were approved by the Ethical Committee in Animal Experiment and Practical Classes of UNIOESTE.

Experimental Groups

Animals were divided randomly in 4 groups of 6 animals each, according to Felipetti, *et al*., 2014[15].

- Group (CS): control and sedentary
- Group (CA): control and active
- Group (PDS): with periodontal disease and sedentary
- Group (PDA): with periodontal disease and active

Inducing of the Periodontal Disease

Animals received anesthesia (xylazine 0,04 mL/100 g and ketamine 0,08 mL/100 g), and placed on a proper operatory table, which allowed a maintenance of the buccal opening of the rats making it easier to have access upon their teeth on the posterior jaw region. With the support of a modified pinch and an explorer probe, cotton ligatures number 40 were placed around the lower right first molar. This ligature acted irritating the gingival margin for 30 days, provoking accumulation of bacterial plaque and consequently development of the periodontal disease[16].

Protocol of Aerobic Activity

Previously to inducing the protocol of the periodontal disease on animals of CA and PDA went through a practice of familiarizing on the swimming activity, initiated one week before, with the duration of 15 minutes, 3 times a week.

Two days after inducing the DP, the groups have done an aerobic activity similar to swimming, during 4 weeks, with progressive intensifying over time, from 15 minutes on the first week, 30 minutes on the second, and successively up until reaching 60 minutes on the fourth week, daily with the break of 2 days between the beginnings of the week. The place used was an oval tank, with capacity of 200 L, depth of 60 cm and controlled temperature of 32º ± 1º C[17].

Analysis of Tumoral Necrosis -α Expression (TNF-α)

By the end of the activity period, all the animals were weighted and anaesthetized with ketamine (50 mg/Kg) and xylazine (10 mg/Kg) and beheaded on a guillotine. A portion of the gingival tissue around the teeth submitted or not to the use of the ligature and the soleus muscle, all the experimental groups were removed and used for the by Enzyme-Linked Immunosorbent Assay (ELISA) analysis on the presence of TNF-α cytokine. The total of proteins was extracted from samples of the gingival tissue and the soleus muscle using an extraction buffer to the detergent basis (T-PER, Tissue Protein Extraction Reagent - Pierce), with a cocktail of proteasis inhibitor (Protein Stabilizing Cocktail - Santa Cruz Biotecnology), according to the manufacturer instructions.

The samples were macerated on the buffer (50 µL/mg of tissue) and centrifuged for 5 min to 13.000 rpm to 4 0C. The overflown contend was quantified using a system of Bradford protein (Bio-Rad). As to detect and quantify the TNF-α were used 100 µL of the sample and 100 µL of the buffer in a length of wave of 450 nm according to the Kits ELISA (TNF-α ELISA Kit Biosource), accordingly to the manufacturer instruction.

Collecting the soleus muscle and the Histological Preparation

The right soleus muscle was dissected, fixed on methacarn solution (70% of methanol, 20% of chloroform, 10% of glacial acetic acid) for 24 hours and stored in 70% of alcohol. After that, it went through a process of dehydration in an increasing alcoholic series, diaphanized in xylol and embedded in paraffin. The transversal cut of 5 µm in thickness were obtained by a microatom (CUT 4055 Olympus®, Mainz, Germany) and colored with Hematoxylin and Eosin (HE) for the general analysis of muscular tissue and with Mallory Trichrome (MT) for the conjunctive tissue analysis.

Morphological and Morphometrical Analysis

The following measurement of the soleus muscle was carried: transversal section area, minor fiber muscular diameter, density of the conjunctive tissue and the evaluation of the muscular tissue.

Obtained slides were analyzed under the light of (BX60 Olympus®, Tokyo, Japan). For the measurement of the transversal section area and minor diametrical muscular fiber 10 images were randomly obtained on the objective of 40x, for each image 10 fibers were measured on the program of Image-Pro-Plus 6.0 (Media Cybernetics®, Silver Spring, USA), totalizing 100 measurements for each animal.

To determine the density of the conjunctive tissue, 10 images were randomly obtained through the objective of 40x and 10 measurements carried for each animal, endomysium and perimysium analysis, the GIMP was used (GNU Image Manipulation Program 2.0 - GNU General Public License®, Berkeley, California). The relative area of the conjunctive tissue (density area) was calculated by dividing the total of pixels of the photomicrography by the total of pixels of the conjunctive tissue highlighted.

Data analysis

The data were analyzed and evaluated using the one-way ANOVA test and, when statistically significant differences were found, the Tukey test was used to determine differences between the groups at a 5% significance level. The quantified results were presented on tables and/or in graphics, while the results about the tissue morphology were displayed in images (photomicrography) followed by their description and discussion.

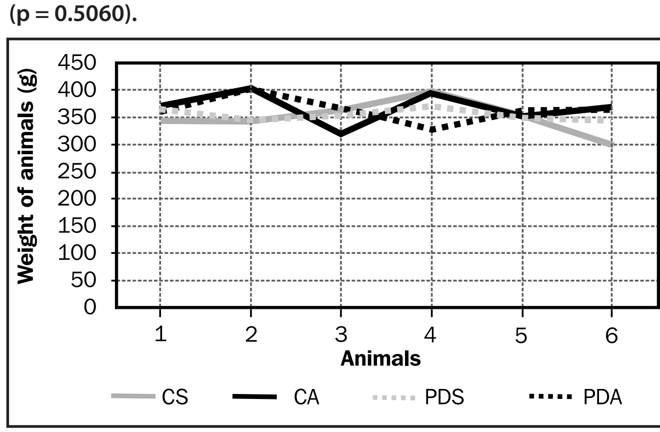
Results

Figure 1 shows the distribution of the weights of the 6 animals of each of the 4 groups. There was no statistically significant difference between the groups at the end of the experimental period (CS = 348.33 ± 30.8, CA = 368.33 ± 28.8, PDS = 353.33 ± 10.5, PDA = 363.5 ± 24.5).

It was observed in Table 1 that, in the groups with induced periodontitis (PDS and PDA), the cement-enamel junction distance was significantly increased, and in the group that performed physical activity on the aquatic environment (PDA) the bone loss was significantly lower than in the group with periodontal disease alone (PDS).

It was possible to observe through Table 2 that the TNF-α expression significantly increased on the gingival tissue happens both on the muscular tissue of rats subjected or not to the physical activity on the aquatic environment, only on the groups with induced periodontitis (PDS and PDA).

Figure 1. Distribution of animal weights of all experimental groups (p = 0.5060).



Results about the area and smaller diameter in soleus muscular fiber, does not show significant statics differences. There was, however, a tendency on the increase of the area and smaller diameter on the CA group and the area of PDA group according to Table 3.

Table 4 shows that the conjunctive is decreasing significantly on the groups of induced periodontitis, subjected or not to swimming activities.

Morphological analysis of the soleus muscle

On the control group and sedentary (CS), the soleus muscle presented an abnormal morphology; the muscular fibers have the polygonal format with nucleus on the periphery position and fascicular pattern (Figure 2A).

The animals subject to the swimming activity (CA) some fiber presented themselves slightly hypertrophic, with a bigger area when compared to the other groups (Figure 2B) and with regular morphological characteristics.

On the periodontal disease group (PDS), the soleus muscle presented morphological changes directly in some fibers. It was observed some fibers with irregular format, disorganization on the cytoskeleton myofibrils (Figure 2C and Figure 2E), with an increase on the number of peripheral nucleus, many of which presented a queued aspect (Figure 2D) and centralized nucleus (Figure 2F). On the conjunctive, it was verified an increase on the endomysium (Figure 2D) and the presence of an inflammatory infiltrate (Figure 2G).

On the periodontal disease associated with activity on the aquatic environment (PDA) was observed few signs of muscle changes, although some fibers present an irregular format with nucleus on the central position and it was not verified any changes on the endomysium, neither the presence of inflammatory infiltrate (Figure 2H).

Table 1. Values of the cement-enamel junction distance to the alveolar bone crest of the established groups.

Groups	Means
CS	47,8 ± 1,2 A
CA	48,7 ± 1,2 A
PDS	84,5 ± 1,2 C
PDA	61,7 ± 2,2 B

Note: Values represent mean ± standard deviation.
Differents letters - statistically different data among the groups (p <0.05).

Table 2. Values of TNF-α expression of all analyzed groups. Values are expressed in pg/ml.

Groups	Gingival tissue	Muscle tissue
CS	4,97 ± 0,17A	3,38 ± 0,88A
CA	4,60 ± 0,43A	3,52 ± 0,59A
PDS	5,35 ± 0,26B	4,39 ± 0,40B
PDA	5,67 ± 0,74B	4,53 ± 0,94B

Note: Values represent mean ± standard deviation.
Differents letters - statistically different data among the groups (p<0.05).

Table 3. Values of area and smaller diameter of muscle tissue of all established groups.

Groups	Area (μm²)	Smaller diameter (μm)
CS	2860,2 ± 210,6	43,1 ± 1,1
CA	3337,7 ± 157,7	45,8 ± 1,5
PDS	2945,2 ± 65,9	40,2 ± 1,5
PDA	3040,4 ± 136,8	43,9 ± 1,9

Note: Values represent mean ± standard deviation.
Differents letters - statistically different data among the groups (p<0.05).

Table 4. Values of conjunctive tissue of all established groups.

Groups	Conjunctive tissue (%)
CS	10,0 ± 3,6 A
CA	6,3 ± 3,1 AB
PDS	3,4 ± 0,5 B
PDA	4,6 ± 1,6 B

Note: Values represent mean ± standard deviation
Differents letters - statistically different data among the groups (p<0.05).

Discussion

The regular physical activity can offer a behavior strategy to limit the inflammation[14]. There is an increasing evidence that, additionally to other benefits, physical activity has an anti-inflammatory effect. The National Health and Nutrition Examination Survey, (NHANES III) verified that the higher the frequency of activity the lesser possibility to increase the C-reactive (PCR) protein and the counting of white cells on the blood[18]. On the other hand, the inverse association between physical activity and several inflammatory biomarkers, including the PCR, were found even in low intensity of activities[19]. Until now, there is no study has explored conjunctively the relation of physical activity with the inflammatory biomarkers during the periodontitis. This was the aim of this study was to evaluate the muscular tissue comportment of rats subjected to experimental periodontitis associated to physical activity.

It was possible to observe that the bone loss evaluated from the distance from the cementum-enamel junction to the alveolar bone crest was significantly increased only in the groups with induced periodontitis (DPS and DPE), demonstrating the efficacy of the experimental periodontal disease induction model (Table 1).

Moreover, it was possible to observe through table 1 that the expression of TNF-α is significantly increased, on the gingival tissue as well as on the muscular tissue of the rats subjected or not to the physical activity, only on groups with induced periodontitis, in accordance to the Arancibia, *et al*. (2013)[7], who reports that the production of TNF-α e TGF-β is increased under inflammatory conditions and could suggest a predisposition to muscular injury or to difficulty to muscular recovering, once the TNF-α inhibits several cellular responses induced by the TGF-β1, including the differentiation of the microfiber blasts, with the activation via Smad signalization (protein involved on inducing the α-SMA under stimulation of TGF-β1) and the production of key-molecules involved on the recovering tissue, such as collagen type 1, fibronectin (FN) and periostin.

Similarly, as to quantified conjunctive tissue (Table 4) it was possible to observe that it decreases significantly on the groups subjected to the experimental periodontitis, independently of physical activity when compared to the controlled group. During the process of wound healing, the initial inflammatory stage is critical to the efficient healing, due to the fact that it promotes the recruitment of phagocytic cells to extinguish the pathogens which could have entered the tissues after reviling. Although the inflammation could delay the process of healing or the wounds or take it to develop an aberrant, granulate formation

Figure 2. Photomicrography of the soleus muscle on the Wistar rats, transversal cut, colored HE (A, B, C, D, F, G, H) and Mallory Trichrome (E). (A), group controlled (CS); (B), group swimming activity (CA); (C, D, E, F e G), Periodontal Disease group (PDS) and (H), Periodontal Disease associated with physical activity (PDA). In A, highlighting the muscle fibers with a polygonal format (F), peripheral nucleus (Np) and blood capillaries (Cs). In B, hypertrophied muscular fibers (F) preserving the structure and muscular organization. In C and E, disorganized presence of myofibrils (Dm). D, increase on the number of peripheral nucleus that presented themselves queued (circle). In F central nucleus (Nu) and G focus on the inflammatory infiltrated (If). In H, pointing out the morphological improvement, but still with the presence of central nucleus on some muscle fibers, with no characteristic format (Nu).

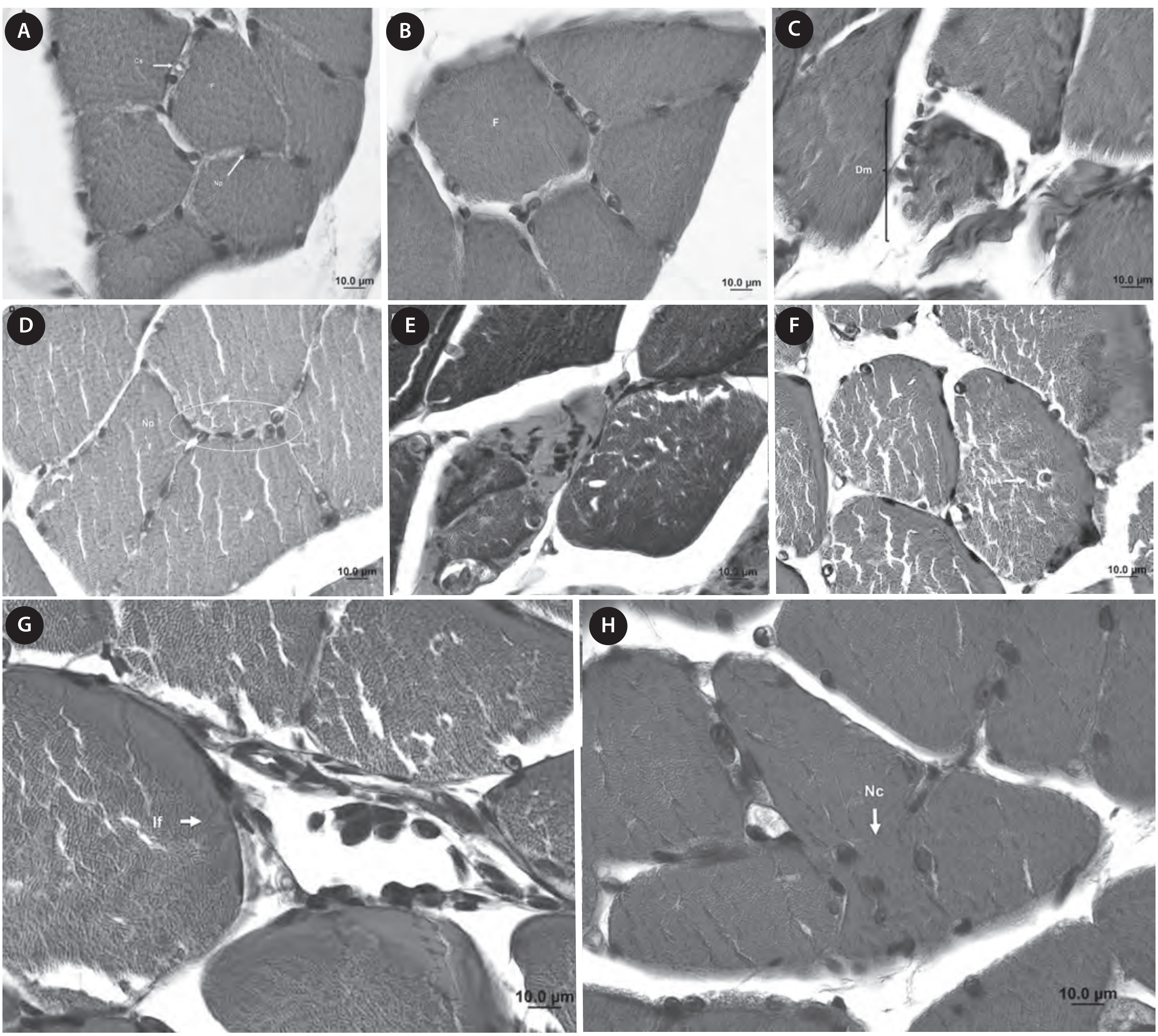
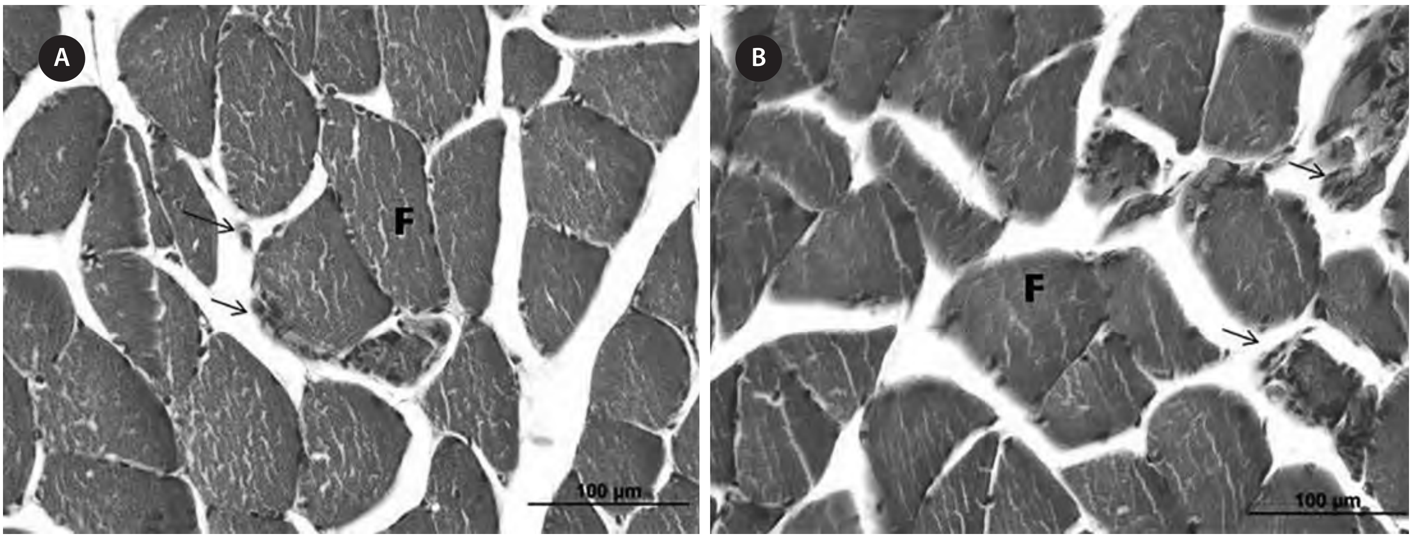


Figure 3. Photomicrography of the soleus muscle on the Wistar rats, colored HE. A – Periodontal Disease group (PDS) and B – Periodontal Disease associated with physical activity (PDA). muscle fibers (F) and inflammatory infiltrated (arrow).



of the tissue. Periodontal disease is considered a chronic inflammatory condition that destroys the tissues and could be prolonged on periods of quiescent or repair. Periodontal inflammation and the recovering of the tissue could actively interact, however, during the natural development of the periodontitis[7].

Morphological data showed that the animals on the group CS presented muscular fibers with regular aspects, as well as the activity group (Figure 2A and Figure 2B)[20,21]. Although the literature brings the swimming activity as to promote morphometric changes, as the increase on the area and the transversal section in muscular fibers[22-24], on this study the used protocol was not able to change significantly this parameters (Table 3). As Oliveira, *et al* (2014)[25] observed on his study, the animals were subjected to swimming activity of high intensity and they did not show morphometric changes on the muscular fibers. The same occurred with the weight of the animals, which corroborated with the same study by Oliveira, *et al*[25], there was no significant alteration between the groups (Figure 1).

Opting for the swimming category is explained because this activity represents an aerobic activity that uses most of the articulation of the body as well as the use of muscles like the soleus, once the animals have to perform an plantar flexion to maintain themselves on the water surface[25], besides the increase on the cardiac and respiratory frequency, due to the viscosity property which offers resistance to movements in any direction, contributing to the muscular resistance training[17]. On the DP group, several morphological detrimental characteristics were found (Figure 2C and Figure 2F). The presence of many big nucleus, as on the periphery as well as centralized on the muscular fibers, presented signs of muscular degeneration, after having suffered changes from the systemic inflammatory process caused by the DP. According to Karalaki, *et al*. (2009)[26], plasticity of the muscular tissue in response to an injury depends on, among other, the functional satellite cells' role. These are on the healthy muscle and are found on the quiescence state. After injury, the support of factor of growing, proliferation and differentiation, create myoblasts that go through the basal sliding the muscular fiber and release enzymes that assist the arrival on the injury place, promoting its recovering[27].

The increase on the inflammatory infiltrated number on the soleus muscle on the group DP (Figure 2G and Figure 3A) confirmed the results found on the analysis of the TNF-α expression (Table 2). These findings point at Souza, *et al*. (2013)[20] who verified on the gastrocnemius and anterior tibia muscles a higher quantity of inflammatory cells on the periodontal disease. The increase of cytokines as the IL-6 and TNF-α indicates that these substances can contribute to the recovering process[28].

The improvement on the morphological parameters on the experimental group DPE (Figure 2H and Figure 3B) can be related to the effects of physical activities upon the muscular physiology, on these animals it was observed that the activity promoted a regeneration on most of the muscular fibers, as well as the decrease on the inflammatory cells number. These data are similar to those found by Faria, *et al* (2008)[29] which evaluated the effect of swimming after a muscular injury, on a protocol of 5 to 8 days with 15 to 45 min sections respectively, and observed an improvement on the muscular morphology. Considering the determined experimental conditions we could observe that muscular tissue's behavior showed an acceleration on the regeneration process after physical activity in aquatic environment in rats with periodontitis experimental and there was a relation between the periodontal disease and the muscular morphological changes.

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Influence of intermittent aerobic performance on the variables of static and dynamic apnea performances

Álvaro Cristian Huerta Ojeda[1,2,6*], Sergio Andrés Galdames Maliqueo[2,3,6], Rafael Guisado Barrilao[4], Pablo Andrés Cáceres Serrano[5,6], Alejandra Paulina Araya Arancibia[6], Petar Danko Sironvalle Argandoña[6]

[1]Universidad de Las Américas sede Viña del Mar, Chile. [2]Centro de Capacitación e Investigación Deportiva Alpha Sports, Chile. [3]Universidad de Playa Ancha de Ciencias de la Educación, Chile. [4]Universidad de Granada. [5]Pontificia Universidad Católica de Valparaíso, Chile. [6]Grupo de Investigación en Salud, Actividad Física y Deporte ISAFYD, Facultad de Educación, Universidad de Las Américas, Chile.

Received: 04.01.2017
Accepted: 24.04.2017

Summary

Over the last years, sport diving has become one of the most studied physical activities. Several physiological responses have been described and analyzed during immersions of static, dynamic, and deep apnea sessions. These analyses, and several others, have been focused mainly on the cardiovascular and hemodynamic changes that this activity produces.
Objective: To determine the influence of aerobic performance of static and dynamic apnea before and after an out-water training based on the average velocity of a 12-minute test.
Material and Method: Eight apnea scuba divers of the Chilean Navy were part of this study. The variables measured were: aerobic performance measured through a 12-minute test, static and dynamic apnea. The protocol used for the aerobic performance development was a study based on the average velocity of a 12-minute test. The statistical analysis was performed with Student's t-test and the size of the effect with Cohen's d test.
Results: The aerobic performance showed a significant increase between the pre and post-test ($p < 0.001$; size of the effect = 1.263). The static apnea did not show significant modifications ($p > 0.05$; size of the effect = 0.025). Lastly, the dynamic apnea showed a significant increase in both measurements ($p < 0.05$; size of the effect = 0.404).
Conclusions: The result of the study showed that increasing the aerobic performance generates a rise in the distance of dynamic apnea. For that reason, it is suggested that apnea scuba divers perform out-water trainings based on the average velocity of 12-minute test as a complement to their immersion training.

Key words:
Aerobic performance. Static apnea. Dynamic apnea. 12-minute test. Average velocity of 12-minute test.

Influencia del rendimiento aeróbico intermitente sobre las variables de rendimiento de la apnea estática y dinámica

Resumen

En los últimos años, el buceo deportivo se ha convertido en una de las actividades físicas más estudiadas. Dentro de estas investigaciones, se han descrito y analizado las respuestas fisiológicas corporales durante las inmersiones de apnea estática y dinámica. De forma específica, los análisis se han centrado principalmente en los cambios cardiovasculares y hemodinámicos que esta actividad deportiva produce.
Objetivo: Determinar la influencia del rendimiento aeróbico en la apnea estática y dinámica antes y después de un entrenamiento fuera del agua basado en la velocidad promedio obtenida a través del test de 12 minutos.
Material y Método: Ocho buceadores de apnea perteneciente a la Escuela Naval de la Armada de Chile fueron parte del estudio. Las variables medidas fueron: rendimiento aeróbico a través del test de 12 minutos, apnea estática y dinámica. El protocolo usado para el desarrollo del rendimiento aeróbico fue basado en la velocidad promedio del test de 12 minutos. El análisis estadístico fue realizado a través de una t de Student y el tamaño del efecto con una d de Cohen.
Resultados: El rendimiento aeróbico mostró incrementos significativos entre el pre test y el post test ($p < 0,001$; tamaño del efecto = 1,263). La apnea estática no mostró cambios significativos ($p > 0,05$; tamaño del efecto = 0,025). Finalmente, la apnea dinámica mostró incrementos significativos entre ambas mediciones ($p < 0,05$; tamaño del efecto = 0,404).
Conclusiones: El resultado del estudio mostró que un incremento en el rendimiento aeróbico genera un aumento en la distancia alcanzada en apnea dinámica. Por tal razón, se sugiere que los buceadores de apnea realicen entrenamientos fuera del agua. Por último, los entrenamientos pedestres basados en la velocidad promedio de la prueba de 12 minutos, son un buen complemento del entrenamiento de inmersión.

Palabras clave:
Rendimiento aeróbico. Apnea estática. Apnea dinámica. Test de 12 minutos. Velocidad promedio del test de 12 minutos.

Correspondence: Álvaro Cristian Huerta Ojeda
E-mail: achuertao@yahoo.es

Introduction

Over the last years, sport diving has turned into one of the most studied physical activities[1]. Several physiological responses have been described and analyzed during immersions of static, dynamic, and deep apnea sessions[2-4]. These analyses, and several others, have been focused mainly on the cardiovascular and hemodynamic changes that this activity produces[5-7]. In connection to these hemodynamic and cardiovascular changes demonstrated in the apnea practice, it has been described that during 215 ± 35 s of immersion the heart rate (HR) decreases by 20 beats per minute, while *systolic and diastolic blood pressure (SBP, DBP)* show an increase of 23 and 17 mmHg respectively during the first 20 ± 3 s (phase I). Then, in the following 92 ± 15 s both the HR and the blood pressure (BP) showed stability (phase II). Later, during the last 103 ± 29 s, SBP and DBP increased in a linear fashion reaching values close to 60% when comparing them with control, while HR remains unchanged (phase III). Lastly, the cardiac output (Q) was reduced by a 35% in phase I, while in phases II and III it did not show significant changes[1]. Several studies have reported that the SaO2 decreased along with immersion[6,8,9].

In this context, a significant decrease has been observed in the arterial oxy-hemoglobin saturation level (SaO2) during maximum immersion and dry apnea. However, decrease in SaO2 is significantly higher during immersion when compared to dry apnea ($p = 0.04$)[8].

Concerning the physiological factors that are more challenging in static and dynamic apnea practice, several studies have described that HR, stroke volume (SV) and Q, SBP, DBP, mean blood pressure (MBP), systemic vascular resistance (SVR), SaO2, spleen and lungs size condition the performance in static and dynamic apnea[10,11]. Similarly, the same hemodynamic, cardiac, and lung variables show a direct relation with aerobic performance and VO_2max[12,13]. Therefore, it is likely that aerobic training enhances apnea performance.

Despite the fact that apnea diving has been widely studied, many of those studies have only been based on the organic responses[6,7]. Studies have also tried to measure some of the pathologies resulted from this practice[14,15], but in only a few opportunities the adaptations to training processes have been visualized[16]. More precisely, only some hemodynamic differences among divers and non-divers have been proved[17]. In this investigation, Tocco *et al.*[17] showed that divers trigger a bradycardia and an adjustment in the systemic vascular resistance faster than the control subjects, in particular in heart rate and systemic vascular resistance[17]. Similarly, Nishiyasu *et al.*[18], showed the relationship present between the degree of bradycardia induced by apnea and the vascular response in arms and legs during knee extension exercises. This research showed that the subject with a greater bradycardia, by breath holding during knee extension during the exercise, also showed a greater vasoconstriction in both active and inactive muscles[18]. It is safe to infer that intermittent endurance training for this population is limited or otherwise the results and adaptations in out-water training have not been published.

In connection to the changes in aerobic and VO_2max performance generated from high-intensity interval training, this training method has shown a significant increase in VO_2max when compared with other training methods of low intensity[19,20]. Unfortunately, there is no enough evidence on intermittent endurance training and/or out-water trainings *being performed* by divers in order to increase aerobic performance and hence increase their performance in static, dynamic or deep apnea tests. The main objective of this research was to determine the influence of the aerobic performance over static and dynamic apnea before and after the performance of an intermittent endurance training based on average velocity of 12-minute test.

Material and method

Experimental approach to the study. Chile is comprised of more than 4.000 meters of coastline making diving practice very attractive for the population. However, the low temperature of the ocean (14 to 17 °C)[21] only allows the sport to be practiced in the warmer months of the year (October – April). That is why divers have to perform two types of training during the colder months of the year (May – September). On one hand, they perform indoor training in swimming pools – *a high-cost alternative of difficult access,* and, on the other hand, *they develop their physical* condition mainly through peak oxygen uptake (VO_2max) performing intermittent endurance training.

Subjects. Eight male apnea divers from the Chilean Navy (age: 20.2 ± 1.22 years, weight: 73.6 ± 5.3 Kg, height: 176.6 ± 5.3 cm, Body Mass Index: 23.6 ± 1.5 Kg/m^2, fat percentage: 15.3 ± 2.9%) were part of the study (Table 1). In *order to define the sample, years of experience performing apnea diving was an inclusion criteria*. Subjects had a minimum of two years of diving practice. During these two years, all subjects were part of 3 weekly session trainings (two session in a heated pool at 26°C and one session in the ocean with temperatures ranging from 14°C to 17°C)[21]. As an inclusion criterion, the selection of participants only included those subjects who did not smoke and were not taking any medication that could risk their health by performing high-intensity tests (12-minute test) and dynamic and static apnea.

All sportsmen and coaches were informed about the aim of the study and the possible risks of the experiment. Subsequently, they all signed a written consent before starting the protocol. The signed consent and the study were approved by the Human Investigation Committee of the University of Granada, Spain (register number 231/CEIH/2016).

Before starting the study, weight, height, and skin folds were measured in all subjects. All subjects of the investigation were asked to not ingest caffeine, any drugs or substances that would increase their metabolism

Table 1. Characteristics of the sample (median ± SD).

Experimental group (n = 8)	
Age (years)	20.7 ± 1.2
Height (cm)	175.0 ± 5.6
Weight (Kg)	72.4 ± 5.4
BMI	23.7 ± 1.7
Body fat (%)	15.5 ± 2.7
Initial distance of 12-minute test (m)	3118.4 ± 148.5
Aerobic performance (mL•Kg^{-1}•min^{-1})	55.0 ± 3.4

BMI (body mass index); SD (standard deviation); mL•Kg-1•min^{-1} (milliliters of oxygen per kilogram of body weight per minute).

48 hours before pre and post test. Also, subjects were asked not to perform any vigorous activities twelve hours before the pre and post test. The time lapse of intake of the last meal before the tests was also controlled. The time registered was two hours for pre and post test.

Protocols and variables. Weight and height were measured with a Health Stadiometer Scale or Meter Professional® to characterize the sample. Skin folds were measured with a F.A.G.A.® caliper. Biceps, Triceps, Subscapular and Supra Supraspinal were measured to determine fat percentage using the Durnin & Womersley method[22].

The application of protocol used a pre-experimental intra-subject design with a pre and post test. *This study used the application of a training session based on average velocity of 12-minute test (AV.12-min test) obtained through a 12-minute test.*

Aerobic performance. The aerobic performance was evaluated with a 12-minute test (Cooper test). This test is able to measure the peak indirect oxygen uptake and the average velocity. This test was selected due to its convenience to assess all subjects simultaneously, *also because* minimum equipment was needed and it proved to be a good cardiovascular indicator. This test shows a strong correlation with the peak oxygen uptake. Therefore, the 12-minute test is an objective test that reflects the cardiovascular condition of an individual[23,24]. The evaluation was carried out in a 400-meter athletic track before and after *the application* of an experimental protocol based on AV.12-min test. Before the evaluation, subjects were encouraged to cover as much distance as possible and, during the test application, all participants received oral cheers from their diving coaches as well as from the researchers. With regard to the time *in which* the 12-minute test was performed, both the pre and post test were performed at 12:00 pm. This allowed the researcher to control specific variables such as temperature, humidity, and wind. The environmental conditions in which the test was performed were: 18°C and 34% relative humidity. The post test environmental conditions were: 20°C and 37% relative humidity.

The distance covered was measured in meters and then converted into kilometers (Km). The VO_2max was obtained with the following equation[23]:

VO_2max (mL•Kg^{-1}•min^{-1}) = (22.351 x distance in kilometers) – 11.288

Also, the average velocity of 12-minute test (AV.12-min test) applied in the experimental protocol was determined through a 12-minute test:

AV.12-min test (m/s) = (distance in meters performed in 12 minutes/ 720 seconds)

Apnea evaluation. In connection to the static and dynamic apnea, these were performed in a pool measuring 50 meters in length and two meters deep with a water temperature of 26°C. Both tests were carried out during the same day (static apnea was evaluated first, and dynamic apnea second). With regard to the time the dynamic and static apnea test was performed, both the pre and post test were performed at 12:00 pm. This allowed the researcher to control specific variables such as temperature and humidity. The environmental conditions were the following: 26°C and 63% relative humidity.

Static apnea. In order to determine the performance of static apnea, the authors measured the longest period of time in seconds (s) that each subject held their breath with their entire body under water. In connection to time recording, the timer started when the subject submerged their airways into the pool and it was stopped when any or all airways would surface. Each subject was evaluated individually, and the protocol considered a resting time of three minutes before starting the test. Also, during the application of the test, all subjects were allowed to be near the edge of the pool.

Dynamic Apnea. In order to determine the performance in dynamic apnea, the authors considered the greatest distance in meters (m) that each subject could swim in a horizontal position after one single immersion at two meters deep in the pool. Subjects were located at one of the ends of the first pool lane with the sole of their feet touching the wall, placing one hand on the starting platform, and looking at

Table 2. Structure of the Aerobic Training Program based on the average velocity of a 12-minute Test.

Months	February											
Week	1			2			3			4		
Type of Microcycle	In			Lo			Lo			Re		
Sessions by Microcycle	3			3			3			3		
Load per session	CT	SA & DA	3x1200 90% re 3:30	5x800 90% re 1:00	6x1000 90% re 1:30	2x2000 85% re 2:30	3x1200 90% re 1:00	1x6000 80%	3x1600 85% re 2:00	3x800 100% re 2:00	2x2000 90% re 3:00	1x3000 85%
Weekly volume (m)	3600			14000			14400			10200		
Average velocity in 12 - minute test	90%			87%			85%			92%		
Periods	Accumulation											
Low intensity												
Middle intensity												
High intensity												

CT (Cooper Test [12-minute test]); SA (static apnea); DA (dynamic apnea); In (introduction); Lo (load); Re (regenerative); Co (competitive); m (meters); MAV (maximum aerobic velocity); % (percent); re (rest).

the direction of the apnea. All movements had to be performed at the bottom of the pool. In relation to the evaluator, he was standing at the edge of the pool. The distance considered for dynamic apnea was measured from the edge of the pool until the point where the head of the divers emerged. In case of reaching the opposite end of the pool, subjects had to touch the end wall with hands and feet and then return in the opposite direction. A metric tape was placed at the edge of the pool in order to determine the exact distance reached in dynamic apnea.

Treatment. The aim of the protocol application was to develop VO_2max in divers connected to the AV.12-min test. This training plan was performed under the application of the periodization model of Block, specifically the Accumulation, Transformation and Realization model (ATR). This periodization model was used since it is one of the most used models to increase VO_2max in endurance sports[25]. The ATR model is characterized by allowing a better concentration of the training load[26]. This load concentration is the most decisive and important component of the block planning[27,28]. In connection to the program, this consisted of three mesocycles. The first mesocycle of accumulation consisted of obtaining the largest possible training volume. Then, in the transformation phase, the volume was reduced and the intensity increased. Finally, in the performance stage, the intensity was increased and the volume was adjusted to the projected distance in the 12-minute test (Table 2 and Figure 1).

Statistical Analysis. The variables of VO_2max, static apnea time, and dynamic apnea time were analyzed with the Kolmogorov-Smirnov (K-S) test. The differences between pre and post test were analyzed with a Student's t-test and intra-class correlation coefficient (ICC), while the size of the effect (SE) was calculated using Cohen's d test. This analysis considered the following values: insignificant ($d < 0.2$), small ($d = 0.2$ hasta 0.6), moderate ($d = 0.6$ a 1.2), big ($d = 1.2$ a 2.0), or very big ($d > 2.0$). The level of significance for all statistical analysis was $p < 0.05$. The data analysis was performed with Graphpad Instat Versión 3.05® software.

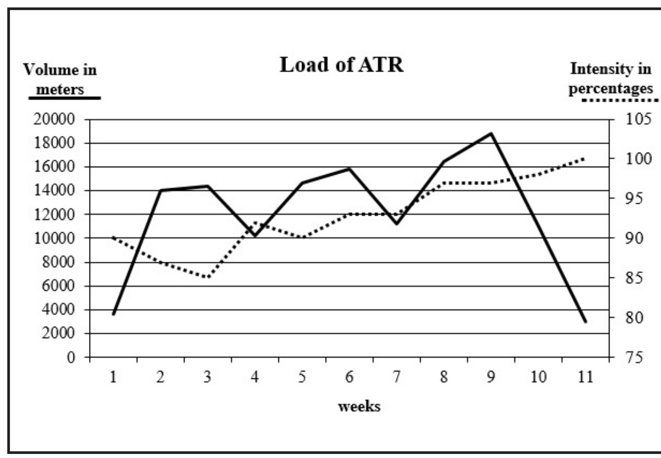
Results

Progressions and differences. The VO_2max obtained through the 12.minute test was significantly higher in post test than in pre test ($p < 0.0001$; SE = 1.263). Static apnea time was not significantly different between the pre and post test training period ($p > 0.05$; SE = 0.025). Finally, dynamic apnea distance was significantly higher in post test than in pre test ($p < 0.05$; SE = 0.404) (Table 3).

Discussion

The main observation in this study was that the dynamic apnea performance significantly increased after post exercise training intervention compared to pre-test, suggesting that the out-water endurance exercise training program in this study enabled the improvement of apnea diving performance.

Figure 1. Volume and intensity distribution of the Aerobic Training Program based on the average velocity of a 12-minute Test.



March												April							
5			6			7			8			9			10			11	
Lo			Lo			Re			Lo			Lo			Re			Co	
3			3			3			3			3			3			3	
4x1200 90% re 1:30	6x800 100% re 2:00	1x5000 80%	6x1000 95% re 1:30	6x800 100% re 1:00	1x5000 85%	6x800 100% re 1:30	2x2000 90% re 2:30	1x4000 90%	6x800 100% re 1:30	7x1200 90% re 1:30	2x1600 100% re 3:30	6x1000 100% re 2:30	4x2000 90% re 2:30	2x2400 100% re 4:30	5x800 100% re 1:30	2x2000 95% re 3:30	1x3000 95%	CT	SA & DA
14600			15800			11200			16400			18800			11000			3000	
90%			93%			93%			97%			97%			98%			100%	
Transformation												Realization							

Table 3. Distance of 12-minute test, estimated VO_2max, time in static apnea and distance in dynamic apnea before and after a training session based on the average velocity of a 12-minute test.

	pre test median ± SD	post test median ± SD	t de Student	d de Cohen	ICC
12-minute test distance (m)	2966 ± 154	3179 ± 182	***	1.263	0.83
Estimated VO_2max $mL•Kg^{-1}•min^{-1}$	55.0 ± 3.4	59.7 ± 4.0	***	1.263	0.83
Static apnea time (s)	91.4 ± 35.7	92.3 ± 11.2	ns	0.025	0.44
Dynamic apnea distance (m)	36.2 ± 11.2	40.9 ± 12.3	*	0.404	0.92

$mL•Kg^{-1}•min^{-1}$ (milliliters of oxygen per kilogram of body weight per minute); s (seconds); m (meters); SD (standard deviation); ns (not significant); ICC (intraclass correlation coefficient); * $p <0.05$; *** $p < 0.001$.

As previously mentioned, most of the research collected has been focused on cardiovascular and hemodynamic changes in static and dynamic apnea[29]. Therefore, the focus of this research is based on the usage of out-water training methods as a companion to the specific training of apnea diving. It is difficult to compare and debate over supplementary intermittent endurance trainings applied to diving practice, since some researchers conducted endurance training that increased aerobic performance with the capacity of static and dynamic apnea. However, the authors could not find information connected to training methods that would increase aerobic performance or VO_2max. In this last case, the evidence is vast and varied[26,27]. Nonetheless, Kapus *et al.*[30] reported that after a one-year intervention there was significant evidence of changes in the diver's capacity to perform maximum static apnea but, due to the fact that the study was a case study, more research was needed to establish the influence of the individual components of apnea training for diving performance.

Only a few studies have reported the increase of static and dynamic apnea performance with a rise in the aerobic performance through intermittent endurance training. Most of the interventions consulted for this research showed hemodynamic and cardiovascular variables[5,6,9], but only a few concluded that static and dynamic apnea can be influenced by other variables. Such is the case of Kjeld *et al.*[31], these researchers evaluated if the ischemic pre-conditioning of the forearm affected static and dynamic apnea under water. The researchers showed evidence that the ischemic pre-conditioning reduced the oxygen saturation of 65 to a 19%. A different study made a correlation between the degree of bradycardia induced by apnea and the degree of vasoconstriction of legs and arms during the dynamic extension of both knees[18]. At the same time, Schiffer *et al*. observed the effects of inorganic nitrate supplementation on the diet over static and dynamic apnea[32]. They concluded that nitrate supplementation reduced oxygen saturation in comparison to the placebo.

Even though the literary review for this article did not include researches connected to out-water treatments for the development of VO_2max, this variable is fundamental to increase the performance in both static and dynamic apnea. That is why the authors have constantly monitored the variables that are directly connected to the VO_2max and their athletic performance[33,34]. There is also a connection to the diver's safety, mainly to the flux of oxygen to the brain in apnea conditions[35-37] or pulmonary edemas post immersion[15].

As evidenced in this research, the out-water treatment together with the diver's apnea training was useful to improve their performance. Likewise, it has been shown that the implementation of field test, such as the 12-minute test, and the determination of the VO_2max[23,24] allowed the design and implementation of personalized exercises based on AV.12-min test.

Finally it is worth mentioning that this research operated only on the VO_2max based on intermittent endurance training. It is suggested to implement extra training to the muscles involved in the respiratory cycles such as the diaphragm[30] and glossopharyngeal insufflations[33]. It is also important to consider a control group for future research. In this study, it was difficult to include a control group since apnea divers population was limited. Furthermore, placing inexperienced subjects under tests of maximum static and dynamic apnea carries risks to their integrity.

Conclusions

The result of the study showed that increasing the aerobic performance generates a rise in the distance of dynamic apnea. For that reason, it is suggested that apnea scuba divers perform out-water trainings based on AV.12-min test as a supplement to their immersion training.

Acknowledgements

We specially want to thank the Chilean Navy School Arturo Prat for allowing us the access to its facilities and work with the Diving team. Also, to all apnea divers who took part in this research.

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TOM-Scale: a new method to programme training sessions loads in football

Alejandro Muñoz López[1], Blanca de la Cruz[2], José Naranjo[1]

[1]*Pablo de Olavide University, Seville.* [2]*University of Seville, Seville.*

Received: 26.01.2017
Accepted: 04.05.2017

Summary

There are several methods to control both the training and match load in football. However, most of these methods do not specify a clear methodology to pre-quantify this training or match load. The aim of this study was to develop a football specific training load monitoring scale (TOM-scale) to programme the session training load. 18 professional football players from the Latvian National Football Team were tracked throughout 466 football sessions, with individual sessions ranging from 13 to 24 for each player. Players were tracked using a multisensor device including a 5 Hz Global Positioning System, a 1000 Hz triaxial accelerometer and a heart rate monitor band. A football specific scale was developed to establish session training load prior to it. This scale is based on commonly football training tasks categories, with specific set parameters for each one. External training load variables involved total running distance, % of high intensity actions (> 14.4 km•h^{-1}), number of accelerations and decelerations, sprints (> 21 km•h^{-1}) and impacts. Internal training load variables were % heart rate maximum, Banister TRIMP and Edwards TRIMP. The results showed positive significant correlations ($p < 0.05$) from moderate ($r > 0.40$) to very large ($r < 0.90$) between TOM-scale training load and all the external and internal training load variables studied. In conclusion, although this new method does not avoid of controlling the real training load to assess the way the football players cope with the individual training loads, TOM-scale may be useful to programme football sessions and adopt a periodization strategy over the season to, in example, avoid non-functional overreaching phases and/or undesirable high isolated performance peaks.

Key words:
Quantification.
Periodization. GPS.
Performance.

TOM-Scale: un nuevo método para programar las cargas de sesiones de entrenamiento en el fútbol

Resumen

En la actualidad, existen diferentes métodos para controlar la carga de entrenamiento y partido en fútbol. Sin embargo, la mayoría de estos métodos no proponen una metodología de programación de dicha carga de entrenamiento o partido. El objetivo de este estudio fue desarrollar una escala específica para programar la carga de entrenamiento en fútbol (TOM-Scale). 18 jugadores profesionales pertenecientes a una selección profesional de jugadores de fútbol europea fueron monitorizados durante un total de 466 sesiones individuales, con una participación individual de 13 a 24 sesiones por jugador. La carga de entrenamiento fue registrada con un dispositivo multisensor el cual incluía un GPS de 5Hz, un acelerómetro de 1000 Hz y una banda de frecuencia cardíaca. Se desarrolló una escala específica en fútbol para establecer la carga de entrenamiento previo a la realización del mismo. Esta escala está basada en la categorización de tareas comunes en los entrenamientos en fútbol, donde posteriormente se especifican en base a parámetros específicos. Las variables de carga externa de entrenamiento contrastadas fueron la distancia total recorrida, el % de acciones de alta intensidad (> 14.4 km•h^{-1}), el número de aceleraciones y desaceleraciones, el número de sprints (> 21 km•h^{-1}) y los impactos. Las variables de carga interna utilizadas fueron el % de la frecuencia cardíaca máxima, Banister TRIMP y Edward TRIMP. Los resultados mostraron correlaciones positivas significativas ($p <0,05$) desde moderadas ($r >0,40$) a muy grandes ($r <0,90$) entre TOM-Scale y todas las variables de carga interna y externa estudiadas. En conclusión, aunque este nuevo método no exime de controlar las cargas de entrenamiento para contrastar las diferentes asimilaciones individuales de la carga de entrenamiento, es útil para programar sesiones de entrenamiento en fútbol y adoptar estrategias de periodización para, por ejemplo, evitar fases de sobre-entrenamientos no funcionales y/o picos altos de forma aislados no deseados.

Palabras clave:
Cuantificación.
Periodización. GPS.
Rendimiento.

Correspondence: Alejandro Muñoz López
E-mail: amunlop@gmail.com

Introduction

In recent years, several training load (TL) quantification (TL-Q) methods have been widely used with the aim of creating positive adaptations through different kind of stimuli[1]. After a fatiguing stimulus, the body falls into a supercompensation phase, turning into an overtraining phase if the stimulus is too fatiguing[2]. Thus, a functional overtraining status may be required to induce positive adaptations[2]; hence, the TL during the programming phase is important and necessary.

TL has been defined as the product of volume x intensity[3], and its quantification has been proposed as an effective strategy to prevent possible injuries[4]. In intermittent sports such as football, TL-Q is complicated since the energy system provides both aerobic and anaerobic energy at different ranges and intensities, and performance depends on different factors such as technique, tactics, physical and psychological team and individual conditions[5].

TL can be divided into external TL (ETL) and internal TL (ITL). The most common and widely studied methods of ITL are based on derived heart rate (HR) such as Banister Training Impulse (TRIMP-B)[6] and summated heart rate zone TRIMP, also known as Edwards TRIMP (TRIMP-E), as well as those derived from rates of subjective perception of the session rate of perceived exertion (s-RPE)[3,7]. Nowadays, Global Positioning System (GPS) and accelerometers are more accurate commonly used to monitor and quantify ETL in football[8], showing significant positive correlations with ITL variables such as TRIMP-B[9], TRIMP-E[10] and s-RPE[11].

s-RPE is probably the most widely accepted and studied subjective method for TL-Q in team sports such as football[9]. The original method proposed by Foster[7] used a Borg CR10 adapted scale to obtain the intensity at the end of training from each football player. TL was finally calculated by multiplying this factor by the training volume (i.e., minutes). Despite this method displayed important and significant correlations with different ETL and ITL variables[3,12], it is used at the end of the training, once the efforts are performed.

Even though some methods have been described to quantify the global TL in football, to our knowledge, there is no method to determine the TL prior to a training session by the technical staff. Thus, the purpose of this study was to propose and validate a simple and practical method to programme the session TL in football.

Material and method

Participants

21 outfield professional football players from the same First Team of the Latvian National Football Association participated in this study (age 26.00 ± 3.35 years, body mass 76.54 ± 4.99 kg, height 181 ± 6.09 cm). The sample consisted on 4 centre defenders (n = 102), 5 fullbacks (n = 111), 4 wingers (n = 89), 5 midfielders (n = 102) and 3 strikers (n = 62). Players provided written informed consent to participate in the study, which was approved by an institutional ethics committee following the Helsinki Declaration guidelines.

Measures

Players were tracked using a multisensor device (details below) throughout different kind of football sessions' (including friendly matches) between May 2014 and March 2015 during the preparation and qualification stages of Euro France 2016. This tracking provided information from a total of 31 team sessions, with individual sessions ranging from 13 to 29 for each player, resulting finally on a total of 466 individual sessions observations. Football sessions were categorized as conditioning training (COND) (n = 6), tactical training (TAC) (n = 13), pre-match training (PR-M) (n = 4), training match (TR-M) (n = 4) or friendly match (FR-M) (n = 4).

Procedures

A football specific scale was developed to establish the intensity of the drills. First, the original Borg Scale 6-20 was transformed into an exponential curve according to some other authors who have proposed similar approaches using other scales, suggesting that the more intensive the exercise, the greater the difference must be between those intensities[6,12-14]. Given that previous researches found associations between the first and second thresholds and the values 12 and 14 respectively on the Borg Scale 6-20[15,16], we factorized as 1 those values from 6 to 11, as 2 from 12 to 14 and as 3 for 15 and above. The initial values of the scale from 6 to 20 were plotted with the factors and the following prediction equation obtained for the model was finally used to adjust the individual factors:

$$y = 0.4497 * e^{\wedge(0.1059x)}$$

Session training load calculation instructions

To calculate each session training load (s-TL), prior to each football session, a group of experts formed by three UEFA PRO coaches and one Sports Science fitness coach with large experience at an international level coach followed several steps. First, the intensity of each session drill was calculated, following the next steps (Figure 1): 1) Select a drill category, 2) Set the drill category specifications depending on the drill specific rules, 3) Exchange obtained intensity value by final factorized value. Second, the drill training load (d-TL) was calculated by multiplying the drill intensity by the total drill volume in minutes. Last, the session training load (s-TL) was calculated by summing each d-TL. The following is a more detailed description of the process:

Step 1: A drill was assigned to one of the possible 13 categories as described below:

Recovery: recovery drill with the main aim of recovering after a high intensity training session or day; *Compensatory*: exercises focusing on improving imbalances with low relative weights, core exercises, range of motion exercises and/or balance exercises on the pitch; *Warm up* (WU): drills designed to prepare the body for the subsequent physical activity; *Set pieces* (StP): set piece situations, including crossing and shooting drills; *Resistance drills:* exercises using external weights or body weight to improve strength on the pitch; *Passing drills* (PD): passing situations where usually many different technical abilities are involved, as well as individual tactical situations; *Tactical work* (TW): drills to improve collective tactical situations usually involving the whole group such as attack-defence situations, game play etc.; *Conditioning circuit* (CC): traditional conditioning circuit with different posts where the objective is to combine different conditional factors with technical and/or tactical abilities; Small sided game (SSG): classic small sided game in any format; Training match (TM); Official match (OM); 1*vs*1: situations where 2 players compete each other; and All out: situations where the player is running at his/her maximum capacity. Those categories were established by the same group of experts as the most commonly categories during the daily trainings in football. Thus, the first step is to choose a drill category to be set during step 2.

Step 2: The drill was set depending on the category selected during Step 1 and the drill own specific rules. The factors used on each of the Step 2 sections were arbitrary decided by the group of experts, similarly to previous[16]. Two points are important at this step: 1) about the relative area per player (Setup D, Figure 1), it was considered that if situated on any limit zone, the higher zone was considered. 2) if the drill category is a training match, it was considered to rest again at the end – 1 if usually pauses or -2 if frequently pauses to the final intensity value

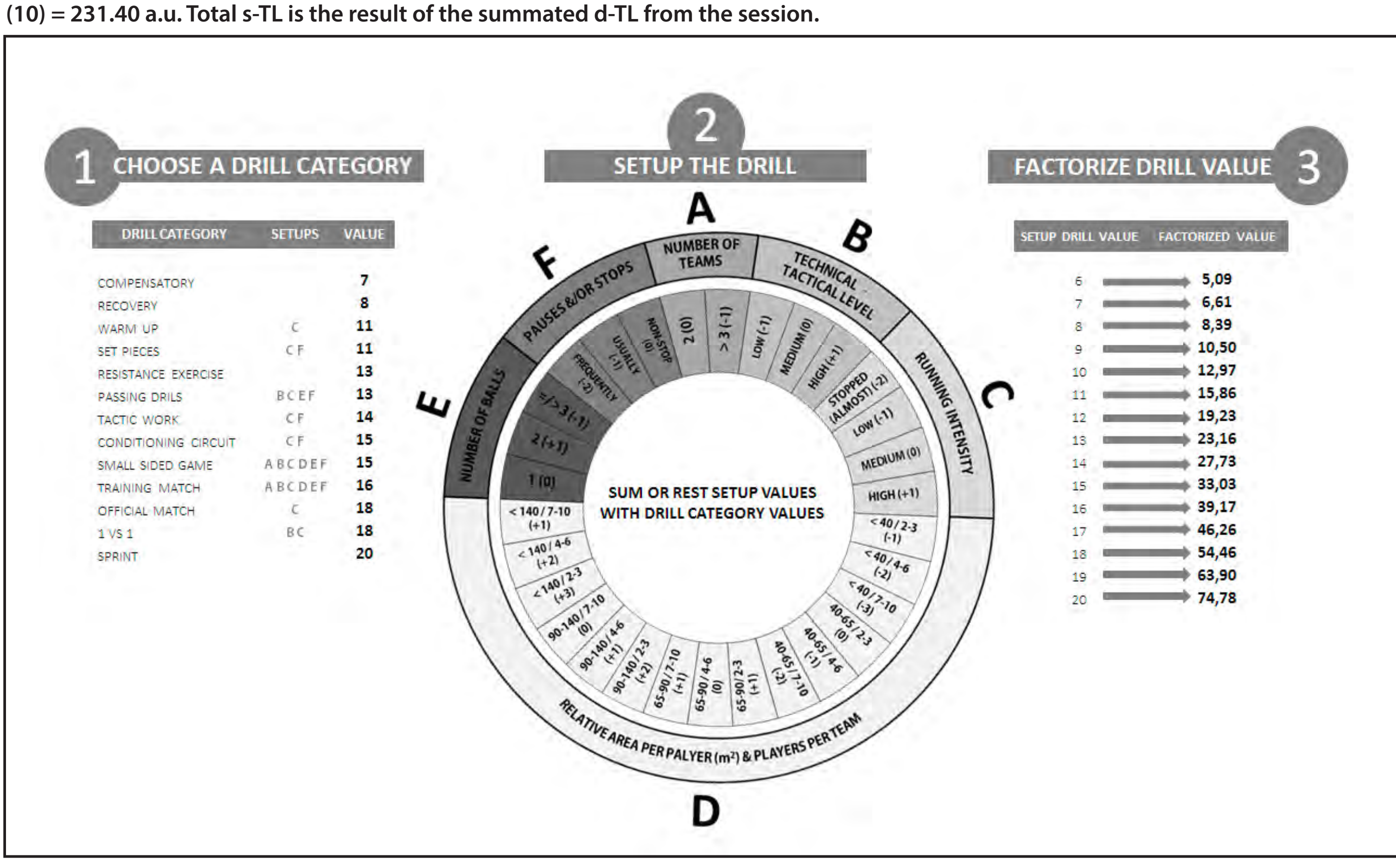
Step 3: the re-converted intensity value obtained after step 2 was exchanged by the corresponding factorized value showed on Figure 1[3]. For drills where resting time is an important factor within the drills themselves, this intensity was also considered into the total load. Rest intensity was calculated following the proposal of Scherr *et al.* (2013)[16], identifying every percentage of maximum heart rate with a given value on the Borg Scale 6-20.

Step 4: The fourth step was to establish the total drill volume. Again, if rest was considered, two different volumes were used. Finally, d-TL was calculated by multiplying the factorized intensity for each volume, also summing each individual effort and rest TL if considering rest.

Step 5: the last step was to calculate the s-TL by summing all the d-TL which would be used during the session. This method will be referred to hereinafter as the "training output monitoring scale" or "TOM-Scale".

During each football session, each player wore a special t-shirt with a multisensor device *(WIMU, Realtrack Systems, Spain)* situated between their two scapulae. This device included a 5Hz GPS, a 1000 Hz triaxial accelerometer and ANT+ that was used to connect with a heart rate monitor band *(Garmin HRM, Garmin, USA)*. Similar devices with same hardware characteristics have been used on previous studies[8,10,18,19]. To test the validity and reliability of the 5HZ GPS device a pilot study was conducted. 2 subjects performed 8 laps (16 laps in total) over

Figure 1. Steps to calculate the drill intensity value. Step 1: choose a drill category for the programmed drill. Step 2: for each drill category, choose its own setup zones (from A to F). Step 3: Exchange the value obtained afer step 2, and factorize with the values from the step 3. Finally, this value (drill intensity) must be multiplied by the total effort time (total drill volume, in minutes). In case of considering the resting, see methods to add the resting drill load.. Practical example: in case of 10 minutes of a passing drill exercise (Step 1= 13), with a medium technical-tactical level for this drill (Step 2, B = +0), high running intensity during the exercise (Step 2, C = +1), 1 ball used at the same time (Step 2, E = +0) and usually stops (Step 2, F= -1), the d-TL = effort intensity (13 + 0 + 1 + 0 – 1 = 13; step 3 = 23.16) * drill volume (10) = 231.40 a.u. Total s-TL is the result of the summated d-TL from the session.



a team sports circuit[20]. Gold standards used were real distance and speed, measured with a dual-beam electronic timing gate *OptoJump System (Polifemo Radio Light, Microgate, Bolzano, Italy)* placed at the start position, 10m and 30m. The mean BIAS for total distance during the circuit, 10m sprint and 30m sprint were -2.73 ± 1.64 m (p <0.001), -0.80 ± 0.58 m (p <0.001) and 0.42 ± 2.50 m (p= 0.515) respectively. The intra-unit reliability was assesed using Bland-Altman plots and coefficient of variation (CV, in %) comparing the results from each lap for all the subjects. The average BIAS compared with each unit mean laps results for total distance, peak speed and average speed were 0.00 ± 1.68, 0.00 ± 1.73 and 0.00 ± 0.33 for the circuit, 0.00 ± 0.49, 0.00 ±0.53 and 0.00 ± 0.77 for 10m sprints and 0.00 ± 2.34, 0.00 ± 0.76 and 0.00 ± 0.74 for 30m sprints, non-significant differences in all cases. The CV for total distance, peak spead and average speed were 1.25%, 2.61% and 3.33% for the circuit, 6.34%, 4.31% and 7.45% for 10m sprints and 8.22%, 2.68% and 4.13% for the 30m sprints.

Data were analyzed after each training session using the manufacturer's analysis software *(Qüiko v. 2.0, Realtrack Systems, Spain)*. If any kind of interference or signal loss were observed on each file, data were removed from the analysis. Different ETL and ITL variables were calculated for the analysis. ETL involved total running distance (TOT-DIST, in meters), % of high intensity actions (% HIA), total number of accelerations (ACC, in $m{\bullet}s^2$) and decelerations (DCC, in $m{\bullet}s^2$) calculated from the GPS, total SPRINTS number and total impacts number (IMPACTS).

HIA speed threshold was considered at speeds > 14.4 $km{\bullet}h^{-1}$[8], whereas the SPRINTS speed threshold was > 21 $km{\bullet}h^{-1}$[8]. For the ACC and DCC, the minimum value considered was 0.55 $m{\bullet}s^{-2}$ [21], whereas for the IMPACTS it was 5G[18]. ITL variables were %HRmax, TRIMP-B[6] and TRIMP-E[17].

Statistical methods

The Statistical Package for the Social Sciences (SPSS, version 20.0 for Windows, SPSS Inc, Chicago, IL) was used to conduct the analysis. Data are shown as mean ± SD. To estimate the minimum sample size for the correlation analysis, G*power software (v. 3.1.7) was used, setting a 0.70 effect size, 80% power and alpha = 0.05. The minimum number of sessions per player determined was 13, in accordance with the minimum number of sessions selected for the analysis.

Correlation analysis was used between each player session ETL or ITL variables and each player s-TL using the Pearson correlation coefficient (r) to analyse each player individual responses against each kind of exercise. Prior to correlation analysis, data were log-transformed to reduce the bias from non-uniform distributions. According to Hopkins, correlation magnitudes were classed as trivial (< 0.10), small (0.10 to 0.29), moderate (0.30 to 0.49), large (0.50 to 0.69), very large (0.70 to 0.89) and extremely large (>0.90)[22]. The confidence limits (CL) were also calculated at 95%.

Results

Figure 2 shows the correlations between s-TL and ITL and ETL variables. Moderate to very large positive significant correlations were observed between ETL and s-TL. The higher correlation was found between s-TL and TOT-DIST (0.87, p = .00, 95% CI = 0.84 - 0.90), while the smallest was found between s-TL and HIA (r = 0.44, p < 0.001, 95% CI = 0.38 – 0.50). Moderate to very large positive significant correlations were also observed between ITL and s-TL. The higher correlation was found between s-TL and TRIMP-B (r = 0.81, p < 0.001, 95% CI = 0.76 – 0.85), with very similar correlations found between s-TL and TRIMP-E (r = 0.79, p <0.001, 95% CI = 0.72 - 0.84). The lowest correlation was found between s-TL and %HRmax (0.46, p < 0.001. 95% CI = 0.32 – 0.60).

Line-dot-line shows the prediction intervals. Short-lines shows the confidence intervals (CI = 95%) for the regression line. Black line shows the fitted linear regression line. R represents the Pearson moment product correlation and r2 the percentage of variance that each variable represents the s-TL variance.

Figure 3 shows a representative load distribution through all football sessions analysed. The higher s-TL were found during Friendly Matches sessions. Accordingly to the correlations values, most of ITL and ETL showed a similar distribution of the load compared to the s-TL. Higher s-TL, ITL and ETL were related to friendly matches, while the lowest loads were found with pre-match training sessions. Distance and %HRmax showed to be the ETL and ITL variables with less variability among the football players (Figure 3, lower SD).

Bars represent each ITL or ETL variables (mean). Black points represent the s-TL for each football session. Error bars represents the standard deviation for each football session (SD) for either the ITL or ETL (bars) and the s-TL (black points). COND = conditioning training. TAC = tactical training. PR-M = pre-match training. FR-M = friendly match. TR-M = training match.

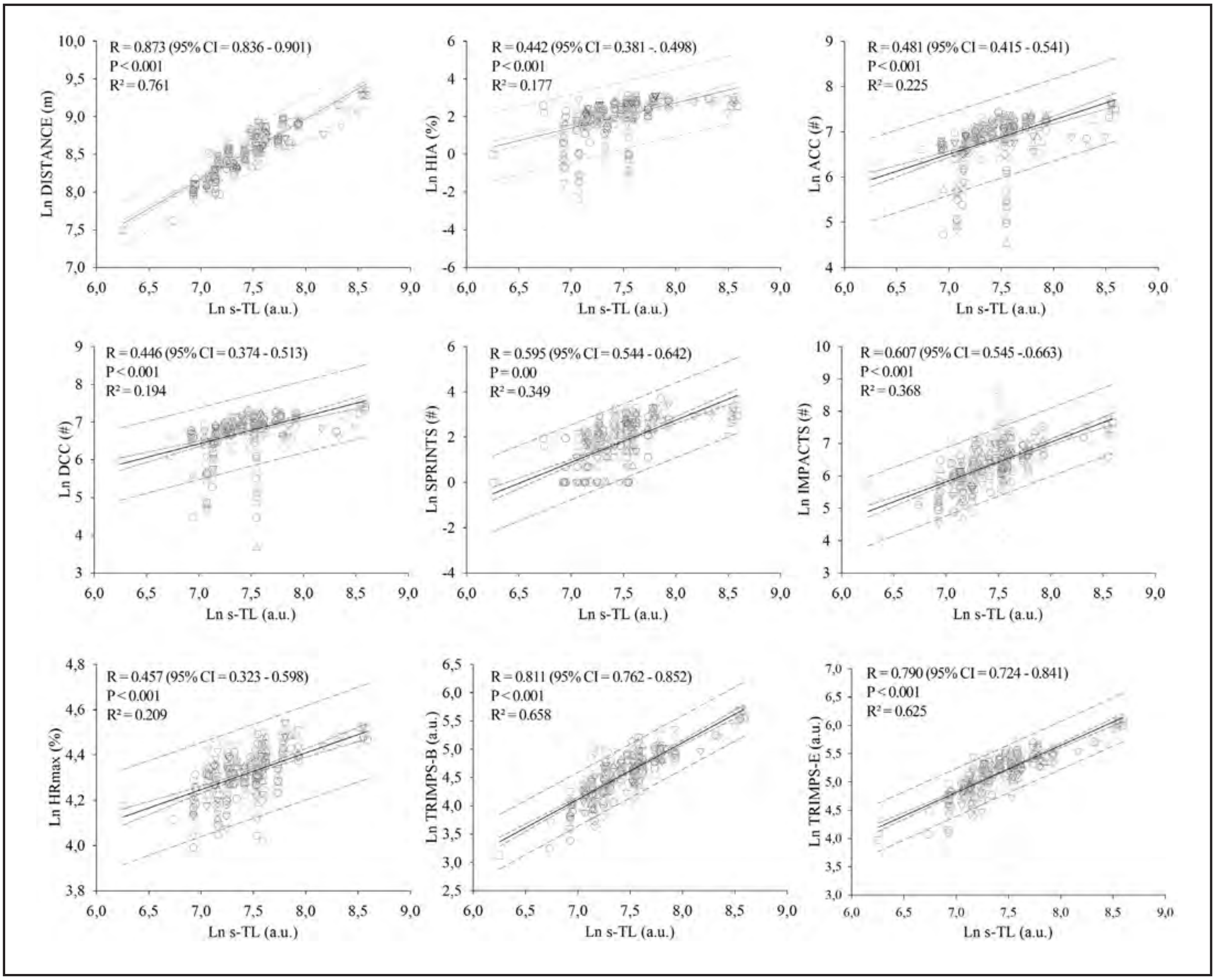
Discussion

The purpose of this study was to propose and validate a simple and practical method to programme the session TL in football. The results showed that TOM-Scale is a valid method to programme the s-TL prior to football training and match sessions.

To our knowledge, this is the first study that observes the relationships between a subjective coach's TL programmed prior to training with ITL and ETL variables among elite football players. The results suggest that the TOM-Scale is a useful method to programme s-TL prior to training. A similar method has been proposed in rowing[23] to predict s-TL in rowing using different factorized values depending on the drill category, together with exponential increment of intensity. In our study, each exercise setup factor was determined arbitrarily (Figure 1, Step 2) by a group of experts (see methods). This approach is supported by some recently research[24].

Several studies have shown positive significant correlations between subjective s-TL (calculated as the TL perceived by the players at the end of training) and similar ITL and ETL variables[9,23]. Previous studies showed that coach's perception of TL after training is not the same as the players[25]. However, our results suggest that using the TOM-Scale, the coach can programme with good relation to some ETL and ITL the s-TL. ITL such as TRIMP-B or TRIMP-E have shown significant correlations with the s-RPE method in football[9,10]. ETL also yielded significant correlations with subjective s-TL perceived by players in football (HIA, r

Figure 2. Scatter plots for ITL/ETL against s-TL. Data are represented as the natural logarithm (ln) for each variable.



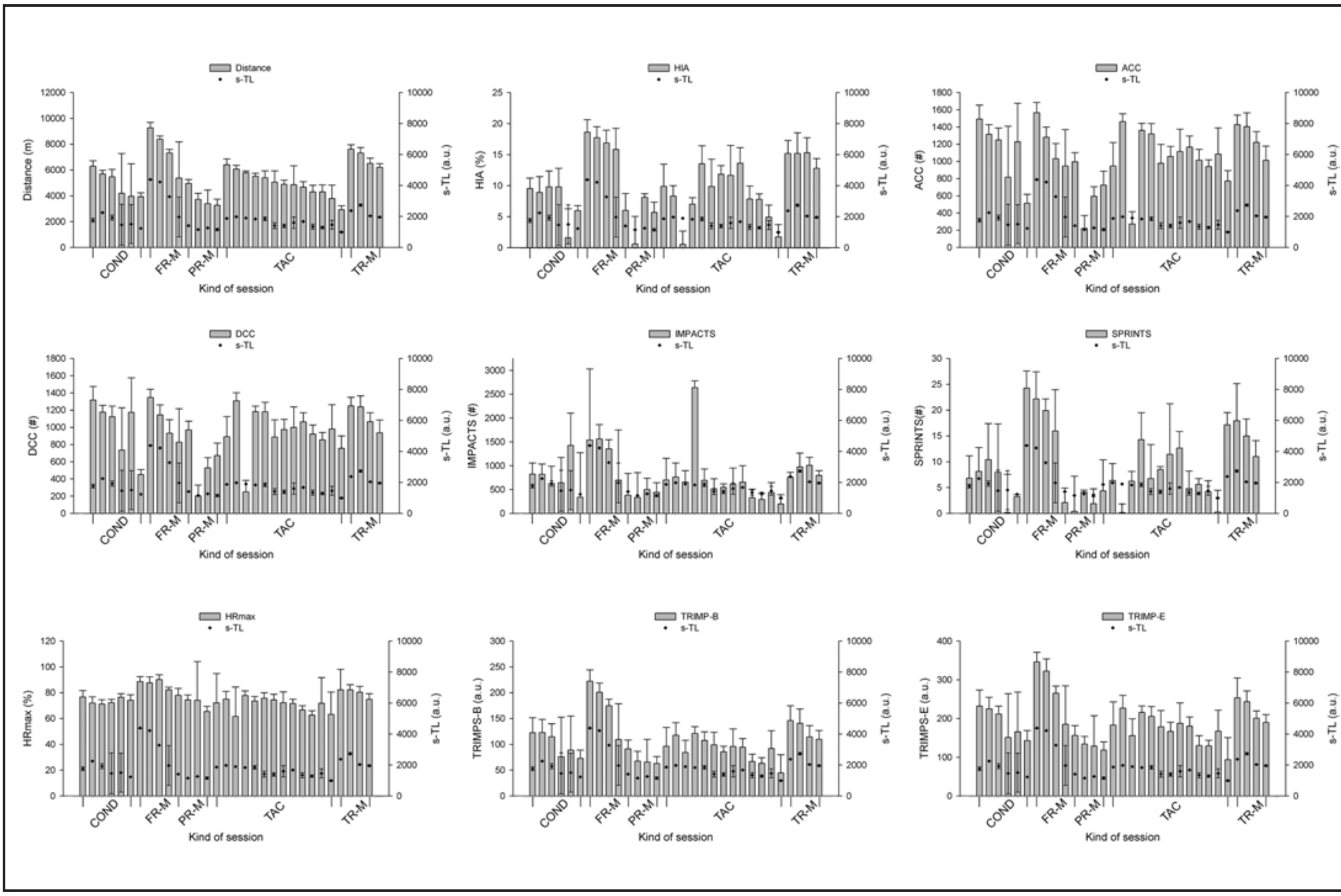
R: pearson moment product; 95% CI: confidence intervals at 95%; HIA: % high intensity actions; ACC: accelerations; DCC: decelerations; HRMAX: % heart rate maximum; TRIMP-B: TRIMP Banister; TRIMP-E: TRIMP Edward.

= 0.54 and SPRINTS distance, r = 0.44), similar to the results presented here[26]. In other similar sports such as Australian football, ETL such as TOT-DIST and HIA showed similar correlations with s-RPE compared to the results of this study ($r \approx 0.80$ and $r \approx 0.70$, $p < 0.05$)[9]. As in the study carried out by Scott *et al.*[9], the correlations found here with TOT-DIST were higher than HIA, SPRINTS, ACC or DCC, and this could be due to a greater dispersion of these variables between different positions on the field[19]. Another probable reason for these lower correlations could be related with the low validity of GPS at higher speeds (over 20 km•h^{-1})[27] or sudden changes of speed[27]. For this reason, HIA, SPRINTS, ACC and DCC correlations must be interpreted with caution.

To date, no information has been located regarding the relationship between impacts obtained by accelerometers and any subjective s-TL. However, some research has examined the relationship with player body load (calculated directly from the accelerometer, as impacts) and s-RPE, with similar results to the correlation with IMPACTS in this study ($r \approx 0.75$)[8,9]. Therefore, this is the first paper to study the relationship between the impacts obtained directly from the accelerometer and a subjective s-TL. In our opinion, IMPACTS is an interesting variable to be controlled since it is directly derived from accelerometers, incorporating information regarding the neuromuscular load. The high correlation found in our study with the TOM-Scale TL suggests that the higher s-TL, higher neuromuscular load will the player present.

In relation to the ITL, the results suggest TRIMP-B or TRIMP-E may be used indistinctly, given that the correlation with TOM-Scale TL was similar. %HRmax, however, resulted on a lower correlation. This could

Figure 3. Representative load distribution of ITL/ETL with s-TL. Data are represented as mean ±SD.



COND: conditioning sessions; FR-M: friendly matches sessions; PR-M: pre-match sessions; TAC: tactical sessions; TR-M: training match; s-TL: session training load; HIA: % high intensity actions; ACC: accelerations; DCC: decelerations; HRMAX: % heart rate maximum; TRIMP-B: TRIMP Banister; TRIMP-E: TRIMP Edward.

be explained by the use of volume to calculate either TRIMP-B or TRIMP-E, whereas %HRmax is just an intensity marker. Some studies have suggested that the use of %HRmax is not valid to quantify TL[29] and has the limitation that it cannot distinguish between high and low intensity exercises when they are performed for just few seconds with enough recovery time between them[30,31].

A limitation of this study is that the GPS device used for the ETL measures was not validated yet in the scientific literature. A recent review[32] has addressed the validity and reliability of similar GPS devices with 5Hz frequency sample, the same of the GPS device used in this study with very comparable results to those found in our GPS validity and reliability test. The evidence largely suggests that 5Hz GPS devices can accurately quantify players' distances during team sports[32]. However, 5Hz GPS devices tends to differ from reality at moderate to high speeds (i.e. >14 km•h^{-1}). Despite of this, the reliability of these devices when changing of direction, curvilinear movements and even high-speed running or sprints from 10 to 40m[32]. has been shown to be moderate and replicable. In addition, 5Hz GPS devices have been shown to be acceptable valid and reliable measuring high-speeds (i.e. >8m•s^{-1})[32]. Finally, to accommodate the potential problems of 5Hz GPS devices when assessing high-speeds, authors propose to group distances based on velocity bands[32], as it has been done in this study. Another limitation is the reproducibility and reliability of the TOM-Scale TL by different coaches and levels. Future research must focus on this aspect, even to analyse more deeply the validity relationships separately across different kind of soccer sessions.

In conclusion, TOM-Scale may be a useful tool to programme and design football field sessions. The TL calculated with TOM-Scale can be managed over a season to avoid non-functional overreaching phases, avoid injuries and/or keep a high level of fitness during all the season. It can help coaches and staffs in general to understand better what kind of stimulus they are going to achieve with the team. Anticipating the session TL may be also interesting to decide which kind of drill suits better to the expected TL. One limitation of this study is that, although the sample comprised elite football players, the data were obtained and the TOM-scale was applied within the context of a 24-h controlled situation (training camps); hence further study is needed to verify whether this method is valid in other kinds of environments.

Acknowledgements

Authors would like to acknowledge the Latvian Football Federation for its support to football sports sciences and especially to the players and technical staff from the National Football Team.

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Review

Anterior cruciate ligament injury in the female athlete: risk and prevention

África D. Lluna Llorens, Bárbara Sánchez Sabater, Isabel Medrano Morte, Elena M. García García, Sara Sánchez López, Juan F. Abellán Guillén

Hospital General Universitario Morales Meseguer, Murcia.

Received: 23.01.2017
Accepted: 23.05.2017

Summary

Background: The anterior cruciate ligament (ACL) injury of the knee is the second most common sports injury after the ankle sprain. It causes knee instability and impacts sport performance. Knowing what predisposes this injury is important to prevent it, specially in women, where the rate is higher. This paper presents the ACL injury epidemiology, making reference to the underlying risk factors and its preventive programmes. The aim of this study was to show the risk factors that predispose to a higher incidence of anterior cruciate ligament injury, as well as to present the effectiveness of the prevention programs.
Methods: A literature review through PubMEd, Cochrane and UpToDate has been performed including the meta-analysis or clinical trials published over the past 10 years.
Results: The injury incidence rate is three times higher in women than in men. It specially develops in sports like football and basketball, i.e., sports where pivoting, sharp running direction changes or abruptly stopping exercises are more common. The risk factors are multifactorial; and the only adjustable ones are the biomechanical risk factors. Prevention programmes are focused in these factors, trying to enhance strength and biomechanical propioception.
Conclusions: After the review we can conclude that ACL injuries are more frequent in women. Prevention programmes focus on neuromuscular training (strengthening exercises, proximal control and plyometric exercises) and they reduces significantly the injury for football and handball players, but not for basketball ones. The programmes focus on strengthening exercises, proximal control and plyometric exercises.

Key words:
Anterior cruciate ligament. Prevention. Athlete. Risk factors.

Rotura del ligamento cruzado anterior en la mujer deportista: factores de riesgo y programas de prevención

Resumen

Objetivo: La lesión del ligamento cruzado anterior de la rodilla (LCA) es la segunda lesión deportiva más frecuente tras el esguince de tobillo. Provoca inestabilidad de la rodilla y afecta al rendimiento deportivo, por lo que es importante saber qué lo favorece y cómo lo podemos evitar. En este trabajo se expone la epidemiología de la lesión del LCA haciendo referencia a los factores de riesgo predisponentes y a los programas preventivos de la misma. El objetivo de este trabajo ha sido mostrar los factores de riesgo que predisponen a una mayor incidencia de lesión del ligamento cruzado anterior, así como presentar la efectividad de los programas de prevención de la misma.
Método: Se ha realizado una revisión de la literatura a través de PubMEd, Cochrane y UpToDate incluyendo los metanálisis o ensayos clínicos publicados en los últimos 10 años.
Resultados: La incidencia de lesión es mayor en la mujer que en el hombre con una relación 3:1, y sobre todo se produce en deportes como el fútbol y el baloncesto, donde se realizan ejercicios como pivotar, cambio brusco de dirección en la carrera o frenar de forma brusca. Los factores de riesgo son multifactoriales, entre ellos los únicos modificables son los factores de riesgo biomecánicos y es en ellos donde se centran los programas de prevención.
Conclusiones: Las mujeres tienen una mayor incidencia de lesión de LCA. Los programas de prevención se centran en factores de riesgo modificable, principalmente en el entrenamiento neuromuscular y disminuyen de forma estadísticamente significativa tanto en el fútbol como en el balonmano, pero no en el baloncesto. Estos programas se centran en ejercicios de fortalecimiento, control proximal y ejercicios pliométricos.

Palabras clave:
Ligamento cruzado anterior. Prevención. Deportistas. Factores de riesgo

Correspondence: África Dakota Lluna Llorens
E-mail: africadakotalluna@gmail.com

Introduction

The anterior cruciate ligament of the knee (ACL) injury rate varies depending on gender, sport, and prevention programmes1. It is higher in women than in men, and a higher rate is seen in sports such as football and basketball[1]. ACL injury is the second most frequent sporting injury after ankle sprain[2], which is why it is important to establish the risk factors, paying attention to those that can be modified, as this is the main focus of prevention programmes.

Epidemiology of ACL injury

Rate

The anterior cruciate injury rate varies in the different studies published, possibly due to the different physical activity performed by the study participants. It is essential to highlight that the data published is heterogeneous, and that the injury rate depends to a great extent on sporting performance[1,2] and therefore on neuromuscular control, which should be considered upon analysing the results.

The female:male ratio of ACL injuries has recently generated considerable attention. In the study by Prodromos *et al.*[1], the ACL tearing rate (female:male) by sport was: basketball, 3.5:1; football, 2.67:1; hockey, 1.18:1. According to the level of female athletes: in secondary school it was 0.09, whilst in men it was 0.02, and the female:male ratio was 4.5:1. At university, the rate was 0.29 for women and 0.08 for men, with a female:male ratio of 3.63:1, and on a professional level it was 0.20 for women and 0.21 for men, with a female:male ratio of 0.95:1. According to this data, as female athletes become more elite, the injury rate reduces to almost the same as the rate for males.

Risk factors

To understand why this injury is more frequent among women and as a means of preventing it, it is important to identify the risk factors. The cause of the increase in ACL injury is multifactorial, and the risk factors have been categorised into: environmental, anatomical, hormonal and biomechanical[3].

Environmental factors

Surface temperatures, friction between materials, and the design of footwear soles can be a risk factor in ACL injury[3]. These factors affect both men and women equally, which is why there is not a higher rate among women.

Hormonal factors

Proof of the effect of sex hormone effects on connective tissue is limited and the results of some studies are inconsistent. However, the synthesis of collagen could be reduced up to 40% due to physiological oestrogen levels, and up to 50% induced by pharmacological oestrogen levels[3].

Anatomical factors

The anatomical difference between men and women could be a factor that contributes to a heightened ACL injury rate. However, these factors cannot be modified, therefore preventive measures cannot be applied to them.

- The breadth of the intercondylar notch in women is smaller than in men, however no differences in the risk of ACL injury have been revealed[3].
- The cross-sectional area of the ACL is greater in men[3], which suggests that a smaller diameter of the ACL in women could be a tearing risk factor.
- The Q angle, whose normal value is from 8 to 17º, is greater in women. This increase is because generally the female pelvis is wider and the femur is shorter[3] or that a high Q angle increases the medial stress on the ligaments of the knee[3].
- The angle between the patellar tendon and the tibia affects the shearing force applied.

Biomechanical factors

The quadriceps muscle displays its greatest muscular activity during knee flexion exercises, whilst the hamstrings tend to relax; moreover, when inducing muscle fatigue and performing sporting manoeuvres in female athletes such as landing on just one leg or changing running direction, a reduction in the hip flex occurs, as well as an increase in the internal rotation of the hip, an increase in knee valgus and an external rotation of the tibia, which leads to less stability in the knee joint3. In men, all of this is less frequent because they have greater muscle control. The lack of this control in women, especially in adolescence, causes a greater predisposition to slipping of the femur over the tibia and greater stress on the ACL.

Biomechanical risk factors are the only ones considered to be modifiable, and this is why prevention programmes focus on this area. Greater neuromuscular control leads to a reduced risk of ACL injury, which explains why as the sporting level increases and training demands are higher, female:male injury ratios are nearly 1:1

Prevention programmes

Biomechanical and biomuscular components are the only modifiable elements. Recent studies reveal that certain risk factors such as an increase in knee valgus, limited hip flex or the internal rotation of the hip predispose the individual to a greater risk of ACL tearing[4,5]. The magnitude of these movements can be controlled by increasing supporting muscle and by alternating this with sporting technique, all through the use of preventive neuromuscular training programmes (PNMT). A recent meta-analysis assessed the overall effectiveness of PNMT and revealed that these programmes reduce ACL injuries in young women[4].

Training promotes muscle development, improves movement patterns and protects from ACL injury; however, it is not clear which kind of exercises are the most effective in preventing this injury. Upon assessing the different studies, in which the groups included are not homogeneous[6-8], it is vital to establish which kind of exercises included in PNMT significantly reduce ACL injury.

A meta-analysis published by Sugimoto *et al.* [4] aimed to establish the exercises that had the greatest prophylactic effect on reducing ACL injury. They examined four exercise categories: balance, plyometric exercises, strength training and proximal control. The results revealed a statistically significant lower number of ACL injuries ($p = 0.001$) with PNMT that focused on strength, proximal control and plyometric exercises.

Neuromuscular risk factors can be modified through training, leading to better sporting performance. Furthermore, given that the majority of ACL injuries occur in non-contact situations, it is necessary to consider the different biomechanical demands of the different sports, as well as to work on the sporting motion depending on the sport.

Different training programmes have been described that reduce the ACL injury rate[9-10]. The most important are included in the systematic review published by Michaelidis *et al*[9] in which 12 PNMT are proposed, which despite their differences, focus their training plan on neuromuscular development and include the types of exercises that have revealed statistically significant reductions in ACL injury.

Training programmes

Different prevention programmes have been proposed with the aim of reducing the ACL injury rate in female athletes. The characteristics and exercises of each of these programmes are summarised in Table 1. To establish whether or not they are effective, they have been assessed using studies with a control group. Below are the main studies used to assess these programmes.

Sportsmetrics

A prospective study to assess the effect of PNMT on the knee injury rate of female athletes. They compared athletes following the

Table 1. ACL injury prevention programmes in female athletes.

Programme	Sport	Characteristics
SPORTSMETRICS	Football Volleyball Basketball	- Session: 60-90 min - Frequency: 3 days/week - Total time: 6 weeks (only pre-season)
FATP	Football	- Session: 75 min - Frequency: 3 days/week - Total time: 7 months
PEP	Football	- Session: 20 min - Frequency: 2-3 days/week - Total time: 12 weeks
KLIP		Football - Session: 20 min - Frequency: 2 days/week - Total time: 4-5 months
FIFA 11	Football	- Session: 15 min - Frequency: 1 day/week - Total time: 7.5 months
SÖDERMAN	Football	-Session: 10-15 min -Frequency: 3 days/week -Time: 6 months
MYKLEBUST	Handball	- Session: 15 min - Frequency: 1 day/week - Total time: 5 months
OLSEN	Handball	- Session: 15-20 min - Frequency: 1 day/week - Total time: 5 months
PETERSEN	Handball	- Session: 10 min - Frequency: 1 day/week - Total time: 8 weeks
PASANEN	Hockey	- Session: 20-30 min - Frequency: 2-3 days/week (intense) alternated with 1 day/week (maintenance) - Total time: 6 months
WALDEN	Football	- Session: 15 min - Frequency: 2 days/week - Total time: 7 months
HTP	Football	- Session: 20-25 min - Frequency: 2 days/weeks (pre-season) / 1 day/week (season) - Total time: 9 months

programme to those without a specific training programme, as well as to a group of male athletes with no training in the different sports (football, volleyball and basketball). The injury rate in the female athletes was not significantly lower than in untrained male athletes; however, a significant difference was observed in the reduction of the ACL injury rate in women to which a PNMT was applied compared to those that did not follow a training programme. This prospective study revealed a lower knee injury rate in women after following a specific plyometric training programme[11].

Frappier Acceleration Training Programme (FATP)

The aim of the study by Heidt *et al.*[12] was to establish the effect achieved with a training programme in the pre-season among female football players compared to those that did not follow a programme. This study led to the conclusion that in this demographic, injuries were more frequent in the lower limbs and that the trained group had a statistically significant lower injury rate. Despite a statistically significant conclusion not being observed, they concluded that the trained group had a lower ACL injury rate. For all of the above, they concluded that physical conditioning entails a reduction in the injury rate of female football players.

Prevent Injury and Enhance Performance programme (PEP)

A clinical trial was carried out in which female football players were randomly separated into a study group and a control group. The aim was to identify the PNMT that reduced non-contact ACL injury rates. The conclusion reached was that with the PEP programme, the ACL injury rate was lower, including among athletes that had a previous history of ACL injury[13].

Knee Ligament Injury Prevention (KLIP)

A prospective study carried out over two years to assess the effects of preventive programmes on female footballers, basketball players and volleyball players at secondary school. The results suggested that a programme focusing on plyometric exercises in which the jump landing mechanism and rapid deceleration were assessed does not reduced the injury rate in female athletes if it is carried out twice a week for 20 minutes[14].

FIFA 11 Injury Prevention Programme

The aim of this randomised controlled clinical trial was to research the effect of the combination of plyometric, strength, balance and proximal control exercises on the risk of injury in adolescent female footballers. No difference was found in the general injury rate between the intervention and control group, or in the rate of any kind of injury. The outcome of the study, in which no effect was found upon applying the exercises, could be due to the poor fulfilment of the prevention programme by the study group[15].

Söderman

Prospective study based on balance exercises. The aim was to establish whether performing these kinds of exercises within a PNMT could reduce lower limb injuries in female footballers compared to those that did not follow the programme. It was established that there were no differences in the study groups, either in terms of the injury rate or the type of injury[16].

Myklebust

A prospective study that assessed the application of a training programme in which the balance exercises used focused on neuromuscular control and landing abilities. Upon finishing the study, a reduction was seen in the injury rate in elite female athletes that had completed the programme compared to those that had not followed it. It was concluded that it is possible to prevent ACL injury with the right PNTM[17].

Olsen

A randomised controlled clinical trial, which assessed the efficiency of a warm-up programme in reducing the rate of knee and ankle injuries in female handball players. The warm-up programme aimed to improve the landing technique, neuromuscular control, strength and balance. It was observed that it prevented injuries and therefore it was established that it should be introduced as part of the PNMT[18].

Petersen

A retrospective study that assessed the effects of PNMT on ankle and knee injury rates, applied to female handball players. It was concluded that neuromuscular training is appropriate and effective in preventing knee and ankle injuries in female handball players[19].

Pasanen

A PNMT that aimed to improve motor skills, body control, as well as to prepare and activate the neuromuscular system. The aim was to prevent non-contact knee injuries, applying the study to female hockey players. After performing the study, the conclusion was reached that the preventive programme is effective and can be recommended in the weekly training routines of these athletes[20].

Walden

A randomised clinical trial that assessed a preventive programme focusing on proximal control exercises, balance and the correct alignment of the knee in football players. It concluded that the warm-up programme statistically significantly reduced the ACL injury rate[21].

"Harmoknee" training programme (HTP)

A clinical trial was carried out on a multifaceted preventive programme in football players. It was concluded that the knee injury rate among young football players was reduced with physical exercise, if this was applied alongside an appropriate sporting education[22].

Furthermore, in a study recently published by Zebis *et al.*[23], the reduction of the ACL injury rate was assessed upon performing rapid direction changes, by applying preventive programmes. A clinical trial was performed with a sample group of 40 female basketball or handball players, aged between 15-16 years, in which 20 were randomly assigned to a control group and the remaining 20 to a neuromuscular training group. The preventive programme consisted of warm-up exercises, analysing the activity (isometric contraction) of the vastus lateralis (VL), of the semitendinosus (ST) and of the biceps femoris using an electromyography (EMG) for 12 weeks. Differences were observed between the follow-up groups in VL-ST activity (43% of difference between the groups; CI 95%, 32% to 55%)[23].

Conclusions

Female athletes suffer from approximately 3 times more ACL injuries in basketball and in football than male athletes. However, female athletes that perform sports to a high performance level have less frequent injuries as they have better neuromuscular control over their lower limbs.

Prevention programmes focus on neuromuscular training and have been shown to cause a statistically significantly reduction in injury rate in both football and handball. However, reductions in injury rates in basketball are not statistically significant. Current analyses of sub-groups indicate that strength programmes, proximal control exercises and plyometric exercises increase the effectiveness of ACL injury reduction among female athletes.

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Review

Breathing at extreme altitudes. Scientific projects "EVEREST" (First part)

Eduardo Garrido[1,2], Oriol Sibila[3], Ginés Viscor[2,4]

[1]Servicio de Hipobaria y Fisiología Biomédica. Universidad de Barcelona. [2]Instituto de Estudios de Medicina de Montaña. Barcelona. [3]Servicio de Neumología. Hospital de la Santa Creu i Sant Pau. Barcelona. [4]Departamento de Biología Celular, Fisiología e Inmunología. Universidad de Barcelona.

Received: 03.03.2017
Accepted: 31.05.2017

Summary

Climbing to the highest height on Earth, the Mt. Everest (8.848 m), without supplementary oxygen equipment involves a physiological demand that is close to the maximum human tolerance. Exposures at extreme altitudes drastically conditions lung function, stores of oxygen and physical performance. This review brings interesting aspects about respiration, blood gases and aerobic exercise reported by those scientific projects that have carried out physiological measurements between 8,000 m and 8,848 m above sea level, under real or simulated altitude: the Operations "Everest I" (1946), "Everest II" (1985), "Everest III-COMEX" (1997), and the Expeditions "AMREE" (1981), "British 40th Anniversary Everest" (1993), and "Caudwell Xtrem Everest" (2007). These fascinating scientific research events, along with other outstanding biomedical expeditions performed above 5,500 m, like especially the "Silver Hut" (1960-61), "Italiana all'Everest" (1973), and "British Everest Medical" (1994), including those pioneer scientific reports made on the XIX century until the most recent research projects performed, have laid the foundations and knowledge on the human tolerance to such extreme levels of hypobaric hypoxia, where the lung, breathing and respiratory chain takes on a major role requiring fine physiological adjustments to ensure cellular oxygenation. Geophysical aspects, climatic factors and other environmental conditions that limit the biological viability and can affect the respiratory health of climbers on the upper troposphere zone at the subtropical latitude where that mountain is located are likewise reviewed and analyzed. Every year, hundreds of climbers try to reach the top of Mt. Everest, but only a few of them achieved their goal without inhaling supplemental oxygen, including some exceptionally gifted Sherpa natives, protagonist on unsuspected exploits in the highest mountain on terrestrial surface, whose summit touch the physiological limit of survival for the human being.

Key words:
Altitude. Oxygen uptake. Hypoxia. Mountaineering. Atmospheric pressure. Respiration.

Respirar en altitudes extremas. Proyectos científicos "EVEREST" (Primera parte)

Resumen

Escalar el punto más alto de la Tierra, el Mt. Everest (8.848 m), sin equipos de oxígeno conlleva una demanda fisiológica que está próxima a la máxima capacidad de tolerancia humana. Exponerse a altitudes extremas condiciona drásticamente la función pulmonar, el nivel de oxígeno y el rendimiento físico. Esta revisión reúne interesantes aspectos respiratorios, de gases sanguíneos y ejercicio aeróbico aportados por aquellos proyectos científicos que han llevado a cabo mediciones fisiológicas entre 8.000 m y 8.848 m, en altitud real o simulada, como las Operaciones "Everest I" (1946), "Everest II" (1985) y "Everest III-COMEX" (1997), y las Expediciones "AMREE" (1981), "British 40th Anniversary Everest" (1993) y "Caudwell Xtrem Everest" (2007). Estos fascinantes eventos de investigación, junto a otros destacados proyectos biomédicos realizados a más de 5.500 m, muy especialmente las Expediciones "Silver Hut" (1960-61), "Italiana all'Everest" (1973) y "British Everest Medical" (1994), incluyendo aquellas pioneras observaciones científicas llevadas a cabo en el s.XIX hasta los más recientes proyectos de investigación realizados, han sentado las bases del conocimiento sobre la tolerancia humana ante niveles de hipoxia hipobárica extrema, donde el pulmón y la cadena respiratoria adquieren un trascendente protagonismo requiriéndose de finos ajustes fisiológicos que garanticen la oxigenación celular. Asimismo, se exponen ciertos aspectos geofísicos, factores climáticos y otros condicionantes ambientales que limitan la viabilidad biológica y pueden afectar la salud respiratoria de los alpinistas situados en las cotas superiores de la troposfera a la latitud subtropical donde se encuentra ubicada dicha montaña. Actualmente cientos de alpinistas intentan alcanzar la cumbre del Mt. Everest todos los años, pero solo algunos consiguen su objetivo sin inhalar oxígeno suplementario, entre ellos algunos excepcionalmente dotados nativos Sherpa, protagonistas de insospechadas hazañas en la montaña más elevada de la superficie terrestre, cuya cima roza el límite fisiológico de supervivencia para el ser humano.

Palabras clave:
Altitud. Consumo de oxígeno. Hipoxia. Montañismo. Presión atmosférica. Respiración.

Correspondence: Eduardo Garrido
E-mail: eduardogarrido@movistar.es

Introduction

Since the peak of Mount Everest (8,848 m) was first reached in 1953 by Edmund Hillary and Tenzing Norgay, its peak has been scaled almost nine thousand times. Both pioneers used oxygen equipment, as climbing the peak with the provision of 3-4 L•min^{-1} of this gas[1] was equivalent to having an oxygen concentration in the body similar to that registered at an altitude 3,000 m lower[2]. The miraculous increase of physical capacity provided by inhaling the contents of the gas cylinders led the native Sherpas to call it "English air"[3]. It was not until 1978 that Reinhold Messner and Peter Habeler set off to conquer the mountain without oxygen equipment, just over half a century after Edward Norton managed to scale 8,570 m in the same way in 1924[4]. This fact proves that the difficulty of ascending the final 300 m of the Everest pyramid is due to environmental factors rather than technical ones. Today, mountaineers that attack the final stretch of this mountain usually inhale 2-3 L•min^{-1} of supplementary oxygen[5], but over the history of this mountain, less than 5% of climbs have been performed breathing environmental air alone. Trying to climb this way, even with previous altitude acclimatisation, entails a three-fold risk of death: Previous data indicates that 8.3% of mountaineers that reach the peak without using oxygen cylinders die, compared to the 3% of climbers that die whilst using them[6]. In general, the greatest probability of successfully scaling Mount Everest and surviving is registered among mountaineers aged between 30-35 years[7]. Having previous experience in climbing at extreme altitude does not seem to reduce mortality rates on this mountain[8]. A third of fatal accidents are associated with hypoxia and low temperatures, generally occurring during the descent, and are attributed to serious traumatisms due to falls, probably furthered by brain swelling which induces neurocognitive dysfunctions, as well as fainting due to extreme fatigue[9,10]. It is worth highlighting that rapid exposure to such extreme heights without previous acclimatisation would cause a severe deterioration of the central nervous system, with the resulting loss of consciousness in less than 2-3 minutes[11].

The lungs are one of the most affected organs at high altitude. Breathing and pulmonary circulation take on a decisive role in adapting to hypoxia, as they must ensure cell oxygen demands are met, and during physical exercise one of the most critical physiological states is reached. Likewise, the respiratory channel is directly exposed to the other harmful environmental altitude factors, such as low temperatures and relative humidity or the presence of ozone. Knowledge available to us today regarding human exposure to height between 8,000m and 8,848m has been provided by numerous scientific publications, essentially produced by the following ambitious research projects carried out in hypobaric chambers: the US 1946 *"Operation Everest I"* and the 1985 *"Operation Everest II"*; the French *"Operation Everest III-COMEX"* in 1997; as well as the following scientific expeditions carried out on Mount Everest: *"American Medical Research Expedition to Everest"* in 1981, the *"British 40th Anniversary Everest Expedition"* in 1993, and the British *"Caudwell Xtrem Everest"* de 2007[12-17]. Despite not having reached the same altitudes, particularly outstanding are the North American scientific expedition *"Silver Hut"* from 1960-1961, the *"Spedizione Italiana all'Everest"* of 1973, and the *"British Everest Medical Expedition"* of 1994[18-20]. Mention should also be made of more recent projects, such as the 1998-1999 North American *"Everest Extreme Expedition",* the 2006 German "*Everest MedEx",* and the 2013 British *"Xtreme Everest 2"*[21-23]. Finally, other noteworthy trials include the one performed in England in 1954 in a decompression chamber, simulating extreme altitudes[24], as well as two pioneering experiments carried out at the end of the 19th century, also in a hypobaric chamber, achieving depressurisation levels similar or greater to those felt at the peak of Mount Everest: in Italy in 1898 and in France in 1874[25,26], with the latter trial later called *"Operation Everest o"* [27]. Similarly, in the first half of the 20th century, two pioneering British alpine expeditions are also noteworthy, carried out on Everest in 1933 and 1938, respectively; despite their main objective not being specifically scientific, parameters were obtained and biomedical samples were taken at extreme altitudes[28,29]. Table 1 details some aspects of all of these biomedical projects and alpine-scientific expeditions.

The main objective of this review article is to bring together and display essential aspects related to respiratory function, the transport of oxygen and/or maximum aerobic power that have been reported in these scientific projects. It is also an opportunity to display and analyse some geo-physical, climatic and meteorological aspects that could interfere in the human capacity to reach the peak of Mt. Everest without an oxygen supplement. To do this, an exhaustive literary search was performed on the PubMed database, with the main search strategy being the following combination: *"Respirat*[TW] OR Everest[TW] NOT mitral AND aerob*".* In addition, the most important recent editions of international Mountain Medicine books and documents to date were reviewed. Furthermore, certain logistical and technical data from some of the alpine expeditions was obtained from official Internet websites published exclusively by the organisers of the respective events.

Atmospheric pressure, respiratory and blood gases at the peak of Mount Everest

The final pyramid that forms the great geological mass of Everest is located in the so-called "death zone"[30]. At its peak the atmospheric pressure (PB) is very low, which entails a severely reduced (~43 mmHg)[31,32] inspired oxygen pressure (PiO_2), but even so, it is possible to climb it without oxygen equipment. This is particularly true during the hottest part of the year, as the PB at the peak is greater (~251-255 mmHg)[33,34]. Pulmonary respiration must be able to ensure the mitochondrial cell respiratory chain, but at 8,848 m of altitude, the partial pressure of oxygen of arterial blood (PaO_2) is just 27-31 mmHg[13,14,31,32,35], average PaO_2 values of below 25 mmHg have even been registered upon descending from the peak[36]. However, the partial pressure of carbon dioxide ($PaCO_2$), secondary to pulmonary hyperventilation, helps keep the partial pressure of oxygen in arterial blood (PaO_2) stable at heights over ~7.000 m[32].

Table 1. Main biomedical scientific projects performed on humans, and mountaineering expeditions in which lung function and/or respiratory gas samples, blood gases and/or ergospirometry samples have been taken at altitudes exceeding 5,300m in the area of Mount Everest, as well as using hypobaric chambers or hypoxic mixes that have simulated very extreme altitudes similar to those of this mountain (8,848m).

Biomedical project or expedition	Year	Country	Scientific director	Max. altitude reached (m)	Max. altitude (m)* samples/parameters
Operation Everest 0 (a)	1874	France	Paul Bert	8,840	8,840
Camera Decompressione (b)	1898	Italy	Angelo Mosso	11,650	10,800
Everest Expedition (c)	1933	England	Raymond Greene	8,580	7,840
Mt. Everest Expedition (d)	1938	England	Charles Warren	8,300	6,400
Operation Everest I (e)	1946	USA	Charles Houston & Richard Riley	15,420	8,848
Himalayan Exped. to Mt. Everest (f)	1953	England	Griffith Pugh & Michael Ward	8,848	7,325
Decompression Chamber (g)	1954	England	John Cotes	8,240	8,240
Silver Hut (h)	1960-61	England	Griffith Pugh	8,362	7,830
Spedizione Everest (i)	1973	Italy	Paolo Cerretelli	8,848	6,500
AMREE (j)	1981	USA	John West	8,848	8,848
Operation Everest II (k)	1985	USA	Charles Houston & John Sutton	9,150	8,848
40th Anniversary Everest Exped. (l)	1993	England	Andrew Peacock & Peter Jones	8,848	8,000
Everest Medical Expedition (m)	1994	England	David Collier	8,848	8,000
Operation Everest III-COMEX (n)	1997	France	Jean-Paul Richalet	8,848	8,848
Everest Extreme Expedition (o)	1998-99	USA	Peter Angood	8,848	6,100
Everest MedEx (p)	2006	Germany	Klaus Mees	8,848	8,763
Caudwell Xtreme Everest (q)	2007	England	Michael Grocott	8,848	8,400
Xtreme Everest 2 (r)	2013	England	Daniel Martin & Edward Gilbert-Kawai	5,300	5,300

*Maximum real or calculated altitude depending on the intra-chamber pressure and/or FiO_2 applied. **(a)** Name given to the first trial performed in a hypobaric chamber; with pressures of below 410 mmHg, equivalent to >5,000 m, oxygen-enriched mixtures were inhaled intermittently, though the FiO_2 used was not specified. **(b)** Project in hypobaric chamber without a specific name; a FiO_2=29.2% to 11,650 m was used. **(c-d)** Not specifically scientific expeditions, though pulmonary gas samples were taken up to the specified respective altitudes. **(e)** The project refers to standard atmosphere in a hypobaric chamber; pulmonary gas samples with PiO_2=43 mmHg, equivalent to 8,848 m. **(f)** Not specific scientific expedition but ventilator parameters were obtained to 6,470 m and pulmonary gas samples to 7,325 m. **(g)** Project in hypobaric chamber with no specific name; belonging to *"The Mount Everest oxygen mask-Medical Research Council High Altitude Committee"*. **(h)** Also entitled *"Himalayan Scientific and Mountaineering Expedition"*; ergometries performed at 7,430 m; pulmonary gas samples at 7,830 m. **(i)** Ergometries performed at 5,350 m and gas samples at 6,500 m. **(j)** International acronym for the *"American Medical Research Expedition to Mt. Everest"*; ergometries performed at 6,300 m with an FiO_2=14%, equivalent to 8,848 m; pulmonary gas samples obtained at a real altitude of 8,848 m. **(k)** The project refers to standard atmosphere in a hyperbaric chamber; ergometries performed at 9,150 m with a PiO_2=43 mmHg, equivalent to 8,848 m. **(l)** Not specifically scientific expedition though alveolar gas and SaO_2 samples were obtained. **(m)** Also called *"MedEx"*; alveolar gas and SaO_2 samples are obtained **(n)** Ergometry performed at 7,000 m in hypobaric chamber; pulmonary gas samples with a PiO_2=43 mmHg, equivalent to 8,848 m**.** **(o)** Also called *"Yale-NASA Mt. Everest Telemedicine Project"* or *"E3"*. **(p)** Also called *"Ludwig Maximilians University Expedition to Mt. Everest"*; polysomnograph is obtained at 7,500 m and SaO_2 at 8,763 m. **(q)** Ergometry performed at 7,950 m and arterial gasometry at 8,400 m. **(r) P**roject depending on the *"Caudwell Xtreme Everest Hypoxia Research Consortium"*; numerous spirometry samples are obtained.

According to the diagram by Rahn and Otis, the average PaO_2 usually remains over 35 mmHg in a subject acclimatised to hypoxia[37] (Figure 1), but the samples obtained at the peak of Mt. Everest, or simulating the same height, oscillate between 21-37.6 mmHg with $PaCO_2$ values between 7.5-14.2 mmHg, demonstrating the extreme hypocapnic hypoxia that occurs under these environmental conditions[14,31,32,35,38,39]. Despite all of these surprising biological figures, the acid-base balance remains within a physiologically viable range, even when faced with such intense respiratory alkalosis with a blood pH that reaches values of up to 7.783[2]. Due to the Bohr effect, this alkalosis reduces the P50 of the oxyhemoglobin to values of ~19 mmHg, achieving a resting arterial oxygen saturation (SaO_2) of 58-70% at 8,848 m[31,32], despite values having been registered of ~50% between 8,400 and 8,763 m[22,36]. Still breathing oxygen at the rate of 2 L•min^{-1}, some subjects at 8,000m do not achieve SaO_2 over 80%[16]. Table 2 displays some average oxygen gas values at sea level and at the peak of Mount Everest.

Figure 1. Alveolar oxygen pressure in correlation to alveolar carbon dioxide pressure under exposure to acute or chronic hypoxia. Based on Rahn and Otis[37].

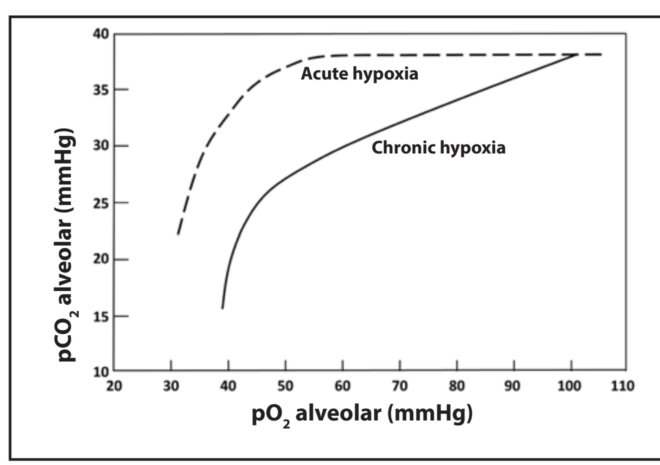

Table 2. Physiological oxygen values at the altitude equivalent to Mount Everest (8,848 m) when resting and during maximum physical exercise. The approximate average values are displayed, calculated based on the data reported from various studies[13-15,31,32,38,39,48,84].

Altitude (m)	PB (mmHg)	PiO_2 (mmHg)	PAO_2 (mmHg)	PaO_2 (mmHg)	SaO_2 (%)	$maxVO_2$ (%)
Sea level*	760	149	100	95	97	100
8,848 resting	252	43	31	29	64	—
8,848 exercise	252	43	35	25	41	25

PB: atmospheric pressure (model atmosphere); PiO_2, PAO_2, PaO_2: partial pressure of oxygen at an inspiratory, alveolar and arterial level, respectively; SaO_2: arterial oxygen saturation; $maxVO_2$: expected rate of maximum oxygen uptake. *Resting PAO_2, PaO_2 and SaO_2 values.

It is worth highlighting that at altitudes near 8,000 m, there are already significant physiological differences between an environmental condition of normobaric hypoxia and hypobaric hypoxia[40], or between measurements carried out at real altitudes on the mountain and the equivalent altitude in a hypobaric chamber[41]. PiO_2 values close to those estimated at the peak of Everest have been registered at 8,400 m and even extreme figures of PaO_2 (19.1 mmHg) have been registered at this height upon descending from the peak[36,42]. As such, it should be considered that there are differences between the type of "international standard atmosphere of aviation" or the "model atmosphere", as in Operation Everest I and II standard atmosphere was used as a benchmark. The intra-chamber PB reached in Operation Everest II (240 mmHg) was equivalent to 9,150 m, a discrepancy corrected by the fraction of inspired oxygen flow (FiO_2) of 22%, a fact that conferred a PiO_2 of 42-43 mmHg[39,43] corresponding to that at the peak of Everest[34].

Lung adaptation and ventilatory function at extreme altitudes

Hypoxia is a powerful pulmonary arteriolar vasoconstrictor, distributing blood throughout the entire vascular bed of the lungs. This physiological response is designed to optimise the ventilation-perfusion (VA/Q) ratio and therefore, gas exchange, despite a major increase of vascular resistance causing pulmonary hypertension (HTP)[44-47]. However, at very extreme altitudes, the existence of a certain imbalance in the VA/Q and the limitation of the alveolar-capillary diffusion seem to play a major role in reducing the PaO_2[45,48,49] and this seems to partly explain the great increase of pulmonary blood volume and the presence of interstitial oedema, precisely due to the rapid hypertension caused by hypoxic vasoconstriction[50]. This situation can set off a spiral of negative effects, in which interstitial oedema, low SaO_2, hypoxic vasoconstriction and HTP can be triggered and worsen reciprocally, causing massive pulmonary oedema with mortal consequences. These facts, as well as the effect caused by hypoxia on the respiratory muscles, are decisive in the early appearance of severe fatigue at great altitude[51].

A determining factor in correctly adapting to altitude is to have a fast and intense hypoxic ventilatory response (HVR)[52-54]. However, in elite mountaineers that are used to high altitudes, a more attenuated HVR seems to be beneficial. Despite this fact causing a certain degree of controversy[55], a lower HVR with greater ventilatory efficiency could help mountaineers to achieve an increased ventilatory store at extremely high altitudes[56]. Today, it is accepted that tolerance to altitude increases with age, and in large part, this is due to an improvement in HVR among males, in accordance with a study performed on 4,675 individuals (2,789 men and 1,886 women aged between 14-85 years), but the cardiac response to hypoxia reduced with age in both sexes. Similar results were found in the same study, in 30 subjects examined with an average interval of 10.4 years, revealing a reduction in heart rate and an increase in HVR with age. These adaptive responses were less marked or absent in post-menopausal women with no physical training[57].

In turn, the maximum voluntary ventilation (MVV) increases progressively with altitude, reaching values of over 200 L•min^{-1} at 8,240 m, due to the low density of the tracheal air. At 8,848 m it is just ~30% compared to at sea level[24]. During a simulated ascent to the peak of Mount Everest, forced vital capacity (FVC) reduces progressively to 14%, the mid-expiratory flow (MEF25-75%) increases to 82%, but the forced expiratory volume in the first second (FEV1) does not reveal significant changes regarding the low level[50]. Other studies do detect slight FEV1 reductions, even at a much lower altitude[58], revealing a direct correlation between subjects that reveal lower FVC and FEV1 levels with lower SaO_2 levels[59]. The FVC reduction is probably due to changes in pulmonary blood volume and/or the presence of interstitial oedema[60]. The resting expiratory volume (EV) at 7,500m is 23 L•min^{-1}[61] and at the peak of Everest it is 40 L•min^{-1}, i.e., some 5 times greater compared to sea level[62]. Mountaineers take on the final pyramid of this mountain at an extraordinarily slow rate, and despite this, they need to keep an EV of ~100-120 L•min^{-1}, hyperventilation characterised by shallow rapid breathing, given that their respiratory frequencies (RF) reach 60 breaths•min^{-1}[48]. But if the exercise is maximum intensity during the ascent to 8,848 m, some subjects can even exceed 200 L•min^{-1}[39] and 80 breaths•min^{-1}[48]. However, such extreme hyperventilation, even with a lower RF, is more common during maximum exertion performed with PiO_2 corresponding to altitudes between 5,000 and 7,000 m, as at higher altitudes the capacity for exercise is considerably depleted[48]. The isocapnic and hypercapnic ventilatory response to hypoxia only increases slightly at altitudes over 8,000 m[61]. It is worth highlighting the high rate of apnoea-hypopnea phases that appear in sleep at high altitudes, and also that at 7,500 m up to 148 phases•h^{-1} have been registered, reaching SaO_2 values of 48%, lower than those recorded in a waking state at 8,763 m in the same subject[22].

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FUTBOL IQ

Por: Dan Blank
Edita: Ediciones Tutor-Editorial El Drac.
Impresores 20. P.E. Prado del Espino. 28660 Boadilla del Monte. Madrid.
Telf. 915 599 832 – Fax: 915 410 235
E-mail: info@edicionestutor.com Web: www.edicionestutor.com
Madrid 2017, 128 páginas, P.V.P: 12,50 euros

Este es el primer libro escrito específicamente para jugadores. Con un lenguaje comprensible y de lectura entretenida y rápida, ofrece soluciones sencillas a los errores y problemas más comunes en el fútbol. Aporta, además, recursos tácticos y estrategias para ayudar a los jugadores jóvenes a pensar y convertirles en futbolistas inteligentes.

Su autor tiene más de 20 años de experiencia como entrenador y ha catalogado los errores y problemas más comunes del fútbol ofreciendo soluciones sencillas. El libro es un compendio de las decisiones futbolísticas inteligentes destinadas a allanar el camino del aprendizaje y a mejorar el resultado de los equipos. Se explica de modo coloquial y sin tecnicismos lo que el autor ha "soportado" en años de estrés entrenando.

FUNDAMENTOS DEL ENTRENAMIENTO DE ULTRAFONDO

Por: Jason Koop y Jim Rutberg
Edita: Ediciones Tutor-Editorial El Drac.
Impresores 20. P.E. Prado del Espino. 28660 Boadilla del Monte. Madrid.
Telf. 915 599 832 – Fax: 915 410 235
E-mail: info@edicionestutor.com Web: www.edicionestutor.com
Madrid 2017, 352 páginas, P.V.P: 31,50 euros

Los ultramaratones no son simplemente maratones más largos. El ultrafondo plantea retos exclusivos en cuanto al entrenamiento y la nutrición, y exige un enfoque totalmente nuevo de la preparación. Este manual es una revolución en el entrenamiento; ha sido probado en competición, se basa en las investigaciones más punteras y en años de experiencia de su autor como entrenador de los mejores ultrafondistas.

Con este libro se pueden adquirir los conocimientos científicos para potenciar al máximo el rendimiento en ultramaratones; a planificar la temporada; el sistema ADAPT para resolver las crisis durante las carreras; los errores habituales y el modo de resolverlos; el entrenamiento interválico para centrarse en la carga de trabajo, dar un salto cuantitativo en las mejoras, reducir el riesgo de lesiones y correr más rápido; estrategias sencillas y eficaces para el aporte energético y la hidratación; y el modo de alcanzar el objetivo, ¡tanto si es terminar una carrera como ganarla!

GUÍA PRÁCTICA DE FISIOLOGÍA DEL EJERCICIO

Por: Bob Murray y W. Larry Kenney
Edita: Ediciones Tutor-Editorial El Drac.
Impresores 20. P.E. Prado del Espino. 28660 Boadilla del Monte. Madrid.
Telf. 915 599 832 – Fax: 915 410 235
E-mail: info@edicionestutor.com Web: www.edicionestutor.com
Madrid 2017, 208 páginas, P.V.P: 35 euros

Esta guía práctica ofrece un contenido fácil de seguir y conduce a sus lectores a través de los conceptos científicos de la fisiología del ejercicio. El texto sirve para aplicar estos conceptos al diseño de programas de ejercicios, dando a los entrenadores personales, a los especialistas de la fuerza y del acondicionamiento de la forma física, así como a otros profesionales del *fitness* y la salud, unos recursos accesibles para utilizarlos con sus clientes y deportistas de cualquier nivel.

El texto se complementa con ilustraciones artísticas de un detallado trabajo médico que facilita la comprensión de los complejos sistemas fisiológicos. Estos sistemas se aplican a la práctica deportiva a través de explicaciones de los ejercicios que benefician a sistemas orgánicos específicos, así como a grupos de población fuera del rango habitual de la competición deportiva: niños, mayores y mujeres embarazadas. Esta guía capacitará a los profesionales para seleccionar y explicar a sus clientes los ejercicios y regímenes de actividad física más apropiados a cada uno.

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54º Congreso Argentino de Ortopedia y Traumatología	2-5 Diciembre Buenos Aires (Argentina)	web: http://www.congresoaaot.org.ar
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12th World Congress of the International Society of Physical and Rehabilitation Medicine (ISPRM)	8-12 Julio París (Francia)	web: http://isprm2018.com/
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XXXV Congreso Mundial de Medicina del Deporte	12-15 Septiembre Rio de Janeiro (Brasil)	web: www.fims.org
5th International Scientific Tendinopathy Symposium (ISTS)	27-29 Septiembre Groningen (Países Bajos)	web: http://ists2018.com/
VII Congreso Asociación Hispanoamericana de Médicos del Fútbol	Octubre Lima (Perú)	web: http://hispamef.com/
28º Congress European Society for surgery of the shoulder and the elbow (SECEC-ESSSE)	Ginebra (Suiza)	web: www.secec.org

2019		
12th Biennial ISAKOS	12-16 Mayo Cancún (México)	web: www.isakos.com
24th Annual Congress of the European College of Sport Science	3-6 Julio Praga (Rep. Checa)	E-mail: office@sport-science.org
14th International Congress of shoulder and elbow surgery (ICSES)	17-20 Septiembre Buenos Aires (Argentina)	web: www.icses2019.org
XV Congreso Nacional de Psicología de la Act. Física y del Deporte	Zaragoza	web: www.psicologiadeporte.org
2020		
25th Annual Congress of the European College of Sport Science	1-4 Julio Sevilla	E-mail: office@sport-science.org
XXXVI Congreso Mundial de Medicina del Deporte	24-27 Septiembre Atenas (Grecia)	web: www.globalevents.gr
2021		
26th Annual Congress of the European College of Sport Science	7-10 Julio Glasgow (Reino Unido)	E-mail: office@sport-science.org

Cursos *on-line* SEMED-FEMEDE

Curso "ENTRENAMIENTO, RENDIMIENTO, PREVENCIÓN Y PATOLOGÍA DEL CICLISMO"

Curso dirigido a los titulados de las diferentes profesiones sanitarias y a los titulados en ciencias de la actividad física y el deporte, destinado al conocimiento de las prestaciones y rendimiento del deportista, para que cumpla con sus expectativas competitivas y de prolongación de su práctica deportiva, y para que la práctica deportiva minimice las consecuencias que puede tener para su salud, tanto desde el punto de vista médico como lesional.

Curso "ELECTROCARDIOGRAFÍA PARA MEDICINA DEL DEPORTE"

ACREDITADO POR LA COMISIÓN DE FORMACIÓN CONTINUADA (ON-LINE 15/10/2015 A 15/10/2016) CON 4,81 CRÉDITOS

Curso dirigido a médicos destinado a proporcionar los conocimientos específicos para el estudio del sistema cardiocirculatorio desde el punto de vista del electrocardiograma (ECG).

Curso "FISIOLOGÍA Y VALORACIÓN FUNCIONAL EN EL CICLISMO"

Curso dirigido a los titulados de las diferentes profesiones sanitarias y a los titulados en ciencias de la actividad física y el deporte, destinado al conocimiento profundo de los aspectos fisiológicos y de valoración funcional del ciclismo.

Curso "AYUDAS ERGOGÉNICAS"

Curso abierto a todos los interesados en el tema que quieren conocer las ayudas ergogénicas y su utilización en el deporte.

Curso "CARDIOLOGÍA DEL DEPORTE"

ACREDITADO POR LA COMISIÓN DE FORMACIÓN CONTINUADA (VÁLIDA DEL 15/10/2016 AL 15/10/2017) CON 8,78 CRÉDITOS

Fecha límite de inscripción: 15/06/2017

Curso dirigido a médicos destinado a proporcionar los conocimientos específicos para el estudio del sistema cardiocirculatorio desde el punto de vista de la actividad física y deportiva, para diagnosticar los problemas cardiovasculares que pueden afectar al deportista, conocer la aptitud cardiológica para la práctica deportiva, realizar la prescripción de ejercicio y conocer y diagnosticar las enfermedades cardiovasculares susceptibles de provocar la muerte súbita del deportista y prevenir su aparición.

Curso "ALIMENTACIÓN, NUTRICIÓN E HIDRATACIÓN EN EL DEPORTE"

Curso dirigido a médicos destinado a facilitar al médico relacionado con la actividad física y el deporte la formación precisa para conocer los elementos necesarios para la obtención de los elementos energéticos necesarios para el esfuerzo físico y para prescribir una adecuada alimentación del deportista.

Curso "ALIMENTACIÓN Y NUTRICIÓN EN EL DEPORTE"

Curso dirigido a los titulados de las diferentes profesiones sanitarias (existe un curso específico para médicos) y para los titulados en ciencias de la actividad física y el deporte, dirigido a facilitar a los profesionales relacionados con la actividad física y el deporte la formación precisa para conocer los elementos necesarios para la obtención de los elementos energéticos necesarios para el esfuerzo físico y para conocer la adecuada alimentación del deportista.

Curso "ALIMENTACIÓN Y NUTRICIÓN EN EL DEPORTE" Para Diplomados y Graduados en Enfermería

ACREDITADO POR LA COMISIÓN DE FORMACIÓN CONTINUADA (NO PRESENCIAL 15/12/2015 A 15/12/2016) CON 10,18 CRÉDITOS

Curso dirigido a facilitar a los Diplomados y Graduados en Enfermería la formación precisa para conocer los elementos necesarios para la obtención de los elementos energéticos necesarios para el esfuerzo físico y para conocer la adecuada alimentación del deportista.

Curso "CINEANTROPOMETRÍA PARA SANITARIOS"

Curso dirigido a sanitarios destinado a adquirir los conocimientos necesarios para conocer los fundamentos de la cineantropometría (puntos anatómicos de referencia, material antropométrico, protocolo de medición, error de medición, composición corporal, somatotipo, proporcionalidad) y la relación entre la antropometría y el rendimiento deportivo.

Curso "CINEANTROPOMETRÍA"

Curso dirigido a todas aquellas personas interesadas en este campo en las Ciencias del Deporte y alumnos de último año de grado, destinado a adquirir los conocimientos necesarios para conocer los fundamentos de la cineantropometría (puntos anatómicos de referencia, material antropométrico, protocolo de medición, error de medición, composición corporal, somatotipo, proporcionalidad) y la relación entre la antropometría y el rendimiento deportivo.

Más información:
www.femede.es

Guidelines of publication Archives of Sports Medicine

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   a. **ORIGINALS REPORTS**: It will contain an introduction, which will be brief and will contain the intention of the work, written in such a way that the reader can understand the following text.
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   Results: They report, not interpret, the observations made with the material and method used. This information can be published in detail in the text or by tables and figures. Information given in the tables or figures must not be repeated in the text.
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Campaña de aptitud física, deporte y salud

La **Sociedad Española de Medicina del Deporte**, en su incesante labor de expansión y consolidación de la Medicina del Deporte y, consciente de su vocación médica de preservar la salud de todas las personas, viene realizando diversas actuaciones en este ámbito desde los últimos años.

Se ha considerado el momento oportuno de lanzar la campaña de gran alcance, denominada **CAMPAÑA DE APTITUD FÍSICA, DEPORTE Y SALUD** relacionada con la promoción de la actividad física y deportiva para toda la población y que tendrá como lema **SALUD – DEPORTE – DISFRÚTALOS**, que aúna de la forma más clara y directa los tres pilares que se promueven desde la Medicina del Deporte que son el practicar deporte, con objetivos de salud y para la mejora de la aptitud física y de tal forma que se incorpore como un hábito permanente, y disfrutando, es la mejor manera de conseguirlo.

UCAM Universidad Católica San Antonio de Murcia

Campus de los Jerónimos,
Nº 135 Guadalupe 30107

(Murcia) - España

Tlf: (+34)968 27 88 01 · info@ucam.edu

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