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Departamento de Ciencias Biomédicas

Efecto de la realización de diferentes programas de entrenamiento sobre la inflamación asociada al envejecimiento: papel de la vía de señalización de los receptores tipo Toll 2 y 4

Memoria presentada por la Licenciada en Biotecnología Paula Rodríguez Miguélez,

para la obtención del grado de Doctor por la Universidad de León

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INFORME DE LOS DIRECTORES DE LA TESIS

Las Dras. Mar Almar Galiana y María José Cuevas González, directoras de la Tesis Doctoral titulada "Efecto de la realización de diferentes programas de entrenamiento sobre la inflamación asociada al envejecimiento: papel de la vía de señalización de los receptores tipo Toll 2 y 4" realizada por la Lcda. Paula Rodríguez Miguélez, en el programa de doctorado de Biomedicina, informan favorablemente el depósito de la misma, dado que reúne las condiciones necesarias para su defensa.

Lo que firman, en León a __ de noviembre de 2013.

Las directoras de la Tesis Doctoral

Fdo. Dra. Mar Almar Galiana

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ADMISIÓN A TRÁMITE DE LA TESIS DOCTORAL

El órgano responsable del programa de doctorado Biomedicina en su reunión celebrada el día ___ de noviembre de 2013 ha acordado dar su conformidad a la admisión a trámite de lectura de la Tesis Doctoral titulada "Efecto de la realización de diferentes programas de entrenamiento sobre la inflamación asociada al envejecimiento: papel de la vía de señalización de los receptores tipo Toll 2 y 4", dirigida por las Dras. Mar Almar Galiana y María José Cuevas González, y elaborada por la Lcda. Paula Rodríguez Miguélez, cuyo título en inglés es: "Effect of different training programmes on the age-related inflammation: Role of Toll like receptor 2 and 4 signalling pathways".

Lo que firmo, en León a ___ de noviembre de 2013

La Secretaria,

Fdo. Dra. Mar Almar Galiana

VºBº

Presidente de la Comisión Académica,

Fdo. Dra. Mª Jesús Tuñón González

Parte de los resultados expuestos en la presente memoria han sido objeto de las siguientes comunicaciones a congreso:

"**Resistance training decreases TLR4 expression via Myeloid differentiation factor 88-dependent pathway in elderly subjects**". P. Rodríguez-Miguélez, R. Fernández-Gonzalo, M. Almar, P.S. Collado, J. González-Gallego, J.A. de Paz, M.J. Cuevas. *18th Annual Congress of the European College of Sport Science (ECSS)*. Barcelona (España), junio 2013. Publicado en, European Database of Sport Science, 2013.

"**El entrenamiento de fuerza disminuye la inflamación sistémica en una población de edad avanzada**" P.S. Collado, P. Rodríguez-Miguélez, M. Almar, R. Fernández-Gonzalo, J. González-Gallego, J.A. de Paz, M.J. Cuevas. *V Congreso Nacional de la Federación Española de Medicina del Deporte (FEMEDE).* Santander (España), noviembre 2012. Publicado en Archivos de Medicina del Deporte, 2012, 29, 151: 898

"**Efecto de diferentes tipos de entrenamiento sobre parámetros hematológicos y bioquímicos en personas de edad avanzada**" P.S. Collado, M.J. Cuevas, M. Almar, P. Rodríguez-Miguélez, R. Fernández-Gonzalo, J.A. de Paz, J. González-Gallego. *IV Congreso Nacional de la Federación Española de Medicina del Deporte (FEMEDE).* Reus (España), noviembre 2011. Publicado en Archivos de Medicina del Deporte, 2011, 28, 145: 353-357.

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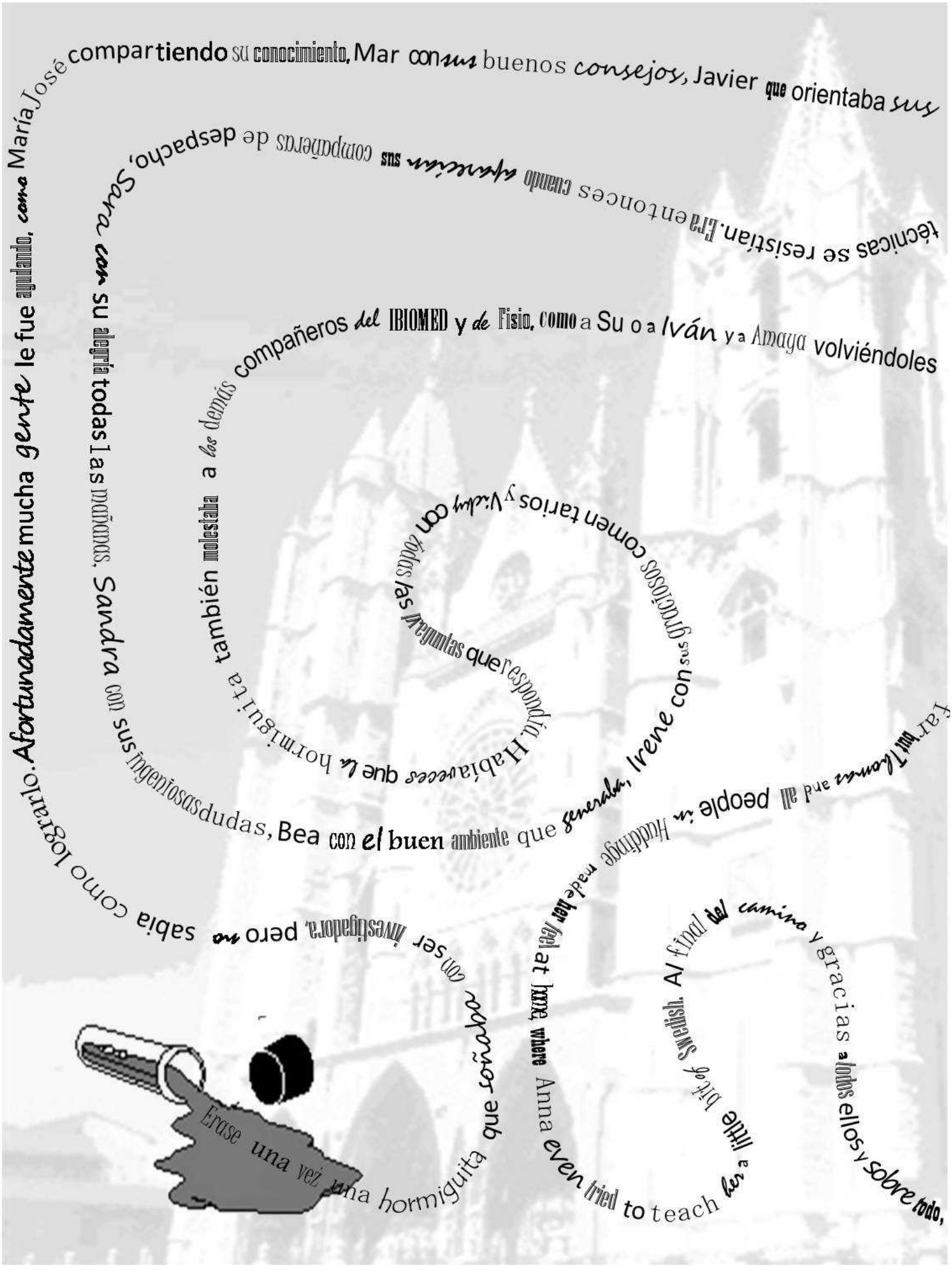
"Hay una fuerza motriz más poderosa que el vapor,

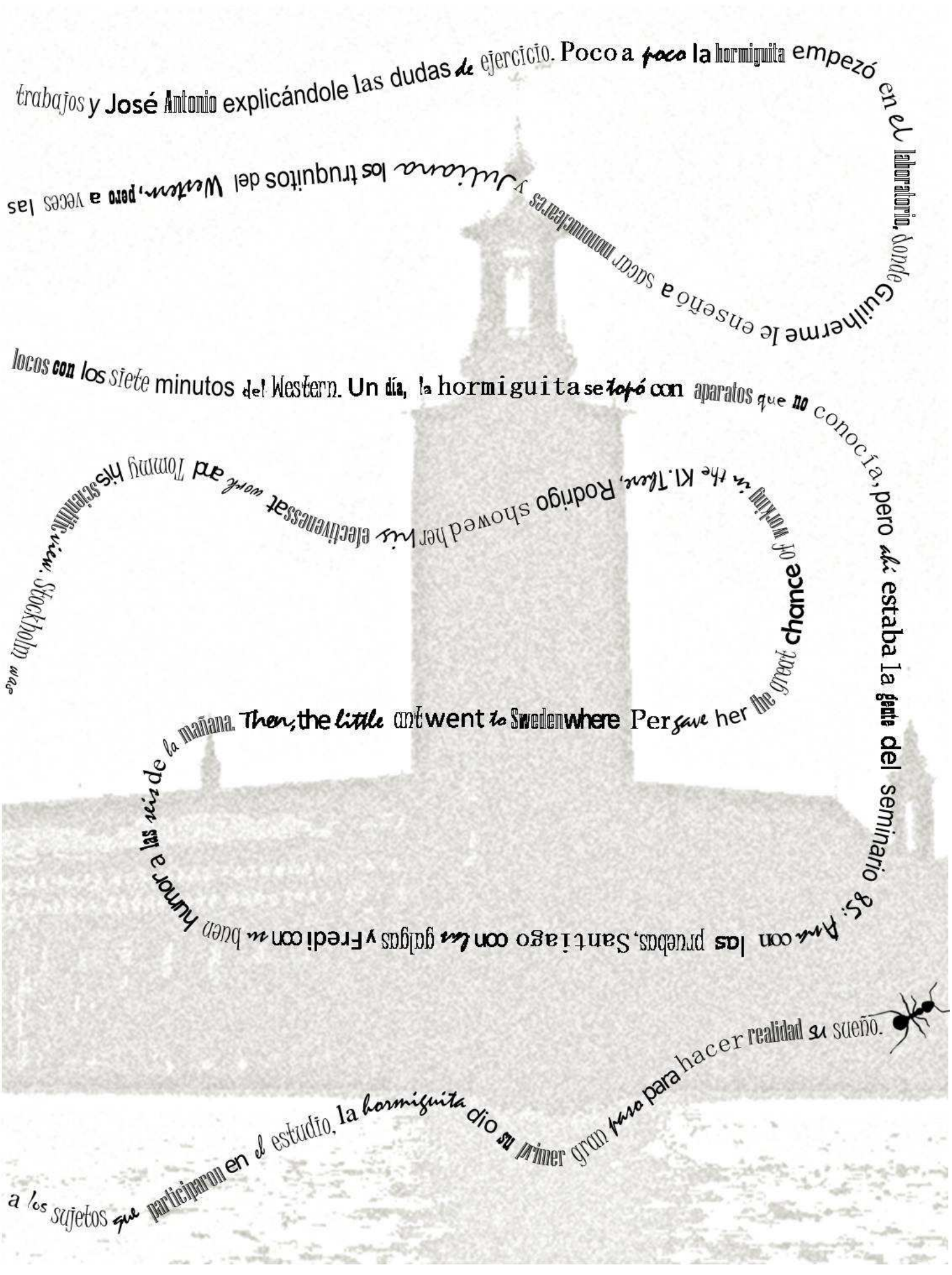
la electricidad y la energía atómica: la voluntad"

Albert Einstein

A mis padres y a mi hermana

Vuestro apoyo ha sido mi impulso


Érase una vez una hormiguita que soñaba con ser investigadora, pero no sabía como lograrlo. Afortunadamente mucha gente le fue ayudando, como María José compartiendo su conocimiento, Mar con sus buenos consejos, Javier que orientaba sus técnicas se resistían. Era entonces cuando aparecían sus compañeras de despacho, Sara con su alegría todas las mañanas, Sandra con sus ingeniosasdudas, Bea con el buen ambiente que generaba, Irene con sus graciosos comentarios y Vicky con todas las preguntas que respondía. Había veces que la hormiguita también molestaba a los demás compañeros del IBIOMED y de Fisio, como a Su o a Iván y a Amaga volviéndoles loca. But Thomas and all people in Hadding made her feel at home where Anna even tried to teach her a little bit of Swedish. Al final del camino y gracias a todos ellos y sobre todo,


a los sujetos que participaron en el estudio, la hormiguita dio su primer gran paso para hacer realidad su sueño.
Ana con las pruebas, Santiago con las gafas y Fredi con su buen humor a las nueve de la mañana. Then, the little ant went to Sweden where Per gave her the great chance
of working in the Kl. Then, Rodrigo showed her his effectiveness at work and Tommy his scientific. Stockholm was
locos con los siete minutos del Western. Un día, la hormiguita se topó con aparatos que no conocía, pero ahí estaba la gente del seminario
trabajos y José Antonio explicándole las dudas de ejercicio. Poco a poco la hormiguita empezó en el laboratorio, donde
Guillermo le enseñó a sacar nanonucleares y Julieta los trquitos del Western, pero a veces las

universidad de león

Departament of Biomedical Sciences

Effect of different training programmes on the age-related inflammation: role of Toll like receptor 2 and 4 signalling pathways

PhD Dissertation

Paula Rodríguez Miguélez

León, 2013

Abbreviations and symbols

1RM	One repetition maximum
ACSM	American College of Sport Science
ANOVA	Analysis of variance
AP-1	Activator protein 1
ATG	Aerobic training group
BMI	Body mass index
bpm	Beats *per* minute
cDNA	Complementary DNA
CG	Control group
CRP	C-reactive protein
ds	Double strand
EDTA	Ethylenediamine tetraacetic acid
ERK	Extracellular signal-regulated kinase
GADPH	Glyceraldehyde 3-phosphate dehydrogenase
HR	Heart rate
HRmax	Maximum HR
HRP	Horseradish peroxidise
Hsp	Heat shock protein
Hz	Herz
IFN	Interferon
IKK	Inhibitor of κB kinase
IKKi/IKKε	Inhibitory κB kinase inducible or epsilon
IL	Interleukin
IRAK	IL-1 receptor-associated kinase
IRF	IFN regulatory factor
ISGF3	IFN-stimulated gene factor 3
IκB	Inhibitor κB
JNKs	C-Jun N-terminal kinases
KO	Knock out
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
MAPKKKs	MAPKK-kinases
MAPKKs	MAPK-kinases
MAPKs	Mitogen-activated protein kinases
MVIC	Maximal isometric voluntary contraction
MyD88	Myeloid differentiation primary response gene 88
NAP	NF-κB-activating kinase-associated protein
NF-κB	Nuclear factor kappa B
NK	Natural killer
NSCA	The National Strength and Conditioning Association
PAMPs	Pathogen-associated molecular patterns
PAR-Q	Physical activity readiness questionnaire
PBMC	Peripheral blood mononuclear cells

PBS	Phosphate buffered saline
PBS-t	PBS-tween
PRR	Pattern recognition receptors
PVDF	Polyvinylidene difluoride
RHD	Rel homology domain
RTG	Resistance training group
RT-qPCR	Real time and quantitative polymerase chain reaction
SARM	Sterile alpha and heat-armadillo motifs containing protein
SDS	Dodecyl sulfate sodium
SEM	Standard error of means
ss	Single strand
sv	Splice variant
TAB	TGF beta-activated kinase 1 binding protein
TADs	Terminal transactivation domains
TAK1	TGF beta-activated kinase 1
TANK	TRAF-associated-NF-κB activator
TBK1	TANK binding kinase 1
TGF	Transforming growth factor
TICAM	TIR-containing adaptor molecule
TIR	Intracellular toll-IL-1 receptor
TIRAP	TIR-containing adaptor protein
TLR	Toll like receptor
TNFα	Tumour necrosis factor alpha
TPY	Threonine-proline-tyrosine
TRAF	TNF receptor associated factor
TRAM	TRIF related adaptor molecule
TRIF	TIR domain-containing adaptor inducing interferon beta
VO_2	Oxygen uptake
VO_2max	Maximal oxygen uptake
VTG	Whole body vibration training group
WBV	Whole-body vibration
wk	Week
κB	Kappa B

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Introduction and aims

Ageing is a natural process characterized by a decline in the normal function of several physiological systems, with a chronic, mostly asymptomatic, low-grade inflammatory state that can lead to a more vulnerable status, increasing the risk of developing chronic illnesses. Indeed, most age-related diseases, such as cardiovascular disease, type 2 diabetes mellitus or osteoporosis, are associated with low-grade chronic inflammation.

Toll-like receptors (TLR), in particular TLR2 and TLR4, may play an important role in the chronic inflammatory situation developing with the senescence process. TLRs bind to specific exogenous or endogenous ligands released from damaged or stressed host tissue, mediating the activation of an early phase that induces the production of pro-inflammatory cytokines, and subsequently, a late phase, which promotes the expression of interferon inducible genes.

Multiple studies have reported an association between low-grade systemic inflammation and physical inactivity. In fact, whole body chronic inflammation appears to be highest in individuals who are sedentary, since physical conditioning seems to correlate with anti-inflammatory effects through the amelioration of classical inflammatory mediators. However, this beneficial adaptation depends on the modality, intensity and length of the exercise protocol carried out.

Traditionally, resistance or endurance training protocols have been the most used to achieve functional benefits in the elderly, although nowadays vibration exercise has been also proposed as a useful method to enhance physical senior's fitness. Hence, exercise could be an efficient countermeasure to either prevent or delay the onset of some chronic diseases associated with this low-grade inflammatory status but there is a lack of knowledge in the inflammatory response associated with common elderly physical activity routines.

Considering these data, the main purpose of this thesis was to assess the effect of three different exercise training programmes on the age-related molecular inflammatory status associated with TLR2 and TLR4 pathways in elderly subjects.

The following specific objectives were proposed:

-To analyze the expression of Toll like receptor 2 and 4 before and after three different training programmes in the elderly participants.

-To go deeply into the activation and the regulation of the MyD88-dependent pathway signalling in all experimental groups.

-To study the effects of the different training protocols on the TRIF-dependent pathway in the elderly.

-To asses the production of several inflammatory biomarkers after eight weeks of training in mononuclear cells of senior participants.

-To determine in the elderly the role of the heat shock proteins Hsp60 and Hsp70 on the Toll-like receptor pathways after completing the three different physical activity programmes.

Literature review

2.1. AGEING

According to the United Nations, the elderly population of the world is growing at its fastest rate ever. By 2050, there will be more than two billion people aged sixty or over, representing 22% of world population (United Nations, 2009). Therefore, life expectancy in the development countries has increased exponentially, leading to an enormous social and economic change.

Ageing is a multifactorial complex process characterized by a progressive inevitable decline in health, partially attributed to the gradual failure of the cells to repair or replace themselves (Chodzko-Zajko and Ringel, 1987). It depends on genetics and influenced by an extensive range of environmental factors such as diet, exercise and exposure to microorganisms, pollutants or ionising radiation (Nigam *et al.,* 2012).

It is characterized by its deleteriousness since most functions in the body tend to deteriorate slowly and progressively as the result of gradual accumulation of damaged substances or the lost of normal tissue (Goto, 2008).

Although this senescence process has tried to be explained by many hypothesis, nowadays exists two major modern biology-ageing theories in humans: the programmed and the non-programme theories. The first, also known as adaptive or active theories, imply that organisms are designed to follow a biological timetable by genetic changes expression regulated by multiple factors such as hormones, signals, external conditions and other characteristics typical of evolved biological functions. This regulation would depend on changes in gene expression that affect the systems responsible for maintenance, repair and defense responses. On the other hand, the non-programmed theories, also known as non-adaptive or passive, support that ageing is an unavoidable adverse side effect of some useful biological functions resulted from the lack of evolutionary force toward continuing life. Therefore, the organism lost the maintenance and repair capabilities needed for living longer (Goldsmith, 2012).

Overall and despite recent advances in molecular biology and genetics, multiple theories of ageing have been proposed but currently there is no consensus on this issue (Goldsmith, 2012).

2.1.2. Physiological ageing changes

As people get older, gradual changes appear in the body resulting in a progressive loss of a variety of physiological functions (Figure 1) (Nigam *et al.,* 2012). Many of these modifications often begin in early adulthood, but thanks to the ability of the organs to adjust and maintain health, the real loss is not experienced until it is extensive.

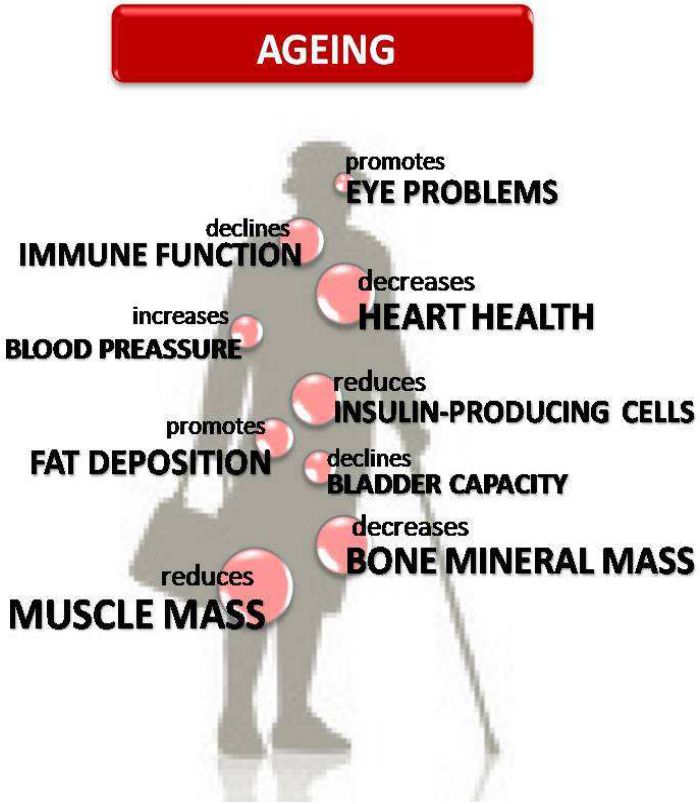

Figure 1. Age-related changes in health may alter the older person's response to illness and depend on the genetic and long-term lifestyle factors

One of the most important alterations that commonly afflict older people is muscle atrophy since their muscle mass starts to deteriorate, decreasing gradually the capacity to assure strenuous efforts. This alteration is closely related with the

mechanical failure of the skeleton and the increase risk of fractures, especially in older women (Syed and Ng, 2010).

Moreover, functioning of all body organs is not as efficient as in young individuals. In the cardiovascular system, the ageing process promotes the decrease of the cardiac output and the increase of the blood pressure due to the thickness and hardening of the arteries (Laurent, 2012). The lungs also show impaired gas exchange with slower expiratory flow rates owing to the decrease in their elasticity (Ketata *et al.,* 2012). In the urinary system, tubular function and bladder capacity also declines with senescence (Kanasaki *et al.,* 2012). Digestive problems also appear such as a decrease in gastrointestinal absorption and intestinal motility, atrophic gastritis and altered hepatic blood flow (Grassi *et al.,* 2011).

Senescence also arise some perturbations in the endocrine system being the most significant the progressive degradation of the number and the function of insulin-producing beta cells (Gunasekaran and Gannon, 2011). Another related considerable modification appears in women when menopause brings enormous physical changes as the natural production of estrogens fall, leading to thinning of the skin and bones (Santoro, 2005).

Additionally, metabolism undergoes remarkable changes in seniors starting to be slower. Fat deposition is promoted since young adulthood to middle age and continues in advanced age with loss of fat-free mass. This is the start to metabolic diseases like type 2 diabetes, hyperlipidaemia, atherosclerosis or hypertension (Roberts and Rosenberg, 2006).

Peripheral immune system is also affected by the ageing process (Gruver *et al.,* 2007). It is widely accepted that the immune function declines significantly with advancing age (Katz *et al.,* 2004) affecting both the innate and the adaptive immune systems (Allman and Miller, 2005). As a result, older subjects are more susceptible to viral or bacterial infectious diseases, even autoimmune diseases (Murasko and Jiang, 2005, Pawelec *et al.,* 2006, Prelog, 2006) and decrease their response to vaccinations (Simpson *et al.,* 2012).

2.2. INFLAMMATION AND AGEING: INFLAMM-AGEING

Inflammation is defined as "a wide variety of adaptive physiological and pathological processes to avoid infection and repair damage, restoring the organism to the usual state of homeostasis" (Majno and Joris, 1995).

Early in life, it constitutes a fundamental positive response to protect against infectious diseases and other damageing agents, achieving a stronger status (Franceschi, 2007). Nonetheless, inflammation in the elderly can be a detrimental process with a major contribution in the development of multiple age-related chronic diseases such as atherosclerosis (Libby, 2006), type 2 diabetes (Asrih and Jornayvaz, 2013), Alzheimer's disease (Holmes, 2013) and rheumatoid arthritis (Ferraccioli *et al.,* 2012).

Inflammation constitutes one of the first responses of the immune system to infection, classically recognized by five symptoms including pain, heat, redness, swelling, and loss of tissue function (Takeuchi and Akira, 2010). Currently it is known that, in response to an injury or abnormal stimulation caused by a physical, chemical or biologic agent, this process initiates a long chain of reactions such as cytological changes, cellular infiltration and mediators release that occurs in the affected blood vessels and adjacent tissues. With these sequences, the inflammatory response is rapidly concluded and damaged tissue is repaired (Akira *et al.,* 2006).

2.2.1. Inflamm-ageing

Age-related changes in the immune system and the increase in life expectancy in world population has promoted that individuals are exposed longer to endogenous and environment antigens which allow an activation of the innate immune system and subsequently, a pro-inflammatory state. This situation has been coined inflamm-ageing or immunosenescence (Franceschi *et al.,* 2000, Franceschi, 2003) and has been defined as a low-grade chronic controlled asymptomatic and non-pathological state of the inflammatory system in the elderly (Giunta, 2006, Goto, 2008).

Although it was suggested that the evolution of the pro-inflammatory status in the elderly was the result of chronic activation of the macrophages (Franceschi *et al.,* 2000), this phenomenon is only part of the whole spectrum of changes characteristic of immunosenescence. Certainly, monocytes and macrophages function are compromised with age (Gómez *et al.,* 2005) but growing evidence suggests that additional detrimental effects appear on other immune cells. This is the case of neutrophils that show a reduce ability in their phagocytic capacity, in their synthesis of reactive oxygen species and in their intracellular killing efficiency (Fulop *et al.,* 2004). Lymphocytes are also affected inducing the expansion of memory cells, the decrease and the exhaustion of naive cells and the shrinkage of the T-cell population (Gómez *et al.,* 2008).

Taken together, the total number of innate immune cells is modified in the elderly and some of their abilities such as phagocytic activity, cytokine secretion, antibacterial defences, or the antigen presentation show impairment in the ageing process (Sebastian *et al.,* 2005).

2.2.2. Cytokines and inflammation

Besides the effects of immunosenescence in the different cell populations described above, it has been clearly shown that inflamm-ageing is also force by the abnormal production of pro-inflammatory cytokines in seniors (Boehmer *et al.,* 2004, Renshaw *et al.,* 2002).

Cytokines (from the Greek *cyto-*, cell and *-kinos*, movement) are a group of intercellular signalling low-molecular weight proteins that play pivotal roles in a variety of responses including the immune system, hematopoiesis, neurogenesis, embryogenesis, and oncogenesis. They are mediators produced widely throughout the body by cells of diverse embryological origin and most of them act in an autocrine or paracrine mode via specific cell-surface receptors on their target cells, although some are able to act far from their production, functioning as hormones (Dinarello, 2007).

Traditionally, cytokines have been classified by several ways being the most broadly division in pro-inflammatory cytokines, responsible for the onset of the inflammatory response, and anti-inflammatory cytokines, generated in the inflammatory process and responsible for its conclusion.

Already in 1993, Fagiolo and co-workers analyzed peripheral blood mononuclear cells (PBMC) from seniors concluding that aged people were able to present higher amounts of pro-inflammatory cytokines that young subjects (Fagiolo *et al.,* 1993). Nowadays there are considerable evidences to accept that these mediators orchestrate the inflamm-ageing response. Certainly, a number of inflammatory markers, especially tumour necrosis factor alpha (TNFα), interleukin (IL) 6 and C-reactive protein (CRP) have the most consistent association with age-related low-chronic inflammation (Singh and Newman, 2011). They seem to be responsible of the cell death regulation in inflammatory tissues, the modification of vascular endothelial permeability, the recruitment of blood cells to inflamed tissues and the production of acute-phase proteins (Takeuchi and Akira, 2010).

Within the pro-inflammatory cytokines, highlight TNFα, an early mediator of local inflammatory processes as well as an initiator of the systemic acute-phase response (Bruunsgaard, 2005). It is a 26 kDa homotrimer transmembrane protein which is cleaved and released into the circulation as a functional 17 kDa soluble form (Speeckaert *et al.,* 2012). Its production is mainly linked to adipose tissue (Hotamisligil, 2006, Krogh-Madsen *et al.,* 2006) but also to other cells such as monocytes and macrophages (Brotas *et al.,* 2012).

This polypeptide is a primary mediator in infection, trauma and inflammation, although its main physiological function is the recruitment of leukocytes to sites of infection by increasing adhesion molecules in endothelial cells (Ishii and Yoshida, 2010). Moreover, it is a key marker of the severity of bacterial infection (Brotas *et al.,* 2012). Thus promotes prostaglandin synthesis by hypothalamic cells leading to fever states. For this reason, TNFα is considered an endogenous pyrogen mediator (Brotas *et al.,* 2012).

Another pro-inflammatory marker is IL-1 responsible of the immunospecific stimulation and the enhancement of mediators such as prostaglandins and leukotrienes (French *et al.,* 1991). It has been identified two isoforms: IL-1α and IL-1β. The first one is expressed on the surface of activated macrophages and is able to increase the local and systemic inflammatory response (Hawrylowicz *et al.,* 1989). The second, IL-1β is a potent mediator in response to infection and injury (Dinarello, 1998), produced mainly by monocytes (Sasaki *et al.,* 2002). Significant elevations of plasma IL-1β have been detected in patients with septic shock (Cannon *et al.,* 1992). It has long been known the role that IL-1 has in the synthesis of another important cytokine, IL-6 (Bochner *et al.,* 1991). IL-6 is a pleiotropic cytokine with a wide range of biological activities in immune regulation or inflammation among others (Pedersen, 2006). It is mainly produced by monocytes, fibroblasts and endothelial cells (Sironi *et al.,* 1989) but its secretion may also be found in other populations of cells (Su *et al.,* 2012). Its expression is induced by a variety of stimuli including IL-1, TNFα, platelet-derived growth factor and microbial elements such as lipopolysaccharide (LPS), major component of the outer membrane of Gram-negative bacteria (Kishimoto, 2005).

Accumulating evidence indicates pathological roles for IL-6 in various disease conditions, such as inflammatory, autoimmune or malignant diseases (Kishimoto, 2005). Hence, it has become a basic predictor of morbidity and mortality in the elderly (Franceschi *et al.,* 2000).

Surprisingly, there is considerable evidence to support an anti-inflammatory and immunosuppressive role of IL-6 (Pedersen, 2006). These properties have been confirmed in several mouse models where IL-6 has induced the regeneration of intestinal epithelial cells after induced damage (Grivennikov *et al.,* 2009).

It is worth noting that, while most young individuals show low or even undetectable levels of IL-6 in plasma, the content of this cytokine starts to increase in healthy people according with age. Even healthy centenarians in good shape show high levels of IL-6 in plasma (Baggio *et al.,* 1998).

On the other hand, and as an example of anti-inflammatory mediators, it has been found IL-10, originally known as the cytokine synthesis-inhibiting factor. It is a small key immunoregulatory mediator that can be produced by almost all leukocytes, including innate immune cells (monocytes, macrophages, NK cells, etc.) and adaptive immune cells (Th1, Th2, etc.) (Yanaba *et al.,* 2009). Although IL-10 is known to have many different roles in the immune system, there are two major activities to highlight that include the blockage of the accessory functions of macrophages during T cell activation and the inhibition of pro-inflammatory cytokine release by macrophages (Iyer and Cheng, 2012). This last action is exerted by clamping the inhibitor of kappa B (κB) kinase (IKK) masking the nuclear localization signal of the nuclear factor κB (NF-κB) and keeping it sequestered in an inactive state in the cytoplasm (Hayden and Ghosh, 2012). Therefore, as NF-κB controls the transcription of various inflammatory cytokines and chemokines, IL-10 may exert its anti-inflammatory properties by inhibiting the action of this transcription factor (Moore *et al.,* 2001).

2.2.3. Pathologies related to inflamm-ageing

There is considerable experimental evidence to support that alterations in the concentration of some cytokines, far from levels observed during acute or severe infections, are associated with the inflammatory situation observed in older adults and with an important number of age-related pathologies (Goto, 2008).

In this regard, it is found cardiovascular diseases, closely related to a low-grade systemic inflammation state owing to enhancements of IL-6 and TNFα (Lambertsen *et al.,* 2012). Indeed, plasma concentrations of these cytokines induce the production of CRP, which has turned out to be a strong and consistent predictor of coronary events (Pepys and Hirschfield, 2003).

Additionally, cardiovascular disease is directly linked to atherosclerosis (Ross 1999). Thus, high TNFα levels are associated with a low arterial pressure index, indicating peripheral atherosclerosis (Van der Valk *et al.,* 2012). Furthermore, this

cytokine has an important role in lipid metabolism (Plomgaard *et al.,* 2008) and lipoprotein lipase (Feingold *et al.,* 1992).

Moreover, the TNFα is used as a predictor of insulin insensitivity with advancing age (Arai *et al.,* 2011, Paolisso *et al.,* 1998), being also involved in type 2 diabetes (Alexandraki *et al.,* 2006) and glucose metabolism (Plomgaard *et al.,* 2007).

Elevated levels of circulating TNFα are also contributing factors for the loss of muscle fibers and hence, the development of the age-related sarcopenia process (Dirks and Leeuwenburgh, 2006). In addition, it is also linked with the death of muscle cells in sarcopenia due to the enhancement of the apoptosis and/or myofibrillar protein degradation (Carbo *et al.,* 2002).

Other diseases are also related with higher concentrations of pro-inflammatory cytokines such as the cognitive decline with ageing and Alzheimer's disease (Akiyama *et al.,* 2000) or bowel disease among others (Nielsen and Ainsworth, 2013).

2.3. TOLL LIKE RECEPTORS

Around us, there are millions of pathogens with enough potential to cause us infections. Moreover, damaged injured or stressed cells also send out alarm signals that must be identify. To avoid these harmful effects, evolution has developed the immune system to identify and remove those agents as well as to trigger an inflammatory response (Kappagoda and Amsterdam, 2012).

The immune system is a collection of cells, tissues and molecules that protect the body from possibly detrimental substances by recognizing and responding to antigens. This defense has been divided into two major subdivisions: the innate or non-specific immune system and the adaptive or specific immune system. Thus, innate and adaptive immunity can be thought of as two equally important responses with the difference of how quickly and for how long they react to pathogens (Alam, 1998).

Focusing in the first response, the innate immune system consists of cells and proteins that are immediately available to combat common structures named pathogen-associated molecular patterns (PAMPs) that are shared by a vast majority of threats (Medzhitov, 2007). PAMPs are recognized by corresponding germline-encoded pattern recognition receptors (PRR) expressed on the surface of innate immune cells. In lieu of the importance of the mechanism, there is no method in the cells of selecting foreign from self substances and there is no memory to change how the system responds to the same threat upon the following exposures (Medzhitov, 2007). Indeed, damaged injured or stressed cells are recognized by the same receptors as those that indentify pathogens (Medzhitov, 2007), triggering an acute inflammatory response leading to the efficient destruction of the invading pathogens or the damaged tissue (Akira *et al.,* 2006, Beutler *et al.,* 2006).

Four main families of PRRs have been shown to initiate inflammatory signalling pathways although the best characterized is one of evolutionarily conserved transmembrane proteins known as TLRs (Takeuchi and Akira, 2010).

The TLR family recognize distinct PAMPs cooperating in the first line of defense and playing a significant role in inflammation, immune cell regulation, survival, and proliferation (Kawai and Akira, 2010).

This family was originally identified in *Drosophila melanogaster* as essentials receptors for host defense against fungal infection (Takeda and Akira, 2005). Then, a mammalian homolog of this fly-receptor (now termed TLR4) was shown to control the expression of genes involved in the inflammatory responses (Medzhitov *et al.,* 1997). Since then, it was indentified thirteen members (TLR1-TLR13): the first nine are conserved in both humans and mice, while TLR10 is expressed only in humans and TLR11 to TLR13 are present only in mice (Kawai and Akira, 2010).

These receptors are type I trans-membrane glycoproteins containing an extracellular leucine-rich repeat (LRR) domain, a transmembrane domain and an intracellular toll-IL-1 receptor (TIR) domain (Pietrocola *et al.,* 2011). Mostly of them are expressed on plasmatic cell membranes though a minority are located almost

exclusively in endosomal compartments (TLR3, 7, 8 and 9) (Pietrocola *et al.,* 2011). They are often linked to innate and adaptive immunity cells as monocytes, neutrophils, dendritic cells, phagocytes, natural killer (NK) cells, or T and B lymphocytes. Furthermore, current evidence suggests the expression of these receptors also in non-immune cells as endothelial cells, fibroblast or adipocytes (Pietrocola *et al.,* 2011).

2.3.1. TLRs ligands

As previously mentioned, TLRs have been revealed to play essential roles in the recognition of a wide variety of specific components as structural motifs of the bacterial cell wall, genetic material from virus, intracellular components of necrotic cells, or signs of stress such as heat shock proteins (Hsp) (Cristofaro and Opal, 2006). Different TLRs recognize different molecular patterns of microorganisms and self-components (Takeuchi and Akira, 2010).

TLR2 is involved in the recognition of a extensive range of PAMPs derived from microbial organisms leading to the formation of heterodimers with either TLR1 or TLR6 (Jin *et al.,* 2007) and enhancing the synthesis of pro-inflammatory cytokines (Takeuchi and Akira, 2010). These components include lipoproteins, peptidoglycan and lipoteichoic acid from Gram-positive bacteria (Takeuchi and Akira, 2010), mycobacterial lipoarabinomannan (Wieland *et al.,* 2004), glycosylphosphatidylinositol from *Trypanosoma cruzi* (Campos *et al.,* 2001) or polysaccharides from *Saccharamysec cerevisiae* (Frasnelli *et al.,* 2005). It is also activated by non-microbial molecules such as Hsp60, Hsp70 (Asea *et al.,* 2002), hyaluronan (Scheibner *et al.,* 2006), or saturated fatty acids (Lee *et al.,* 2004).

With regard to TLR4, it has an essential role in the transduction of the signal from LPS, consider one of the main immune stimulator (Hoshino *et al.,* 1999). TLR4 is also capable of identified fusion protein from respiratory syncytial virus and the envelope protein from mouse mammary tumour virus (Rassa *et al.,* 2002).

Endogenous molecules such as Hsp60 and Hsp70 (Ohashi *et al.,* 2000), fibrinogen or hyaluronic acid (Termeer *et al.,* 2002) are also ligands of TLR4.

Respecting to TLR3, TLR7, TLR8 and TLR9, they recognize nucleic acids derived from viruses and bacteria and lead to the production of type I interferon (IFN) in addition to pro-inflammatory cytokines (Akira *et al.,* 2006). Specifically TLR3 located in the endolysosomal membrane is also able to detect viral double-stranded (ds) RNA, while TLR7 and TLR8 identify single-stranded (ss) RNA (Takeuchi and Akira, 2010).

2.3.2. TLR signalling pathways

Stimulation of TLRs by PAMPs triggers expression of several genes that are involved in immune responses (Takeda and Akira, 2005). After ligand recognition, TLRs undergoes oligomerization and the recruitment of downstream mediators through interactions with TIR domains of adaptor proteins (Figure 2) (Takeuchi and Akira, 2010). There are five TIR domain-containing adaptor proteins: myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adaptor inducing IFN-β (TRIF), TIR domain-containing adaptor protein (TIRAP), TRIF-related adaptor molecule (TRAM) and sterile α and heat-Armadillo motifs containing protein (SARM) (Takeuchi and Akira, 2010). Different TLRs use different combinations of adaptors proteins with the exception of TLR4 that is the only known able to use all of them (Lu *et al.,* 2008).

Recent accumulating evidence indicates that those TIR domain-containing adaptor proteins subdivided TLRs signalling pathway at least in a MyD88-dependent pathway, common to all TLRs, and a MyD88-independent pathway or TRIF-dependent pathway that is peculiar to the TLR3 and TLR4 (Lu *et al.,* 2008). The first pathway is responsible for the expression of pro-inflammatory cytokines and the TRIF-dependent cascade also controls the expression of IFN-inducible genes (Takeuchi and Akira, 2010).

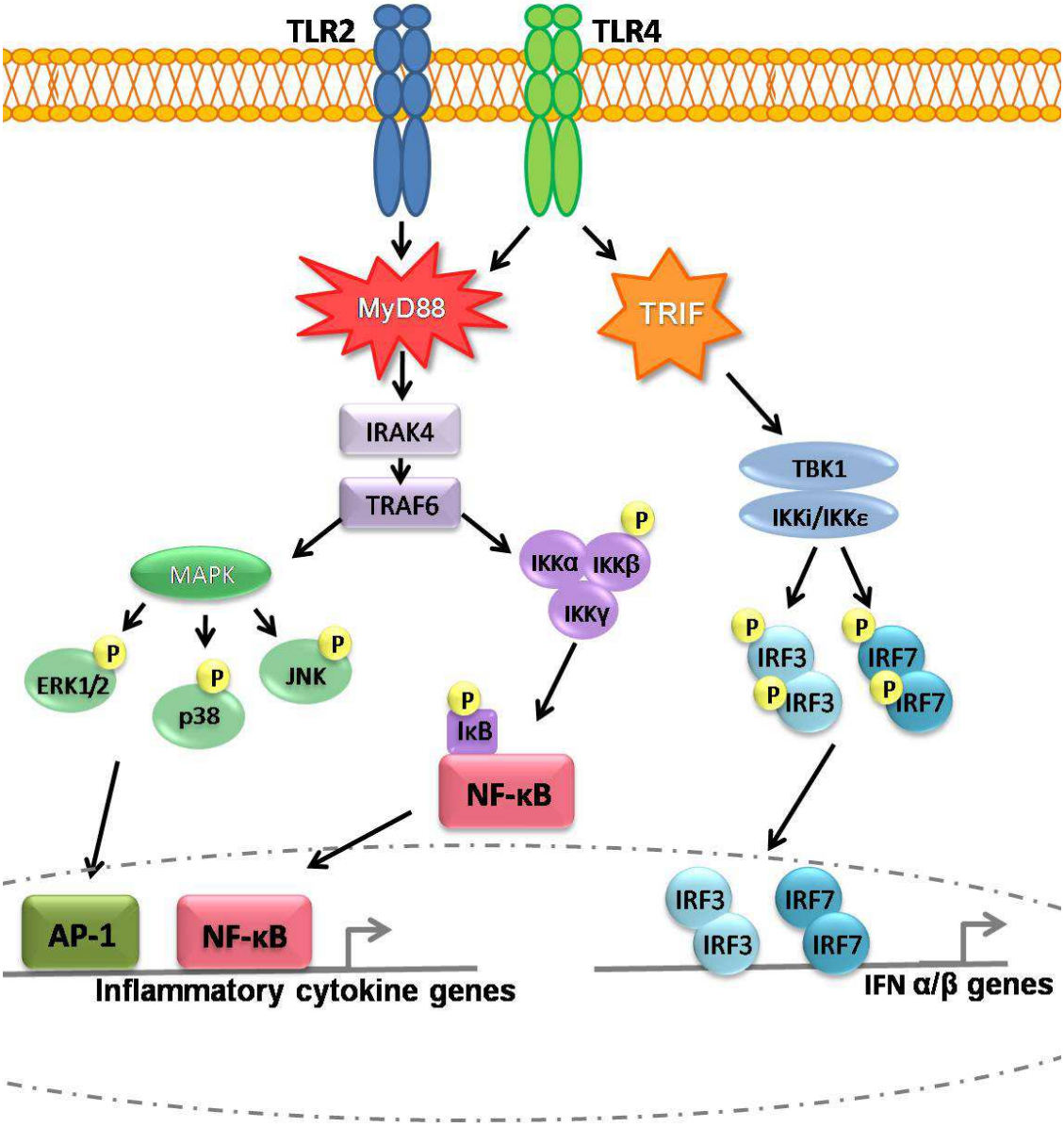

Figure 2. TLR2 and TLR4 signalling pathways. MyD88 is an essential TIR domain-containing adaptor for the induction of inflammatory cytokines. TRIF, a second TIR domain-containing adaptor, mediates the MyD88-independent pathway via TLR4, leading the production of IFN-α and IFN-β

2.3.2.1. The MyD88-dependent signalling pathway

MyD88 is a key signalling molecule implicated in the regulation of wide physiological mechanisms such as the innate and the acquired immunity, the inflammatory process, the bone metabolism or the development in some types of tissues as the central nervous system, the skin or the mammary glands (Wu and Arron, 2003).

MyD88 harbours a TIR domain in the C-terminal and a death domain in the N-terminal portion, that upon stimulation, establishes an interaction with another homologous death region located in an IL-1 receptor-associated kinase (IRAK) protein (Kawai and Akira, 2010). Studies conducted to examine the role of this protein in TLR pathways have demonstrated how MyD88 knockout (KO) mice presented a blockage in the production of inflammatory mediators after LPS induction (Takeda and Akira, 2003). This finding confirms MyD88 as a key component in TLR cascades.

As previously mentioned, IRAK is the next family protein to continue with the cascade. Four members comprise this group of mediators, originally identified as serine-threonine kinases: IRAK-1, IRAK-2, IRAK-M and IRAK-4 (Kawai and Akira, 2006). MyD88 established the interaction with IRAK-4, which subsequently activates IRAK-1 and IRAK-2 (Takeuchi and Akira, 2010).

Intrigued by the relevance of these proteins in TLR signalling pathways, IRAK-1 KO mice and IRAK-4 KO mice were used. Results inferred that IRAK-1 showed defective responses to LPS stimulation (Swantek *et al.,* 2000) while IRAK-4 confirmed an almost complete impairment in the response to this microbial components (Suzuki *et al.,* 2002).

Afterwards, the complex composed by MyD88, IRAK-4 and IRAK-1 initiates the recruitment of TNF receptor associated factor (TRAF) 6, an important mediator in cytokine production with two TRAF domains in the C-terminal (TRAF-N and TRAF-C) and a zinc finger domains in the N-terminal (Kawai and Akira, 2010).

TRAF6 acts as an E3 ubiquitin protein ligase (Takeuchi and Akira, 2010) associating with several proteins as transforming growth factor (TGF) β-activated kinase 1 (TAK1) and TAK-1 binding protein (TAB) 1, 2 and 3 to constitute an ubiquitin-conjugated enzyme complex (Xia *et al.,* 2009). The heterodimer moves into the cytoplasm to phosphorylate downstream mediators of two different signalling pathways: NF-κB and mitogen-activated protein kinases (MAPKs) pathways (Lu *et al.,* 2008).

NF-κB pathway

After TRAF6 activation and the formation of the TAK1-TAB1-TAK2/3 complex, the cascade advances to the phosphorylation of an IKK heterodimer comprised of three IKK subunits, α and β with the regulatory protein NEMO or subunit γ (Hayden and Ghosh, 2012). This complex prompts the phosphorylation of the inhibitor κB (IκB) and thereby, its degradation by the action of the ubiquitin proteasome 26S complex (Karin and Ben-Neriah, 2000). This allows unmasking the nuclear localization sequence of the NF-κB and its translocation to the nucleus.

The NF-κB is an essential transcriptional factor with a conserved role in the immune system, implicated in the expression of the pro-inflammatory cytokine genes. In addition, it also acts broadly to influence other gene expression events that affect cell survival, differentiation and proliferation (Hayden and Ghosh, 2012). As a result of such wide effects on physiology, aberrant activation of NF-κB has lead to multiple consequences, including some major illnesses such as cancer, autoimmune diseases or diabetes (Dolcet *et al.,* 2005, Gilmore, 2006). Consequently, a number of positive and negative regulatory elements govern NF-κB.

In mammals, there are five NF-κB family members: p65 or RelA, p50 or NF-κB1, p52, NF-κB2, RelB and c-Rel (Hayden and Ghosh, 2012). All of them are characterized by the presence o an N-terminal Rel homology domain (RHD) that allows the binding with DNA and supports subunit dimerization. Besides, p65, c-Rel and RelB possess C-terminal transactivation domains (TADs) that confer the ability to initiate transcription. It is worth noting that p50 and p52 can also positively regulate transcription through their heterodimerization with TAD-containing NF-κB subunits, regulating different transcriptional programmes (Hayden and Ghosh, 2012).

MAPK pathway

In addition to NF-κB driven transactivation, the TAK1-TAB1-TAK2/3 triggers also the activation of MAPK family involved in the regulation of another

transcriptional factor that also control the expression of pro-inflammatory cytokines; the activator protein 1 (AP-1) (Sakurai, 2012).

MAPKs are a family of serine-threonine specific protein kinases largely expressed in all cell types, which connect cell-surface receptors to regulatory targets within the cell. Therefore, they regulate numerous cellular events associated with the inflammation as well as cell proliferation and survival mechanism in response to a variety of external stimuli such as cytokines, mitogens, or Hsp (Chang and Karin, 2001).

The signalling cascades of these proteins are organized hierarchically into three-tiered modules: MAPKs are phosphorylated and activated by MAPK-kinases (MAPKKs), which in turn are phosphorylated and activated by MAPKK-kinases (MAPKKKs). In mammals, downstream of all of these kinase proteins, it is found other four subfamilies of regulated MAPKs: the extracellular signal-regulated kinase (ERK) 1/2, the p38, the c-Jun N-terminal kinases (JNKs) and the ERK5. Prevailing data in the literature supports the idea that after ligand stimulation, ERK1/2, p38 and JNK are activated by different TLRs (Li *et al.,* 2010).

ERKs were the first MAPKs to be identified as serine-threonine kinases (Boulton and Cobb, 1991). Several kinds of ERKs have been described, although the main role has been attributed to ERK1 and ERK2 involved in cell proliferation processes. Once they are phosphorylated and therefore activated, they can be translocated to the nucleus where they stimulate an array of targets, including mediators that activate the AP-1 transcription factor (Shaulian, 2010).

Aside from ERKs, the JNKs family constitutes a group of proteins involved in the MAPK cascade that are activated by a variety of environmental stresses, inflammatory cytokines and growth factors. This family comprises JNK1 and JNK2, ubiquitously expressed, and JNK3 that is restricted to some tissues as brain or heart.

The JNK kinases have threonine-proline-tyrosine (TPY) motifs in their kinase domains that are required for activation (Roux and Blenis, 2004). After their

stimulation, they are translocated into the nucleus where can regulate the activity of multiple transcription factors such AP-1 (Guha and Mackman, 2001).

With regard to p38, it was identified as a 38 kDa protein that became tyrosine phosphorylated in response to LPS stimulation and involved also in the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNFα (Huang *et al.,* 2010). Nowadays, it has been described four different isoforms of p38 MAPK (α, β, γ, and δ), which show different tissue expression patterns (Roux and Blenis, 2004). It should be noted that p38α is ubiquitously expressed at significant levels in most cell types and most of the published studies refers to p38α as general p38.

In mammalian cells, p38 is strongly activated by environmental stresses and inflammatory cytokines being critical for normal immune and inflammatory responses (Ono and Han, 2000). As other MAPKs previously described, p38 is activated by specific MAPKKKs, which subsequently catalyze the phosphorylations of a conserved motif inducing conformational reorganizations and its stabilization binding specific substrate to lead the activation of different transcription factors (Roux and Blenis, 2004).

The AP-1 transcription factor family is a sequence-specific transcriptional activator composed of members of the Jun and Fos families. Both families contain basic leucine zipper domains allowing them to constitute homo or heterodimers. These complexes control a broad range of genes involved in the immediate-early cellular response of a wide gamut of physiological and pathological stimuli including pro-inflammatory cytokines, stress signals, infections or oncogenic signals (Vaiopoulos *et al.,* 2010).

Consequently, AP-1 is regulated on multiple levels commonly through posttranslational modifications as phosphorylation by MAPKs or ubiquitination by proteasome-mediated degradation. In short, the biological role of each signal determines which MAPK pathway is activated and how AP-1 is going to act (Sakurai, 2012).

2.3.2.2. The TRIF-dependent signalling pathway

As described above, MyD88 is essential for the production of inflammatory cytokines in response to a variety of components. Nonetheless, TLR stimulation in MyD88-deficient macrophages resulted in activation of NF-κB and MAPK pathways with delayed kinetics in spite of no production of cytokines (Kawai *et al.,* 1999).

Consistent with this idea, it was proposed the existence in some TLRs of a MyD88-independent component that could also lead the synthesis of cytokines. This was the role give to TRIF, also known as TIR-containing adaptor molecule 1 (TICAM). It is a large protein consisting of 712 amino acids in humans with a central role in the synthesis of IFN-β inducible genes and the late-phase activation of NF-κB and MAPK cascades (Moynagh, 2005).

The mechanisms by which the TRIF-dependent pathway leads the activation of those aforementioned pathways are now under investigation. Oshiumi and co-workers have proposed that both N and C terminal portions of TRIF are involved in NF-κB promotion, while only the N terminal takes part in the IFN-β-gene induction (Oshiumi *et al.,* 2003). Thus, TRAF-binding motifs in TRIF N-terminal portion are the responsible of the interaction with TRAF3 (Hacker *et al.,* 2006) and TRAF6 (Sato *et al.,* 2003), leading the late production of pro-inflammatory mediators (Tseng *et al.,* 2010).

In the TRIF-dependent pathway, the cascade continues with the interaction between the N-terminal portion of TRIF, TRAF-associated NF-κB activator (TANK) binding kinase 1 (TBK1) and the inhibitory κB kinase inducible or epsilon (IKKi/IKKε), an important activation IKK-related kinases (Guo and Cheng, 2007, Hacker *et al.,* 2006, Oganesyan *et al.,* 2006). This complex is important for the dimerization and translocation of the interferon regulatory factor (IRF) 3 (Lu *et al.,* 2008). Indeed, there are few reports supporting that the inhibition of TBK1 or IKKi/IKKε expression lead to impaired induction of IFN-β in response to viruses and dsRNA (Fitzgerald *et al.,* 2003). Conversely, the overexpression of these two proteins has been related with the

phosphorylation and therefore, increased transactivation of the NF-κB subunit p65 (Moynagh, 2005).

Although is not completely elucidated yet, it is worth highlight the differential expression between TBK1 and IKKi/IKKε which may reflect a non redundant role in the activation of IFN signalling (TenOever *et al.,* 2004). As of yet, TBK1 is constitutively and ubiquitously expressed (Bonnard *et al.,* 2000) and closely correlates with the expression of IRF3 (TenOever *et al.,* 2004). However, IKKi/IKKε, which have two novel splice variants (sv) (IKKi/IKKε-sv1 and IKKi/IKKε-sv2), presents cell type- and stiumulus-specific protein expression (Koop *et al.,* 2011).

IRF3 and IRF7 are structurally related proteins present in the cytoplasm in an inactive condition. While IRF3 is constitutively expressed, the expression of IRF7 is weak in unstimulated cells and dramatically increased after the correct stimuli. Thus, initial induction of IFN-β is largely dependent on IRF3 activation (Kawai and Akira, 2006).

Collectively, the activation of some TLRs with the appropriate stimuli coordinate the response through the TRIF-dependent pathway, leading to the activation of IRF3 and IRF7 and therefore, to the production of type I IFN (Theofilopoulos *et al.,* 2005). Notably, only TLRs located in the endosomal membrane can induce this response, with the exception of TLR4 (Takeda and Akira, 2003).

In contrast to NF-κB activation, which relies on the degradation of a cytoplasmic inhibitor, IRF3 and IRF7 activation occur directly through phosphorylations in the C-terminal. IRF3, as before described, is phosphorylated by the action of TBK1 and IKKi/IKKε in a serine-threonine cluster of its C-terminal region. Nevertheless, it is not completely elucidated yet which kinase is required for the IRF7 activation. While some studies suggest the same process of activation mediated by TBK1 and IKKi/IKKε (Moynagh, 2005), others support the theory that IRF7 is IKKα dependent (Hoshino *et al.,* 2006, Kawai and Akira, 2006).

These modifications promote conformational changes revealing IRF-association domain and DNA-binding domain and therefore, enabling the homodimerization of IRF3 and shuttle it into the nucleus (Chau *et al.,* 2008) where activate the transcription of IFN-β genes. Then, IRF3 subsequently activates an IFN-stimulated gene factor 3 (ISGF3) complex which in turn induces the expression of IRF7 and its activation in a similar manner as IRF3 (Moynagh, 2005).

Both transcriptional factors mediate the synthesis of IFN-α and β genes, as well as other IFN-induced genes (Moynagh, 2005). Type I IFNs present potent antiviral functions but additionally, they also have an important role in bridging innate and adaptive immunity by mediating the induction of co-stimulatory molecules on antigen-presenting cells (Hoebe and Beutler, 2004).

2.4. EXERCISE

During the last decades, multiples scientific evidence has described the benefits of practicing exercise regularly in health and quality of life (De Lemos *et al.,* 2012, Viña *et al.,* 2012). Physical activity is defined as "any bodily movement produced by skeletal muscles that result in energy expenditure" (Caspersen *et al.,* 1985). Although exercise and physical activity have been considered interchangeably terms, they are not exactly the same. Exercise is described as "a type of physical activity that is planned, structured and repetitive to improve or maintain one or more components of physical fitness" (Caspersen *et al.,* 1985).

Regular workout provides multiple health benefits for all ages, both sexes and all physical abilities. It is a popular notion that exercise can prevent excess weight gain, preserve lean body mass, increase resting metabolic rate and raise the ability of the body to use fat during physical activity (Strasser *et al.,* 2012). In addition, it improves and strengthens the cardiorespiratory system and combats health conditions including stroke (Nocon *et al.,* 2008), type 2 diabetes (Tuomilehto *et al.,* 2001), certain types of cancer (Paffenbarger *et al.,* 1994, Tuomilehto *et al.,* 2001) and arthritis (Tierney *et al.,* 2012). Additionally, regular workout enhances muscle tone,

muscular strength, muscular flexibility and maintain bone mass, helping in posture and physical appearance and preventing chronic back pain (Crocker *et al.,* 2013). Exercise is also characterised by promoting psychological well-being through better mood, decreasing the symptoms of depression and anxiety (Paffenbarger *et al.,* 1994).

2.4.1. Exercise in the elderly

All these benefits that regular exercise can exert are largely attributable to physically active seniors (Figure 3) (Physical Activity Guidelines Advisory Committee Report, 2008, Paterson *et al.,* 2007). Generally, as people get older, activity levels tend to decrease. In fact, statistics infer that more than 30 % of older adults are completely sedentary (Allen and Morelli, 2011).

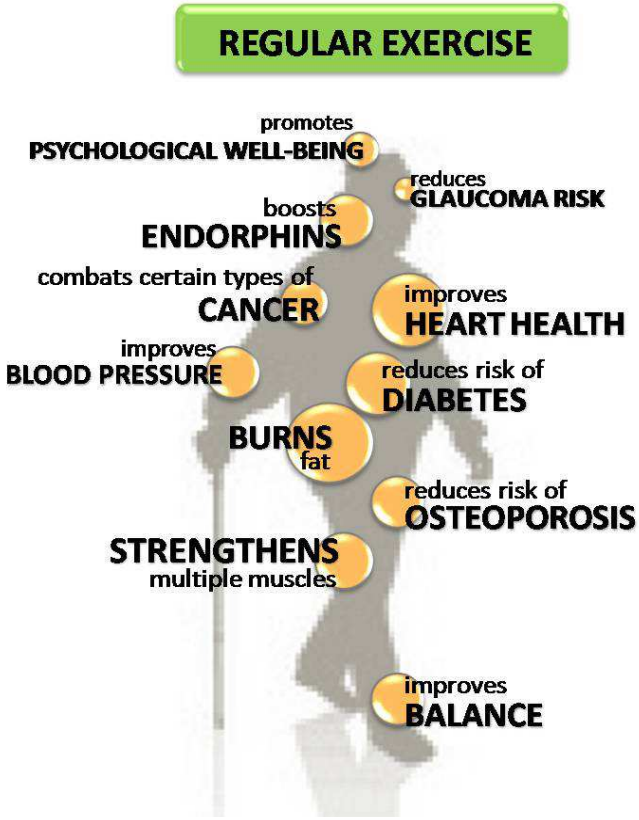

Figure 3. Regular physical activity benefits older adults. Regular and moderate exercise can help seniors to prevent or delay many physical and psychological age-related changes, decreasing mortality and morbidity in older adults

Ageing is denoted by many physical and psychological changes such as slower basal metabolic rate, an increase in blood pressure, a decrease in maximum heart rate, cardiac output, or maximal oxygen uptake (VO_2max) (Concannon *et al.,* 2012). Additionally, the loss in muscle mass and the deterioration of cardiorespiratory function (Harris, 2005) have serious impact on the ability of elderly subjects to perform daily activities, declining their quality of life, promoting deconditioning and dependency of others. For all of these reasons, it is worth noting that physical activity provides an easy tool to improve personal fitness, health and independency in older adults (Cress *et al.,* 2005).

2.4.1.1. Immunological benefits of exercise in the elderly

In addition to the general benefits mentioned above, multiple studies support a positive role that exercise may exert in the immune system. It is well accepted that physical conditioning is correlated directly with the induction of anti-inflammatory effects that counteract the age-associated increases in pro-inflammatory cytokines, reducing the risk of developing diseases related with a sedentary lifestyle and the rate of morbidity and mortality (Greiwe *et al.,* 2001, Pedersen *et al.,* 2000).

Specifically, strong evidence supports an alteration of the number and function of circulating cells of the innate immune system after acute and chronic exercise (Walsh *et al.,* 2011).

For instance, after an acute exercise, blood neutrophils levels increase accordingly to the intensity and duration performed (Peake, 2002). However, studies conducted to analyze this cell population after a training period show different results, inferring no change in neutrophils counts after regular training (Gleeson and Bishop, 2005).

Monocytes are also affected by exercise supporting the idea of a transient increase of this cell population in the circulating pool after acute bouts (Okutsu *et al.,* 2008) but a real reduction after exercise training (Flynn *et al.,* 2003, McFarlin *et al.,* 2004, McFarlin *et al.,* 2006, Sloan *et al.,* 2007, Stewart *et al.,* 2005). In accordance

with this, it has been also report an exercise phenotype change in monocytes triggering alterations of cell surface proteins (Lancaster *et al.,* 2005, Simpson *et al.,* 2009) and cytokines expression (Lancaster *et al.,* 2005, Starkie *et al.,* 2000).

Alterations in other blood leukocytes concentration were also reported after both acute and prolonged exercise such as an increase in the number of dendritic cells (Chiang *et al.,* 2007, Liao *et al.,* 2006) and modest enhancements of NK cells after moderate exercise training (McFarlin *et al.,* 2005, Woods *et al.,* 1999).

Overall, it appears that both acute and chronic exercise have the potential to alter the number and function of circulating cells of the innate immune system, modifying the susceptibility to infectious diseases.

2.4.1.2. Anti-inflammatory effects of exercise

Given the important role of innate immune cells in inflammatory states and the relationship between inflammation and chronic illness, it has been proposed physical activity as an efficient therapeutic approach to either prevent or delay the onset of certain diseases closely related to inflamm-ageing (Baltgalvis *et al.,* 2008, Pawelec *et al.,* 2006). In fact, cross-sectional studies have established an association between low-grade systemic inflammation and physical inactivity in healthy subjects (Colbert *et al.,* 2004, Kullo *et al.,* 2007, Pedersen and Bruunsgaard, 2003).

Multiple longitudinal experiments have probed that regular workout in seniors is able to diminished circulating levels of LPS-induce cytokine release (Nicklas *et al.,* 2008, Phillips *et al.,* 2010). Evidence suggests that, to yield these positive effects, each bout of exercise promotes an anti-inflammatory environment (Mathur and Pedersen, 2008, Petersen and Pedersen, 2005).

Recent reviews have inferred two major mechanisms through exercise encourage these anti-inflammatory effects (Flynn and McFarlin, 2006, Mathur and Pedersen, 2008, Petersen and Pedersen, 2005): an exercise-induced reduction in visceral fat and an exercise-induced anti-inflammatory environment.

Reduction of visceral fat mass

An inactive lifestyle leads to the accumulation of visceral fat turning into a problem of health that leads to reduce life expectancy and to enhance the likelihood of some pathologies, mainly heart disease or type 2 diabetes (Haslam and James, 2005).

The increase in body fat, especially in the abdomen, liver or muscles is closely related with the infiltration of immune cells in the adipose tissue (Ouchi *et al.,* 2011). As a result, it has been described an accretion in the production of pro-inflammatory mediators and a decrease in the content of anti-inflammatory markers (Ouchi *et al.,* 2011). This imbalance induced a low-grade chronic inflammatory state that could be substantially improved with regular physical activities (Yudkin, 2007) since exercise increases energy expenditure, reduces abdominal and visceral fat (Ross and Bradshaw, 2009) and reduces levels of pro-inflammatory mediators (Ben Ounis *et al.,* 2009, Mujumdar *et al.,* 2011).

Evidence is now emerging that these beneficial effects could have a closely relation with body weight loss (Church *et al.,* 2010). However, cross-sectional and longitudinal data indicate that exercise is able to promote this anti-inflammatory effect independently from reduction body size (Balducci *et al.,* 2010, Fischer *et al.,* 2007).

Establishment of an anti-inflammatory environment

Multiple evidences support that circulating IL-6 levels are exponentially increased during and immediately after exercise (Fischer, 2006, Meckel *et al.,* 2009). The magnitude by which plasma IL-6 increase seems to be directly linked with duration, intensity and muscle mass involved in the physical activity (Febbraio and Pedersen, 2002, Pedersen *et al.,* 2003, Wilund, 2007). Although active skeletal muscle is the major source of this cytokine, increasing both cellular and circulating levels of IL-6 (Keller *et al.,* 2005, Pedersen, 2009), adipose tissue also contributes with more than a quarter part of the total plasma level of this mediator (Fried *et al.,* 1998).

As previously has been reviewed, IL-6 is often classified as a pro-inflammatory cytokine but also has many anti-inflammatory and immunosuppressive effects. This is closely link with the inhibitory effect that IL-6 can exert in the TNFα production, with the stimulation of anti-inflammatory cytokines such as IL-1ra and IL-10 (Pedersen and Fischer, 2007, Steensberg *et al.,* 2003a, Steensberg *et al.,* 2003b), and with increments in cortisol levels, a steroid hormone that downregulates LPS-induced production of cytokines (Cupps and Fauci, 1982).

In accordance with the link between TLR2 and TLR4 and cytokine production, some exercise training studies have compared the inflammatory response of blood monocytes and TLR4 expression from physically active and inactive participants. The conclusion was that exercise may induced a decrease in both cell population and protein expression of the receptor closely associated with an amelioration in the inflammatory status of the active participants (Flynn and McFarlin, 2006, Gleeson *et al.,* 2006). Thereby, it has been infer an axis between TLRs, exercise and low-chronic inflammation (Gleeson *et al.,* 2011).

Afterwards, other TLRs have been also analyzed following both acute (Gleeson *et al.,* 2011, Oliveira and Gleeson, 2010, Stewart *et al.,* 2005) and prolonged (Lancaster *et al.,* 2005) exercise interventions, showing a similar response that the one observed in TLR4. As of yet, it is not clarified if these reductions are linked with a downregulation in cell surface expression or however, these receptors are internalized. Besides, another key point is what promotes this exercise-induced decrease in cell surface TLR expression. Several possible signals act as candidates, including anti-inflammatory cytokines, stress hormones or Hsp (Gleeson *et al.,* 2006). It is worth noting that plasma free fatty acids released after prolong exercise also seems to have an important role in this process (Nguyen *et al.,* 2007).

2.4.2. Exercise training programmes

Taken together all mentioned above, a large number of studies support the beneficial effect that practicing exercise induces in the elderly, but it is not yet clarified the optimal training modality or the intensity that should be employed.

2.4.2.1. Resistance exercise

Resistance or strength training is described as the voluntary activation of specific skeletal muscles against some forms of external resistance (American College of Sports Medicine *et al.,* 2009). There are considerable experimental evidences to support its capacity to induce profound effects on the musculoskeletal system contributing to the maintenance of functional abilities by preserving muscle mass and avoiding decreases in strength (Krebs *et al.,* 2007, Reeves *et al.,* 2006). Moreover, it is able to prevent osteoporosis and lower back pain (Sinaki *et al.,* 2010).

Muscle strengthening activities may be also efficient to ameliorate visceral adipose tissue and therefore, improve obesity status (Strasser *et al.,* 2012). It has been also demonstrated the positive effect of strength training in the protein-lipid profile (Costa *et al.,* 2011), resting metabolic rate (Hurley and Roth, 2000, Straight *et al.,* 2012), blood pressure (Kelley and Kelley, 2000), cardiovascular disease (Brogardh and Lexell, 2012, Perez-Terzic, 2012), and cancer (Strasser *et al.,* 2013).

All these problems affect the capacity of seniors to carry out daily living skills independently. For this reason, public authorities have emphasised on developing appropriate resistance-training programmes and promoting the regular practice of physical activity in this sector of the population. Therefore, health guidelines have included strength training as a safe and relative simple method that takes minimal time to encourage and maintain good health in the elderly (American College of Sports Medicine *et al.,* 2009). In fact, it has been record the possibility of accrue most of these benefits with only 15 or 20 min twice *per* week (wk) (Feigenbaum and Pollock, 1999).

Exercise prescription

In order to obtain improvements in functional capacity and strength, it is important to incorporate progression and variation into the resistance training intervention designed. Indeed, modifications in frequency, duration, exercises and intensity are recommended for achieve the best results (Nelson *et al.,* 2007).

In older adult, the resistance training programme normally are focused on lower-limb muscles, since their strength and power are directly needed for daily activities such as walking or rising from a chair. Nonetheless, it is important to determine a protocol with one or two exercises for each of the major muscle groups (Nelson *et al.,* 2007). The American College of Sport Science (ACSM) also suggests focus primarily on multi-joint exercise (bench press, shoulder press, leg press, etc.), although some uni-joint exercise (biceps curls, triceps extensions, leg extensions, etc.) are not discouraged for seniors.

Furthermore, for improving muscle strength in seniors, current guidelines advocates an average of two sets of each exercise with an appropriate resting period between sets of two or three min to avoid excess fatigue (Hautier and Bonnefoy, 2007).

Intensity is also an important parameter to define in the strength protocol. It refers to the percentage of the momentary degree of effort that is required (Feigenbaum and Pollock, 1999). Literature proposes training procedures under conditions of moderate to high intensity, without setting the precise level that yields optimal outcomes, since the appropriate amount of resistance depends on the strength level of each individual (Nelson *et al.,* 2007). Intensity is often expressed as a percentage of the one repetition maximum (1RM) that is the maximal amount of weight that can be lifted in a single repetition for a given exercise. For older adults, health authorities suggest intensities ranging from 65% to 75% 1RM to acquire significance increases in muscle strength (American College of Sports Medicine *et al.,* 2009).

Additionally, to achieve these aims, few reports also indicate the importance to maintain the highest speed possible (Cress *et al.,* 2005, Petrella *et al.,* 2007). However, this suggestion has been controversy because moving quickly undermines effectively overloading, fatigues muscles and stresses joints (Westcott *et al.,* 2001). Certainly, studies using slow repetitions show greater strength outcomes and higher increases in bone mineral density (Layne and Nelson, 1999, Westcott *et al.,* 2001).

Another important factor to determine in strength protocols is the number of repetitions that an individual performs. There is an inverse relationship between intensity and repetitions, indicating that as the intensity increases the repetitions should decrease (American College of Sports Medicine *et al.,* 2009). In view of the previously intensity recommended, some evidences attribute strengthen with a range between 8 to 12 movements per exercise with 3 sets (Harris *et al.,* 2004), although traditional studies were used to employ 10 to 15 repetitions in 3 sets (Feigenbaum and Pollock, 1999, Galvao and Taaffe, 2005). These data corroborate that longer training sessions are not necessarily more effective. In fact, older adults should avoid lengthy training sessions, which increase their risk of injury, manifested by extreme fatigue. Present guidelines for resistance training in seniors recommend a range of approximately 20 to 45 min per session (American College of Sports Medicine *et al.,* 2009).

Additionally, in a training protocol it should be also defined frequency, in other words the number of exercise sessions *per* wk. Elderly strength training reports recommend two or three training sessions *per* wk for improving the overall health and fitness capacity and to achieve muscle strength increases (American College of Sports Medicine *et al.,* 2009). Notable, it has been proposed that if intensity remains high, only one training session *per* wk may be enough for increasing strength and muscle mass (Nelson *et al.,* 2007) but it is not yet clear if other health-related changes attributable to resistance exercise would be maintained with such reduced training frequency.

Resistance exercise and inflamm-ageing

Despite the multiple studies that have confirmed the positive adaptations of practicing regular resistance exercise in the functional abilities among the elderly, its effects on the immune system have been poorly investigated although strength training has been also proposed as a therapeutic approach to ameliorate low-grade chronic inflammation associated with senescence. Indeed, the majority of publications were carried out in young individuals (Fernández-Gonzalo *et al.,* 2012, Jiménez-Jiménez *et al.,* 2008).

Hence, several researcher works have associated different programmes of chronic resistance exercise with reductions of circulating levels of pro-inflammatory cytokines in the elderly (Phillips *et al.,* 2010). Consequently, correlations with TLR expression and NF-κB have been also proposed. Literature describes how a resistance exercise training intervention was able to decrease cell-surface TLR4 content (Flynn *et al.,* 2003, McFarlin *et al.,* 2004) and how eccentric training programmes in older participants attenuated the activation of NF- κB (Jiménez-Jiménez *et al.,* 2008).

Discrepancies are also founded in the literature with some experiments that described no changes in the inflammatory biomarkers after a resistance exercise protocol (Kapasi *et al.,* 2003, Netea *et al.,* 1996), but different intensities used could explained the divergences.

Overall, it has been proposed that elderly individuals can engage in resistance training protocols without immune negative effects for exercise performed between 50 and 80% 1RM, several times *per* wk and at low-intensity (Neves Sda *et al.,* 2009, Ogawa *et al.,* 2010).

2.4.2.2. Aerobic exercise

Aerobic or endurance exercise is the ability to maintain a given level of physical activity over time or to perform a given task repeatedly increasing heart and breath rates (Frankel *et al.,* 2006). It must recruit a muscle mass sufficient to adjust

the cardiovascular system to the increase in demand (Hautier and Bonnefoy, 2007). Typical endurance training activities include walking and cycling or other everyday housework activities.

Ageing may adversely affect endurance fitness level. They often present irregular oxygen-carrying capacity of the cardiovascular system, modifications of both enzymes and mitochondrial capacities in muscle (Charles *et al.,* 2006). In addition, an endurance programme also affects diseases or general deconditioning processes.

There is a general consensus that body weight and fatness increase with advanced age, associating it with an augmented risk of morbidity and premature mortality (Kohrt *et al.,* 1992). Multiple evidences support that individuals who exercise regularly, accumulate less adipose tissue particularly in central body regions (Horowitz and Klein, 2000, Poirier and Despres, 2001). This promotes a modification of the abdominal fat distribution profile in older adults, reducing the risk to develop metabolic disorders associated with obesity (Strasser, 2013).

Moreover, cardiovascular problems are the second most common disease and the leading cause of death in older people. General deconditioning from inactivity is common in older adults, contributing to poor endurance. Various ageing studies reveal a direct relationship among cardiac fitness and the degree of independence (Charles *et al.,* 2006, Woo *et al.,* 2006). So, aerobic activities can help seniors to maintain and improve their cardiovascular health (Hautier and Bonnefoy, 2007, Short *et al.,* 2004).

In accordance with this, arterial blood pressure increases with advancing age, contributing to age-related increases in the risk of a number of cardiovascular disorders (Anonymous, 1997). Prevailing data in the literature support a decrease of systolic and diastolic blood pressure after regular aerobic exercise (Bean *et al.,* 2004, Kokkinos *et al.,* 2001). Additionally, it has been described this reduction of 11 mmHg in systolic and 8 mmHg in diastolic blood pressure with lowered total cholesterol content and upper levels of high density lipoproteins (Mazzeo and Tanaka, 2001). Nevertheless, plasma low-density lipoproteins are not obviously modulated by

regular exercise unless accompanied by significant reduction in bodyweight (Kiens, 2006, Mazzeo and Tanaka, 2001).

Additionally, it is also known the connection between aerobic exercise and diabetes. Studies conducted to examine the insulin sensitivity after endurance exercise training in humans have noted significant improvements (Hamdy *et al.,* 2001). For this reason, the enrolment in an aerobic activity has shown enhancements in lipid profile, blood pressure and energy expenditure in diabetic patients (Frankel *et al.,* 2006).

Exercise prescription

Considering all mentioned things, to promote and maintain health and physical function, deconditioned older adult need to practice moderate-intensity aerobic exercise in addition to routine tasks of daily living (Frankel *et al.,* 2006). The selection of the activity should be based on the fitness level of the participants and their preferences. Typical endurance training activities include cycling, running, walking and swimming. It is worth emphasized that current guidelines recommend practicing these activities with a moderate intensity.

Aerobic exercise intensity has commonly expressed using the oxygen uptake ($VO_{2)}$ which represents the capacity to transport and use oxygen during incremental exercise and reflects the physical fitness of an individual. Another option to appreciate the intensity of an aerobic programme is evaluating the percentage of heart rate (HR), corresponding the optimal intensity between 55% and 70% of maximum HR (HRmax) (Mazzeo and Tanaka, 2001).

Moreover, according to the current recommendation by the ACSM (Haskell *et al.,* 2007) to maintain and prolong health benefits associated with practicing aerobic exercise, seniors should accumulate at least 30 min of moderate intensity physical activity on most days of the wk (Haskell *et al.,* 2007). Gradually increasing the duration of each session should be a priority and then, increasing the intensity for short periods (Hautier and Bonnefoy, 2007).

Aerobic exercise and inflamm-ageing

Data gathered from the literature regarding the effects of aerobic physical training on inflamm-ageing are still limited and report either advocating benefits or asserting a lack of evidence.

Some studies carried out in older adults have described how in response to a long-term moderate intensity aerobic exercise training, participants showed a lower production of pro-inflammatory cytokines and a higher secretion of anti-inflammatory mediators (Borges *et al.,* 2013, Nunes *et al.,* 2013). Other shorter training periods have also demonstrated such beneficial adaptations in elderly individuals (Gano *et al.,* 2011, Goldhammer *et al.,* 2005, Kadoglou *et al.,* 2007). Those changes described in the concentration of circulating inflammatory mediators have been also related with decreases in TLR expressions (Oliveira *et al.,* 2011) and in NF-κB activation (Durham *et al.,* 2004, Ma *et al.,* 2013). However, controversial results also exist indicating no changes (Ferrier *et al.,* 2004, Ho *et al.,* 2005, McKenzie and Goldfarb, 2007, Nickel *et al.,* 2011, Reyna *et al.,* 2013, Zbinden-Foncea *et al.,* 2012) These divergences may rely in the variability between intensity and time of the different endurance programmes.

It is important highlight that some works have proposed that all of these positive effects induced by endurance activities on circulating inflammatory cytokines are related with weight loss (Simpson *et al.,* 2012). However, several cross-sectional experiments have indicated that the enhancement of this anti-inflammatory situation is independently from reductions in adiposity (Balducci *et al.,* 2010, Fischer *et al.,* 2007).

Taken together these valuable adaptations induced by chronic aerobic exercise, it has been proposed this physical activity as an inexpensive measure to improve elderly quality of life and reduce age-related inflammation.

2.4.2.3. Whole-body vibration exercise

Traditionally, resistance or endurance exercise has been the most popular training interventions since they are shown well-known positive effects in elderly. Nowadays, whole-body vibration (WBV), another physical activity modality, has received considerable attention since it seems to be a potentially safe, low-impact alterative to combat some age-associated deteriorations (Mikhael *et al.,* 2010). Despite the number of studies that have evaluated this alternative in young participants (Cochrane and Stannard, 2005), there is a little support evidence of the effects of WBV could exert in older adults (Merriman and Jackson, 2009).

WBV uses low to moderate multidimensional mechanical oscillations generated by a vibrating platform and transmitted through the body (Hazell *et al.,* 2010, Wilcock *et al.,* 2009). Some authors have proposed this exercise as an alternative for enhancing muscle activity (Abercromby *et al.,* 2007, Ronnestad, 2004), muscle strength (Pel *et al.,* 2009), muscle power and flexibility (Fagnani *et al.,* 2006), muscle cross sectional area (Machado *et al.,* 2010, Osawa and Oguma, 2013), bone mineral density (Verschueren *et al.,* 2004), and for decreasing abdominal fat (Vissers *et al.,* 2010). However, others have found little or no effect (Cormie *et al.,* 2006, De Ruiter *et al.,* 2003). These differences may rely in the divergence between protocols depending on the type of vibration platform, frequency displacement and training duration (Marin and Rhea, 2010, Osawa and Oguma, 2013).

Additionally to the benefits above mentioned, in older adults WBV training has great potential as a safe, low-impact training method with a low starting threshold (Tsuji *et al.,* 2013). In particular it has been reported benefits in balance, mobility and risk factors for falls in older adults (Cheung *et al.,* 2007, Kawanabe *et al.,* 2007) as well as enhances in postural control in patients with Parkinson's disease (Turbanski *et al.,* 2005) and reductions in muscle plasticity in patients with cerebral palsy (Ahlborg *et al.,* 2006).

Exercise prescription

Vibration is defined as any motion that repeats itself after a given period of time (Cochrane, 2011). Mostly it is practiced as whole body vibration standing on oscillating platforms. There are three main ways of energy generation: direct mechanical transmission, electromagnetic transmission and oscillating mass-spring systems although nowadays, the majority of vibration platforms use this last one system producing periodic sinusoidal oscillations, where energy is transferred from the vibratory machine to the human body (Rittweger, 2010).

Moreover, devices are also divided by different types of energy transference through the platform: vibration synchronously and vibration in a side-alternating way (Rittweger, 2010). In the first type, both legs extend and stretch at the same time, moving predominantly in the vertical direction. Conversely, in the side-alternating mode the right and left leg operate anti-phase, introducing a rotational movement around an ateroposterior horizontal axis (Cochrane, 2011).

Besides these differences between platforms, to establish an appropriate protocol it is important to characterize several parameters such as frequency, amplitude, acceleration or duration of the exposure.

Frequency is referred to the number of cycles *per* unit time and it is measured in hertz (Hz) that are one cycle *per* second (Bullock *et al.,* 2008, Luo *et al.,* 2005). It has been reported that vibration frequency below 20 Hz induces muscular relaxation, whereas values above 50 Hz promote muscle soreness (Rittweger *et al.,* 2003). In fact, the most effective range to activate the muscle has been described between 30 and 50 Hz (Luo *et al.,* 2005).

Amplitude is considered the half difference between the maximum and the minimum value of the oscillation and is expressed in mm (Kosar *et al.,* 2012). It can be also defined by the peak-to-peak amplitude, which is referred to as the displacement from the lowest to the highest point of the vibration wave (Rauch *et al.,* 2010). It depends on the type of vibration device: while for a side-alternating vibration

platform the amplitude is smaller closer to the axis and larger near the edge of the platform, for a synchronous vibration device foot placement is independent of amplitude and has a pre-setting of low (2 - 4 mm) or high (4 - 6 mm) (Cochrane, 2011).

Besides, another key parameter is the acceleration. Mathematically it is derived from the product of angular velocity and amplitude and expressed as terrestrial gravitation *g* (1 *g* = 9.81 m/s^2) or m/s^2 (Cochrane, 2011). Direct relationship has been described among acceleration and the force applied. Consequently, increasing acceleration relies on changes in frequency and amplitude (Cardinale and Wakeling, 2005). It should be noted how high levels of acceleration can be potentially harmful the organism (Wakeling *et al.,* 2002).

Although there is not a consensus in the most beneficial intervention that should be used, it has been inferred that low amplitude (ranging from 0.7 to 14 mm), moderate frequency (ranging from 20 to 50 Hz) and short periods of exposure, are safe and have beneficial effects on muscles (Verschueren *et al.,* 2011, Von Stengel *et al.,* 2012), bone mineral density (Mikhael *et al.,* 2010, Totosy de Zepetnek *et al.,* 2009) and body balance (Bogaerts *et al.,* 2007, Cheung *et al.,* 2007).

Moreover, although currently there is little scientific evidence on the optimal duration of a WBV session, it seems that intermittent protocols (30 - 60 s exposure) may be preferred to continuous (3 - 5 min) exposure because it stimulates muscle while limiting fatigue (Cochrane, 2011).

Vibration exercise and inflamm-ageing

Studies conducted to examine the influence of WBV exercise in an inflammatory status are limited and show controversial results. Some experiments report increases of creatine kinase and IL-6 (Naghii *et al.,* 2011). Certainly, a 5 wk WBV programme induced a significant decrease in the concentration of IL-10, inferring a possible trend for increased levels in pro-inflammatory cytokines (Nowal *et al.,* 2012).

However, Hazell and co-workers have demonstrated how a moderate intensity WBV session adding to a dynamic exercise intervention resulted in no muscle damaged and no enhance of muscle inflammatory markers (Hazell *et al.,* 2013). Subsequently, Simao and colleagues also proposed a significant reduction in plasma concentration of inflammatory biomarkers after combined squat exercise with vibratory stimulus (Simao *et al.,* 2012).

It is worth highlight that total duration of the WBV intervention may be a crucial factor in metabolic response since recently, Pawlak and co-workers explained how longer periods of application of short vibratory stimuli (3 or 6 months) did not influence blood parameters (Pawlak *et al.,* 2013). Therefore, repeated cycles of short vibration seem to be safe and can diminish muscle soreness and IL-6 blood concentration (Broadbent *et al.,* 2010).

Methods

3.1. GENERAL DESIGN

All the physical activities took place in the facilities of the Biomedical Department in the Sport Science and Physical Activities School of the University of León. Respecting to the molecular biology analysis took place in the Institute of Biomedicine (IBIOMED) of the University of León.

The study design is showed in Figure 4 and was planned to complete in 10 wks. The first wk was assigned to pre-training descriptive data and pre-training baseline data collection, pre-training testing and blood samples taken. Before the training intervention, subjects from the training groups carried out a session to practice and become familiar with the protocols and the devices. After that, subjects belong to the control group continued with their daily routines while participants of the three training groups started the exercise protocols. At the end of those 8 wks, post-training descriptive data compilation, post-training testing and blood samples taken were repeated.

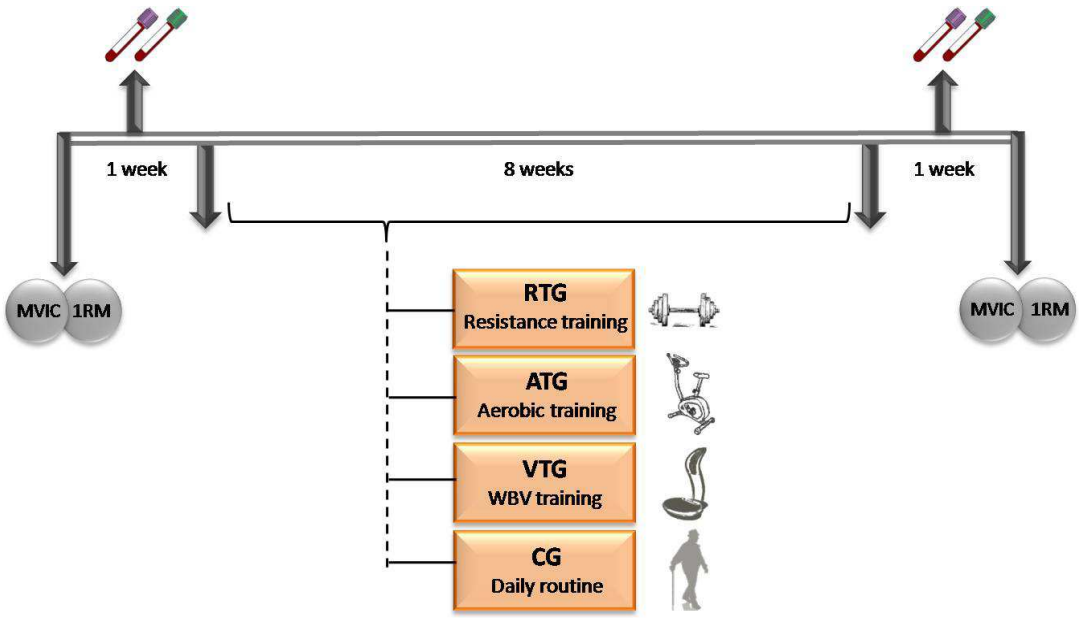

Figure 4. Study designed to complete the experiment. One wk before and after the training period, maximal isometric voluntary contraction (MVIC) and one repetition maximum (1RM) tests were carried out and blood samples collected. In the 8-wk training period, the control group continued with its daily routine, while the three training groups started the exercise protocols

3.2. SUBJECTS AND PROCEDURES

Firstly, 150 potential subjects from Senior Recreation Centres of the León City Hall received general information about the research. Subjects with no contraindications to exercise such as cardiac disorders, pacemaker, metal implants of the spine or cataracts were allowed to participate. In addition, seniors who had consumed any inflammatory medication one month before the study were not allowed to be involved in the study. Only lipid-lowering therapy was permitted. Then, the selected participants were informed in detail of the purposes and possible risks associated with the involvement in the study as well as potential benefits linked with the participation.

Finally, fifty-nine healthy active participants, between the ages of 65 and 87 years old, were recruited and decided to be volunteers in an 8-wk training study. All eligible subjects were asked to provide informed written consent after explaining them the procedures, risks and premises associated with the study, which was approved by the University of León ethics committee in accordance with the Declaration of Helsinki (World Medical Association General Assembly, 2001).

Afterwards, subjects were randomly assigned to four different groups: 13 assigned to control group (CG), 15 to the resistance training group (RTG), 16 to the aerobic training group (ATG) and 15 to the WBV training group (VTG). There were no significant differences between groups for age, height or body mass. None of the participants had any background in regular training and they were asked to make no major changes in their physical activity routines during their involvement in the study.

3.3. DESCRIPTIVE AND BASELINA DATA COLLECTION

Descriptive and baseline data were collected during a laboratory session carried out 1 wk before the first training session. Firstly, each participant completed a personal data sheet and a questionnaire about risk factors. Moreover, they also filled out the physical activity readiness questionnaire (PAR-Q) to determine the safety or

possible risk of being much more physically active. Those documents were completed by each participant without the help of a specialist.

Before any other activity, an extensive medical screening was performed by a physician who checked the inclusion and exclusion criteria and the blood pressure in all the participants. Right after and at rest state, it was made an electrocardiogram test followed by an ergospirometry test with a prolonged submaximal incremental cycling protocol to evaluate HRmax and its relation with VO_2. Then, an anthropometric evaluation (Table 1) was made always by the same experienced researcher, measuring height (metric stadiometer Detecto D52™, Webb City, MO, USA) and weight (scales Cobol 20™, Barcelona, Spain). In addition, body max index (BMI) was determined using the followed formula:

$$BMI = \frac{mass}{(height)^2} = \frac{Kg}{m^2}$$

	n	Age (years)	Height (m)	Weight (kg)	BMI (kg/m^2)
Control group (CG)	13	70.7 ± 0.9	1.58 ± 0.09	68.1 ± 2.5	27.0 ± 0.8
Resistance exercise training group (RTG)	15	69.1 ± 1.1	1.57 ± 0.14	67.4 ± 2.3	27.2 ± 0.6
Aerobic training group (ATG)	16	69.6 ± 1.0	1.61 ± 0.12	70.6 ± 3.1	26.9 ± 0.7
WBV training group (VTG)	15	71.0 ± 1.5	1.56 ± 0.17	65.7 ± 3.1	26.8 ± 1.0

Table 1. Descriptive characteristics of the participants. Age (years), height (m), weight (kg) and body max index (kg/m^2). Data are expressed as mean ± SEM

3.4. MAXIMAL STRENGTH ASSESSMENT

Approximately 1 wk before and 1 wk after the training period, maximal strength was assessed in all the volunteers. The participants carried out a standard 10-min warm-up between 80 and 100 beats *per* min (bpm) on a cycle ergometer

(Tunturi F35, Tunturi®, Turku, Finland). Then, the strength was measured by a maximal isometric voluntary contraction (MVIC) test in a standardized manner: knee flexors and extensors were tested in a 45°-inclined leg-press (Gervasport™, Madrid, Spain) at 110° knee flexion, pushing against a fixed platform as hard as possible for 5 s. Elbow flexors were also evaluated in biceps curl position, in a biceps curl bench device (Gervasport) at 90° elbow flexion, pulling on a fixed resistance also for 5 s. Maximal strength was registered by a strain gauge system (Globus Ergometer™, Globus, Codogne, Italy) placed between the chains used to fix the platform or the resistance device in each case. Subject's angles were measured with a manual goniometer (TEC™, Madrid, Spain). The participants were informed about the test purpose and procedure before the start of the study and verbal encouragement was given during the tests. Each subject performed two attempts interspersed with 3 min recovery, and the best attempt was considered for further data analysis.

After approximately 30 min of rest, 1RM test was performed in the same leg-press and biceps curl bench previously explained and, in addition, in a seated pec deck machine (BH Fitness Nevada Pro, BH, Vitoria, Spain) following "The National Strength and Conditioning Association" (NSCA) protocol (Baechle and Earle, 2000). In the bilateral leg-press already described, subjects performed one repetition from 90° to full extension (180°) with an estimated load that was increased with 10 kg if the participant succeeded or decreased 5 kg if failed. Testing ended when participants failed to overcome a given resistance in 2 successive trials. Participants achieved their 1RM between 3 and 5 attempts. To avoid possible injuries and to standardize body position, participants were asked to keep back, head and bottom against the padded supports, avoiding raised them from the platforms and to keep their hands on the handle-bar at all times. Similar protocols were followed in the biceps curl bench previously described, with a range movement from 120° to maximal flexion and, in addition, in a seated pec deck fixing the position in 90° of elbow flexion and the arms parallel to the midaxillary line of the thorax.

3.5. EXERCISE TRAINING PROTOCOL

3.5.1. Resistance exercise training

Subjects from the RTG completed 16 resistance exercise training sessions over 8 wks (2 sessions *per* wk), with at least 48 hours between sessions. The protocol, initiated 1 wk after the evaluation, started with 10-min warm-up on a cycle ergometer followed by three different resistance exercises to upper and lower body: leg press, biceps curl and pec deck, in the same exercise devices previously used for maximal strength assessment. The repetitions and load for the three exercises were progressively increased as it is shown in Table 2. The recovery time between exercises was 5 min and between repetitions was 2 min.

Week	1	2	3	4	5	6	7	8
Sessions per wk	2	2	2	2	2	2	2	2
Sets x repetitions	3x8	3x10	3x12	3x8	3x10	3x12	3x8	3x10
Load (% 1RM)	60%	60%	60%	70%	70%	70%	80%	80%

Table 2. ***Resistance exercise training programme.*** Each session was divided in leg-press, biceps curl and pec deck exercises with the appropriate sets and repetitions and the suitable load

3.5.2. Aerobic exercise training

Aerobic exercise training consisted of cycling with a progressive increase in the exercise load weekly. This protocol was performed twice per wk on alternating days, for 8 wks straight. Each volunteer wore a heart monitor (Polar, Lake Success, NY, USA) calibrated to ensure the range of training heart rate achieved. Each session consisted of three distinct stages: 5-min progressive warm-up until reach 70% of HRmax followed by alternating periods of maximal effort with less-intense phases and then, a cool-down of 5 min to enable the recovery from the activity. The training programme is in detailed in Table 3.

Week	Session	Duration of session	Intensity	Warm-up	Interval 1	Time between intervals	Interval 2	Time between intervals	Interval 3	Time between intervals	Interval 4	Time between intervals	Cool-down
		min	%HRmax	min	min	min	min	min	min	min	min	min	min
1	1	25	70%	5									5
	2	25	70%	5									5
2	3	25	70%	5									5
	4	25	70%	5									5
3	5	26	75%	5	1	7	1	7					5
	6	25	75%	5	1	6	1	6	1				5
4	7	27	75%	5	1	7	1	7	1				5
	8	25	75%	5	1	6	1	6	1				5
5	9	23	75%	5	1	5	1	5	1				5
	10	28	75%	5	1	5	1	5	1	5			5
6	11	29	75%	5	1	5	1	5	1	5	1		5
	12	28	75%	5	1	5	1	5	1	4	1		5
7	13	27	75%	5	1	5	1	5	1	4	1		5
	14	27	75%	5	1	4	1	5	1	4	1		5
8	15	26	75%	5	1	4	1	4	1	4	1		5
	16	30	75%	5	1	4	1	4	1	4	1	4	5

Table 3. Aerobic exercise training programme. Each session was divided in an initial warm-up of five min, a subsequent maximum effort interval training with less-intense periods based on the percentage of HRmax and a progressive cool-down of five min

In addition, to clarify the aerobic training protocol carried out, figure 5 illustrates one random session profile based on three maximal-effort interval periods and two less-intense phases.

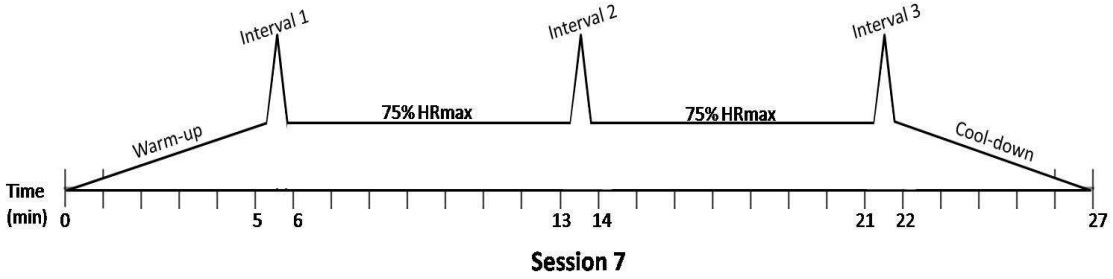

Figure 5. Representative aerobic exercise training profile. The session number 7 was carried out in the 4th wk of the programme and was based on 27 min of cycling with 5-min warm up, three 1-min maximal effort interval periods, two stages at 75% of the heart rate and a final 5-min cool down

3.5.3. WBV exercise training

Participants from the VTG performed an 8-wk WBV training programme on a vibration platform (Fitvibe, Gymna Uniphy NV, Bilzen, Belgium). Following a standardized warm-up of 10 min in a cycle ergometer, each session consisted of static or dynamic exercises including half-squat (Figure 6a) between 120° and 130° knee angle, deep squat (Figure 6b) with 90° of knee angle, wide-stance squat (Figure 6c) and calves (Figure 6d) with knee angle between 120° and 130°. Training volume (repetitions and time per exercise) and frequency were increased weekly. The two sets performed in each session were interspersed by 5-min rest periods (Machado *et al.,* 2010). Protocol is described in detailed in Table 4.

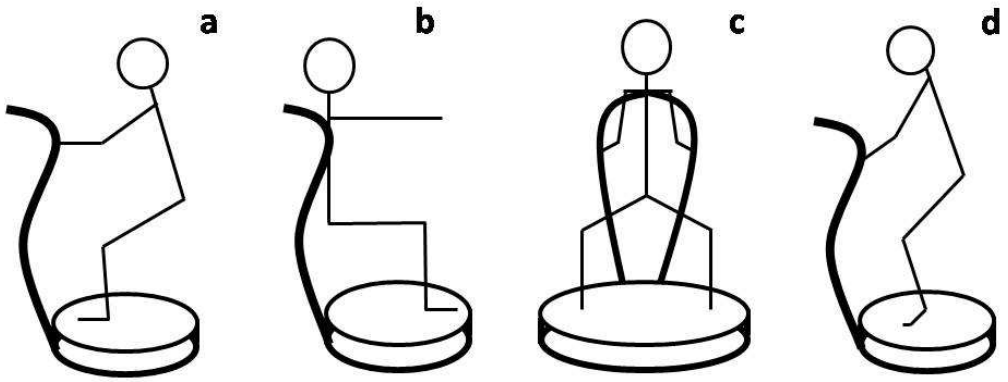

Figure 6. Whole-body vibration training exercises. Each session was based on either static or dynamic exercises such as half-squat (a), deep squat (b), wide-stance squat (c) and calves (d)

Week	Volume							Intensity		
	Training frequency	Repetitions per series of each exercise				Duration of each exercise	Rest between series	Amplitude	Frequency	Modality
	sessions/wk	a	b	c	d	s	min	mm	Hz	
1	2	1	1	1	-	30	5	4	20	Static
2	2	1	1	1	1	30	5	4	25	Static
3	2	2	2	1	1	30	5	4	30	Static
4	2	1	1	2	2	30	5	4	30	Dynamic
5	2	2	2	1	1	45	5	4	35	Dynamic
6	2	1	1	2	2	45	5	4	35	Dynamic
7	2	2	1	2	2	60	5	4	35	Dynamic
8	2	1	2	2	2	60	5	4	35	Dynamic

Table 4. Whole-body vibration training programme. Each session consisted of a defined number of static or dynamic exercises with a determined duration of each one. Exercises included half-squat (a), deep squat (b), wide-stance squat (c) and calves (d)

3.6. BLOOD SAMPLE PREPARATION

Theoretical basis:

Separation of PBMC from whole blood is most commonly accomplished through density gradient centrifugation using Ficoll to isolate lymphocytes and monocytes under a layer of plasma (Cuevas *et al.,* 2005).

Reagents needed:

-Phosphate Buffered Saline (PBS): NaCl 136 mM, KCl 2.7 mM, Na_2HPO_4 7.8 mM, KH_2PO_4 1.7 mM, pH 7.4

-Ficoll separation solution (Biochrom AG, Berlin, Germany)

Procedure:

Venus blood samples (30 ml) were collected from the brachiocephalic vein using heparin and ethylenediamine tetraacetic acid (EDTA) anticoagulants Vacutainer™ system (BD, Franklin Lakes, NJ, USA) 1 wk before (pre) and after (post) the training period.

Whole-blood collected in heparin anticoagulant was used to analyze the total leukocyte count. All samples were counted in triplicate using a particle counter (Beckman- Coulter, Miami, FL, USA).

In addition, just immediately after its collection, total blood in EDTA anticoagulant were centrifuged at 1500 xg for 10 min (4°C) to separate plasma. Subsequent, a density gradient centrifugation on Ficoll separation solution was used to isolate PBMC. After diluting the blood sample with an equal volume of PBS, six 15-ml centrifuge Falcon™ tubes (BD Biosciences, Bedford, MA, USA) with 4 ml of Ficoll each one were used to layer with 6 ml of the reconstitute blood. The tubes were centrifuged for 40 min at 240 xg (room temperature). Then, the interphase corresponding to the mononuclear cell layer was aspirated by manual pipetteing and carefully transferred to new 15 ml Falcon™ tubes where cells were washed with PBS until a final volume of 10 ml, followed by a 10 min centrifuged at 890 xg (room temperature). After that, PBS was decanted and cell pellet was resuspended in 1 ml of PBS and centrifuged again 5 min at 2630 xg at room temperature. Finally, mononuclear cells were dried manually by pipetteing and frozen at -80°C.

3.7. QUANTIFICATION OF mRNA USING REVERSE TRANSCRIPTION AND QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION

Theoretical basis:

Quantitative real-time polymerase chain reaction (RT-qPCR) amplifies DNA exponentially, doubling the number of molecules present in each amplification cycle and detecting the product as the reaction progresses by measuring fluorescence

signals generated when the fluorophore binds to the target sequence (Livak and Schmittgen, 2001).

Procedure:

Total RNA was isolated from PBMC using a RiboPure™-Blood Kit (Ambion, Paisley, UK) and quantified by spectrophotometry (Nanodrop 1000, Thermo Scientific, Waltham, MA, USA). DNase I (RNase-free) (Ambion, Paisley, UK) was used to removed residual genomic DNA. First-standard complementary DNA (cDNA) was synthesized using High-Capacity cDNA Archive Kit (Applied Biosystems®, Paisley, UK) and then, it was amplified using TaqMan® Universal PCR Master Mix (Applied Biosystems®) on a StepOnePlus Real-Time PCR Systems (Applied Biosystems®). TaqMan® primers and probes for IL-10 (Genbank M57627.1 and Hs00961622_m1), TNFα (Genbank M10988.1 and Hs00174128_m1) and GADPH as housekeeping gene (Genbank M33197.1 and Hs99999905_m1) were derived from the commercially available TaqMan® Assays-on-Demand Gene (Applied Biosystems®). Relative changes in gene expression levels were determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The cycle number at which the transcripts were detectable (CT) was normalized to the cycle number of GAPDH detection, referred to as ΔCT.

3.8. PROTEIN CUANTIFICATION USING WESTERN BLOT ANALYSIS

Theoretical bases:

This technique is based on the transferred to a solid-phase membrane support of size-separated proteins leading them accessible for immunological reactions with specific antibodies (Mahmood and Yang, 2012). The analyses used in this study were made following the technique described by Laemmli (Laemmli, 1970).

Reagents:

-Sonication buffer: sucrose 0.25 mM, EDTA 1 mM, Tris 10 mM, pH 7.4

-Lysis buffer: NaCl 140 mM, EDTA 15 mM, glycerol 10% and Tris 20 mM, pH 8

-Loading buffer: H_2O, Tris-HCl 2 M, glycerol 60%, SDS 10% and pyrroline 0.5%

-Electrophoresis buffer: Tris 25 mM, glycine 0.2 M, SDS 3.5 mM, pH 8.0

-Transfer buffer: Tris 25 mM, glycin 0.2 M, methanol 20%

-PBS: NaCl 0.14 M, KH_2PO_4 1.4 mM, $NaHPO_4$ 8 mM, KCl 2.7 mM, pH 7.4

-PBS-tween (PBS-t): 0.05% tween

Procedure:

For western blot analysis, PBMC were homogenized in 150 µl sonication buffer containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) and sonicated to physical disrupted them with an ultrasonic processor (UP100H, Hielscher, Teltow, Germany). Protein content of each sample was measured by the technique described by Bradford (Bradford, 1976). Lysate samples containing 50 µg of protein were fractionated on three different percentage gels: 9% SDS-polyacrilamide gels for the analyses of Hsp60, Hsp70, TLR2, TLR4, TRIF and IKKi/IKKε, 12% SDS-polyacrilamide gels for the analyses of MyD88, p65, ERK1/2, phospho-ERK1/2, p38, phopho-p38, IRF3, phospho-IRF3, IRF7 and phospho-IRF7 and 15% SDS-polyacrilamide gels for TNFα and IL-10. In addition, β-actin were analyzed in each blot to verify equal protein loading.

Separated proteins were transferred to PVDF Immobilon-P (Millipore, Billerica, MA, USA) membranes by a Trans-Blot Turbo Transfer System (Biorad, Hercules, CA, USA). Non-specific binding was blocked by pre-incubation of the PVDF membranes in PBS-t containing 2.5% non-fat milk for 30 min at 37°C. After that, incubation with specific primary antibodies was performed overnight at 4°C (Table 5).

After the overnight incubation, membranes were washed with PBS-t 6 times for 5 min and incubated one hour with an appropriate horseradish peroxidase (HRP) conjugated secondary antibody (Dako, Glostrup, Denmark). Bound primary antibody was detected using a chemiluminescent-HRP substrate (Luminol Reagent, Santa Cruz Biotechnology). Finally blots were exposed to autoradiography films (Amersham Hyperfilm ECL, Amersham, Little Chalfont, UK) and developed. The density of the

specific bands was quantified with an imaging densitometer (Image J, Bethesda, MD, USA).

Antibody	Molecular weight	Commercial	reference
Hsp70	70 kDa	Abcam®	ab1428
TRIF	66 kDa	Abcam®	ab13810
IKKi/IKKε	80 kDa	Abcam®	ab7891
p38	43 kDa	Cell Signalling Technology®	9212
Phospho-p38	43 kDa	Cell Signalling Technology®	9215
Phospho-IRF3	50 kDa	Cell Signalling Technology®	4947
Phospho-IRF7	54 kDa	Cell Signalling Technology®	5184
TLR2	90-100 kDa	Santa Cruz Biotechnology	sc-8690
TLR4	95 kDa	Santa Cruz Biotechnology	sc-293072
MyD88	33 kDa	Santa Cruz Biotechnology	sc-11356
p65	65 kDa	Santa Cruz Biotechnology	sc-372
IRF3	50 kDa	Santa Cruz Biotechnology	sc-9082
IRF7	54 kDa	Santa Cruz Biotechnology	sc-74471
ERK1/2	42-44 kDa	Santa Cruz Biotechnology	sc-93
Phospho-ERK1/2	42-44 kDa	Santa Cruz Biotechnology	sc-7383
Hsp60	60 kDa	Santa Cruz Biotechnology	sc-376240
TNFα	17 kDa	Santa Cruz Biotechnology	sc-8301
IL-10	20 kDa	Santa Cruz Biotechnology	sc-7888
β-actin	42 kDa	Sigma-Aldrich	A5060

Table 5. Primary antibodies used in the Western blot technique to detect and quantify the specific proteins. Abcam® (Cambridge, UK, USA); Santa Cruz Biotechnology (Santa Cruz, CA, USA); Cell Signalling Technology® (Beverly, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA)

3.9. STATISTICAL ANALYSIS

Values are presented as mean ± standard error of means (SEM). Post-training values were normalized to pre-training values. All data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures for group (CG, RTG, ATG and VTG) and time (pre and post). A Bonferroni *post hoc* analysis was used to adjust the *p* value in relation to the numbers of contrast that were performed. Differences were considered significant when $p < 0.05$. All statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA).

Results

4.1. MAXIMAL STRENGTH

Results for maximal dynamic (1RM) strength analyzed in the leg-press, in the biceps curl bench and in the seated pec deck previously described are shown in Table 6. Data obtained after the isometric (MVIC) test for all the groups are also presented in Table 6. All values are represented as mean ± SEM.

Values obtained from the CG showed unchanged strength measurements after the 8 wks in leg-press and in biceps curl bench for both 1RM and MVIC and in seated pec deck for 1RM.

4.1.1. Effects of a resistance training programme

After the resistance training programme, RTG showed an increase from pre- to post-training measurement in leg-press 1RM ($p < 0.03$), in biceps curl bench 1RM ($p < 0.04$) and in a seated pec deck 1RM ($p < 0.04$). Values of maximal strength measured by MVIC test were also significant higher in the RTG before and after the 16 sessions of resistance exercise in leg-press ($p < 0.05$) and in biceps curl bench ($p < 0.04$). Significant differences were also obtained after contrasting all these data of the RTG with the data elicited from the CG (leg-press 1RM, $p < 0.05$; biceps curl bench 1RM, $p < 0.05$; pec deck 1RM, $p < 0.05$; leg-press MVIC, $p < 0.04$; biceps curl bench MVIC, $p < 0.05$).

4.1.2. Effects of an aerobic training programme

Measurements obtained from the ATG illustrated a significant enhancement comparing pre- to post- training data in the MVIC leg-press test ($p < 0.05$), while the rest of measurements were statistically unchanged when contrasted them with the pre values. Similar results were obtained after contrasting these data with values obtained from the CG showed only significant differences in the MVIC leg-press test ($p < 0.04$).

4.1.3. Effects of a WBV training programme

The two maximal strength test carried out in the VTG showed significant higher levels between pre- and post-training in leg-press 1RM ($p < 0.04$) and MVIC ($p < 0.04$), in biceps curl bench MVIC ($p < 0.05$) and pec deck 1RM ($p < 0.05$). Significant differences were also obtained after contrasting all these data of the VTG with the data elicited from the CG (leg-press 1RM, $p < 0.05$; pec deck 1RM, $p < 0.05$; leg-press MVIC, $p < 0.03$; biceps curl bench MVIC, $p < 0.05$).

		Leg Press		Biceps Curl Bench		Pec Deck
		1RM	MVIC	1RM	MVIC	1RM
Control group (CG)	Pre	160.4 ± 13.5	96.3 ± 13.1	19.0 ± 3.2	15.1 ± 2.1	25.7 ± 4.0
	Post	171.3 ± 18.2	98.1 ± 15.8	21.2 ± 4.8	16.3 ± 3.3	27.6 ± 5.4
Resistance exercise training group (RTG)	Pre	157.0 ± 13.1	109.8 ± 12.2	19.7 ± 3.1	13.2 ± 1.1	24.1 ± 4.3
	Post	198.4 ± 14.3*#	137.4 ± 12.8*#	26.0 ± 3.2*#	19.4 ± 2.0*#	31.5 ± 5.0*#
Aerobic training group (ATG)	Pre	168.6 ± 12.4	108.8 ± 10.4	16.5 ± 2.5	16.1 ± 2.6	25.4 ± 4.6
	Post	177.1 ± 10.1	127.9 ± 11.3*#	17.4 ± 2.2	17.7 ± 2.2	27.0 ± 4.3
WBV training group (VTG)	Pre	160.0 ± 13.5	106.7 ± 15.8	15.3 ± 2.0	14.1 ± 2.0	25.7 ± 3.5
	Post	190.4 ± 18.7*#	140.4 ± 16.7*#	16.6 ± 2.2	18.8 ± 2.2*#	30.1 ± 4.8*#

Table 6. Maximal strength before (pre) and after (post) the training period of 8 wks. Values are given as mean ± SEM. Control group (CG), resistance training group (RTG), aerobic training group (ATG) and whole-body vibration training group (VTG) data for one repetition maximal (1RM) and for maximal voluntary isometric contraction (MVIC) in Kilograms (Kg). *Significant differences compared to basal value, same group; #Significant differences compared to CG

4.2. OXYGEN CONSUMPTION

Results for peak VO_2 analyzed in the prolonged submaximal incremental cycling test are shown in Table 7. Values obtained from the CG did not showed any changed in VO_2 measurements before and after the 8 wks of normal daily routine.

		Peak VO_2
Control group (CG)	Pre	30.5 ± 1.3
	Post	30.7 ± 1.5
Resistance exercise training group (RTG)	Pre	30.3 ± 0.9
	Post	32.3 ± 1.1
Aerobic training group (ATG)	Pre	31.0 ± 1.3
	Post	37.5 ± 1.7*#
WBV training group (VTG)	Pre	30.7 ± 1.1
	Post	31.1 ± 1.3

Table 7. Peak oxygen consumption before (pre) and after (post) the training period of 8 wks. Values are given as mean ± SEM. Control group (CG), resistance training group (RTG), aerobic training group (ATG) and whole-body vibration training group (VTG) data in litre/min (L/min). *Significant differences compared to basal value, same group; #Significant differences compared to CG

4.2.1. Effects of a resistance training programme

Values of peak oxygen uptake were unchanged in the RTG before and after the 16 sessions of resistance exercise. Non-significant differences were obtained after contrasting these data of the RTG with the data elicited from the CG.

4.2.2. Effects of an aerobic training programme

The prolonged submaximal incremental cycling test carried out in the ATG showed significant higher levels between pre-and post-training measurements in VO_2 ($p < 0.03$). Significant differences were also obtained after contrasting these data with the data elicited from the CG ($p < 0.03$).

4.2.3. Effects of a WBV training programme

Measurements obtained from the VTG were unchanged when contrasted them with the pre values. Similarly, results were obtained after contrasting these data with values from CG, regarding a non-significant difference.

4.3. EFFECTS OF PHYSICAL TRAINING ON THE TLR2 AND TLR4 EXPRESSION IN PBMC OF THE ELDERLY

Literature described how physical activity induces adaptations in the inflammatory response through the TLRs due to the pivotal role that these receptors play in the initiation of the inflammatory and immune response. Hence, a breakdown in the appropriate regulation or an exaggerated response of the TLR pathway can cause common chronic inflammatory diseases in which high levels of cytokines and inflammatory mediators become destructive (Mookherjee *et al.,* 2006).

After analyzed the protein content for both TLR2 and TLR4 in PBMC, data elicited from the CG showed unchanged values before and after the 8 wks of normal routine that these participants carried out.

It is worth highlight that total PBMC count was unchanged before and after the different training protocols so, the results were not corrected by these values.

4.3.1. Effects of a resistance training programme

The results of the protein expression of TLR2 and TLR4 after the 8 wks for the RTG are shown in Figure 7. How it can be seen, the protein content for both receptors were significantly reduced (TLR2, $p < 0.03$ and TLR4, $p < 0.04$) after the resistance exercise protocol employed here when they were compared with the value before performed the resistance training programme. Significant differences also appeared after contrast post results for both receptors with the precise value obtained from CG (TLR2, $p < 0.03$ and TLR4, $p < 0.04$).

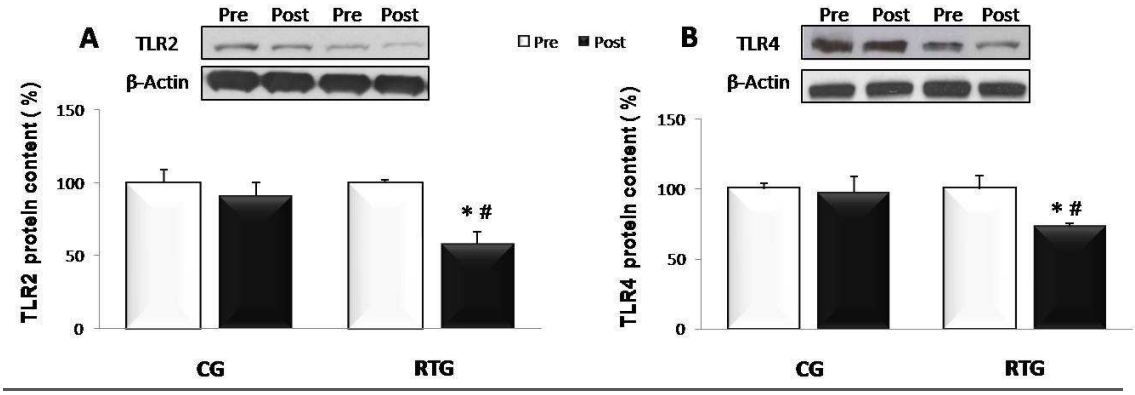

Figure 7. TLR2 (A) and TLR4 (B) protein expression before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.3.2. Effects of an aerobic training programme

Data elicited from the TLR2 and TLR4 protein content after the 16 sessions of aerobic exercise that the ATG carried out, are indicated in Figure 8. It is shown that the content of those two receptors in PBMC of the elderly participants were unchanged, compared them with the pre values of the training group and same results are obtained after contrasted them with the data elicited from the CG.

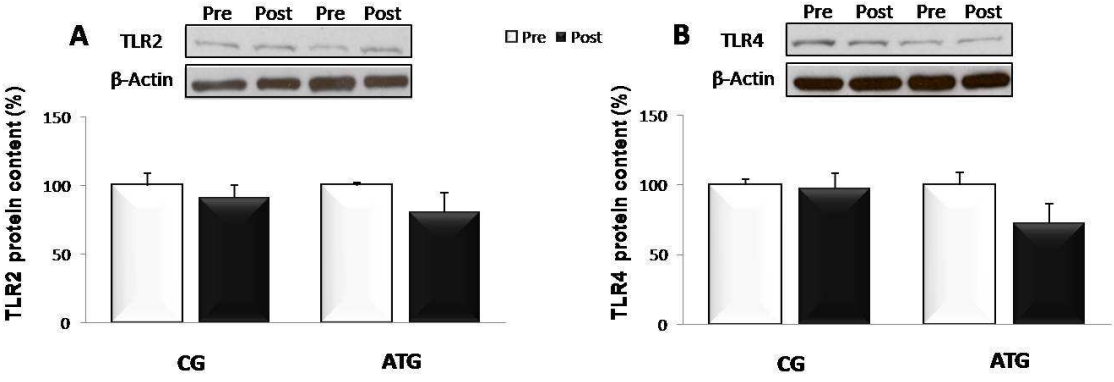

Figure 8. TLR2 (A) and TLR4 (B) protein expression before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM

4.3.3. Effects of a WBV training programme

Figure 9 shows the values obtained after the 8 wks of WBV training for both TLR2 and TLR4 in the vibration and the control group. They illustrate a significant decrease of TLR2 ($p < 0.03$) after the training protocol when these values are contrasted with pre data. Same results are shown in protein content of TLR4, which showed a significant decrease ($p < 0.03$) after the 16 sessions of WBV exercises. Statistically significant differences were also observed after compared these results with the appropriate values of CG (TLR2, $p < 0.04$ and TLR4, $p < 0.04$).

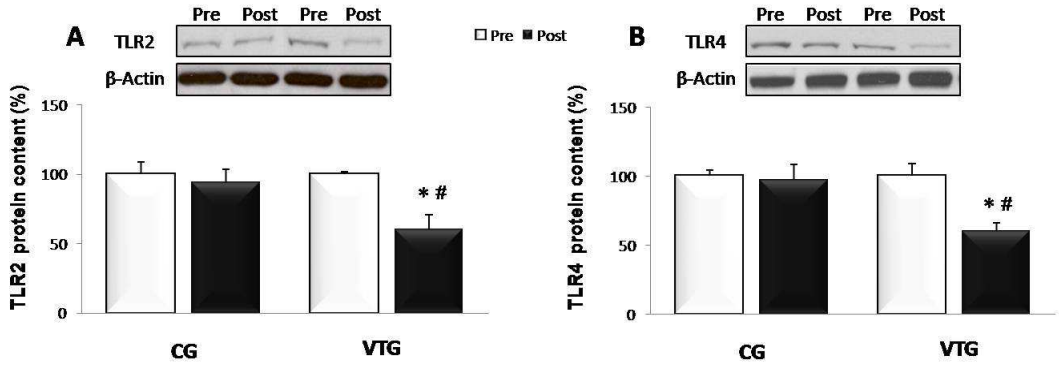

Figure 9. TLR2 (A) and TLR4 (B) protein expression before and after the 8 wks of WBV training for VTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4. EFFECTS OF PHYSICAL TRAINING ON THE MyD88-DEPENDENT PATHWAY IN PBMC OF THE ELDERLY

4.4.1. MyD88 expression

Upon stimulation, TLR2 and TLR4 start the recruitment of different TIR domain-containing signalling adaptors such as MyD88, which leads the activation of a number of downstream mediators to conclude the cascade in the activation of NF-κB or MAPKs (Lu *et al.,* 2008).

Data obtained after the evaluation of this adaptor for the CG revealed that its expression was unchanged when pre and post values were compared.

4.4.1.1. Effects of a resistance training programme

Protein content for MyD88 is shown in Figure 10. Concurring with the molecular pattern showed in the TLRs receptors previously described, RTG showed a considerable diminish in MyD88 protein content ($p < 0.05$) after the 8 wks of resistance training carried out in this study. A significant reduction was also observed (MyD88, $p < 0.04$) after contrasted its values with the data elicited from CG.

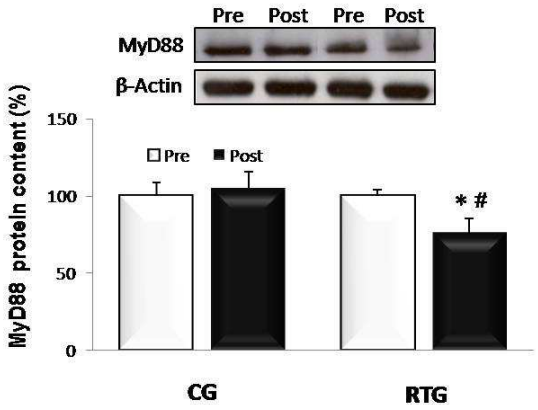

Figure 10. MyD88 protein expression before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4.1.2. Effects of an aerobic training programme

Although MyD88 showed a downward trend in the ATG (Figure 11) after the 8 wks of aerobic training, the value was not statistically significant when it has been contrasted with the pre value.

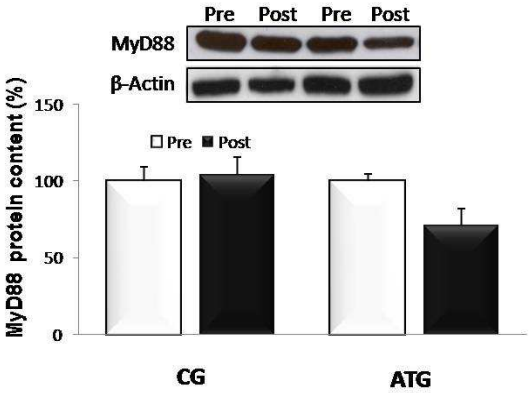

Figure 11. MyD88 protein expression before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM

4.4.1.3. Effects of a WBV training programme

The same pattern of TLR2 and TLR4 expression showed after the WBV training employed here is repeated in the MyD88 protein level of the VTG. Figure 12 illustrates the expression of this adaptor after the 16 sessions of WVB exercises, showing a significant downregulation ($p < 0.04$) compared with the data before performing the protocol and also after contrasting this value with CG (MyD88, $p < 0.05$).

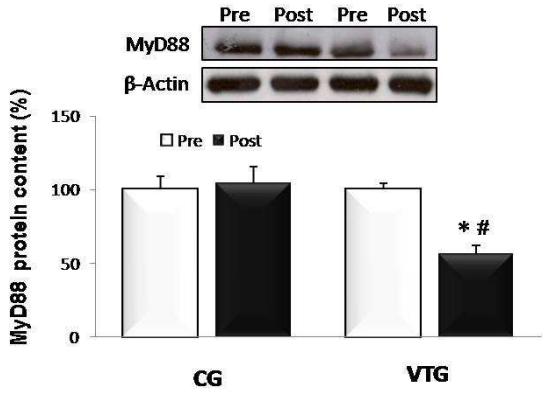

Figure 12. MyD88 protein expression before and after the 8 wks of WBV training for VTG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4.2. NF-κB expression

MyD88 recruits different proteins in the cytoplasm to constitute an enzymatic complex that phosphorylates IκB and thereby, allows the translocation of the NF-κB to the nucleus (Hayden and Ghosh, 2012).

The analysis of p65, one of the key members of the NF-κB family, revealed that its protein content for the CG was unchanged when pre and post values were contrasted.

4.4.2.1. Effects of a resistance training programme

The same response identified previously in the TLRs and in MyD88 is shown in p65 protein content after the 8 wks of resistance training (Figure 13). Indeed, data indicated a significant decrease ($p < 0.03$) of its expression contrasted with pre data and with the results elicited from CG ($p < 0.04$).

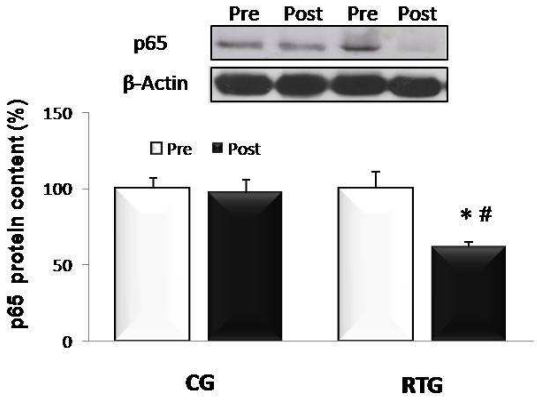

Figure 13. p65 protein expression before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4.2.2. Effects of an aerobic training programme

Figure 14 showed the protein content of p65 after the 8 wks of aerobic training. The protein expression of p65 was unchanged after the physical activity

programme that this group carried out. Contrasting this result with data from CG, any statistically difference has been shown.

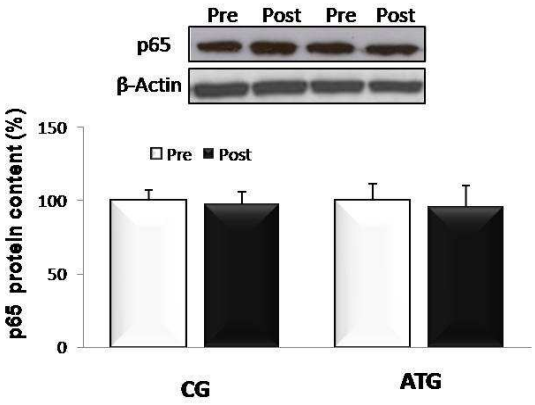

Figure 14. p65 protein expression before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM

4.4.2.3. Effects of a WBV training programme

Figure 15 illustrates the expression of p65 after complete the 16 sessions of WVB exercises, showing a significant downregulation of the protein content ($p < 0.05$) between data before and after performing the protocol, and also after contrasting these values with CG ($p < 0.05$).

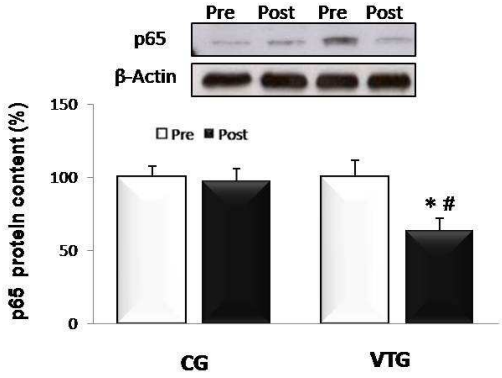

Figure 15. p65 protein expression before and after the 8 wks of WBV training for VTG and the same period of normal daily routine for CG and representative Western blot. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4.3. MAPK pathway

The activation of MyD88 adaptor can also trigger the stimulation of several mediators that are involved in the MAPK signalling pathway. This is the case of ERK1/2 and p38, involucrate in the activation and translocation of AP-1. This nuclear factor is involved in the expression of multiple inflammatory mediators through interactions with NF-κB (Sakurai, 2012).

PBMC samples before and after the 8 wks of normal daily routine of CG not showed any differences in the protein analysis of phospho-ERK1/2, ERK1/2, phospho-p38 and p38.

4.4.3.1. Effects of a resistance training programme

Data shown in Figure 16 indicate that phosphorylate p38 content in PBMC diminished significantly ($p < 0.03$) in response to the resistance exercise protocol when they were compared with total values of p38 of this group.

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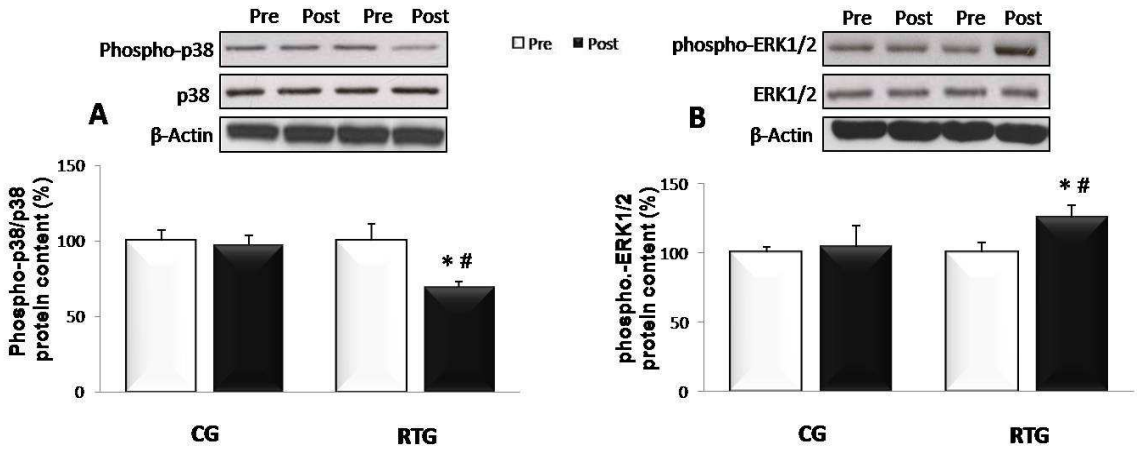

Figure 16. Phospho-p38 / total p38 (A) and phospho-ERK1/2 / total ERK1/2 (B) protein expression before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

On the contrary, a training effect was observed in the protein expression of phospho-ERK1/2 as it can be seen in the figure. A significant upregulation ($p < 0.02$) of

phospho-ERK1/2 was detected when this data was compared with the total values of ERK1/2, which remain unchanged.

In addition, significant differences appeared after contrast both groups of proteins analyzed, phospho-p38/p38 ($p < 0.03$) and phospho-ERK1/2/ERK1/2 ($p < 0.03$) with the data elicited from the CG

4.4.3.2. Effects of an aerobic training programme

The results of the protein expression of some mediators of the MAPK signalling pathway for the ATG are shown in Figure 17. Values bring out for these analyses indicated that the phosphorylated state of p38 was unchanged in response to the WBV exercise protocol. However, phospho-ERK 1/2 showed significant ($p < 0.03$) up-regulation in response to the 16 sessions of aerobic exercise that this group carried out. It is noteworthy that total values of p38 and ERK1/2 were not modified. Significant differences ($p < 0.03$) were also observed after contrasted phospho-ERK1/2/ERK1/2 values from the ATG with the CG.

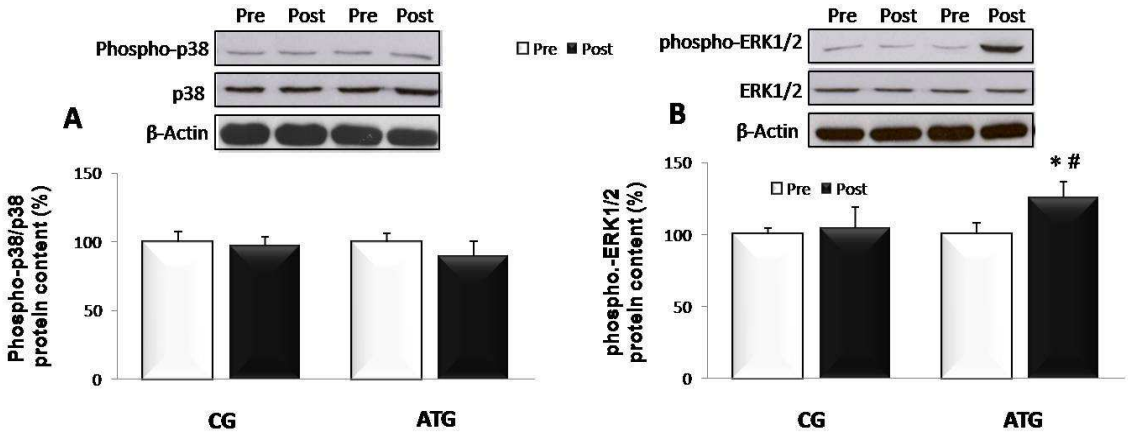

Figure 17. Phospho-p38 / total p38 (A) and phospho-ERK1/2 / total ERK1/2 (B) protein expression before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.4.3.3. Effects of a WBV training programme

Data elicited from the response of phospho-p38 and phospho-ERK1/2 to a WBV training programme is shown in Figure 18. It represents the significant reduction ($p < 0.01$) triggered by the 8 wks of vibration stimulus in the expression of phospho-p38, comparing it with total values of p38 before and after the 16 sessions of the physical activity programme. Statistically differences ($p < 0.03$) also appeared after contrast these values with the data elicited from the CG.

On the other hand, phospho-ERK1/2 showed another different pattern than the one described previously. It can be seen how values of this protein were unchanged in response to this training protocol and total ERK1/2 was not modified.

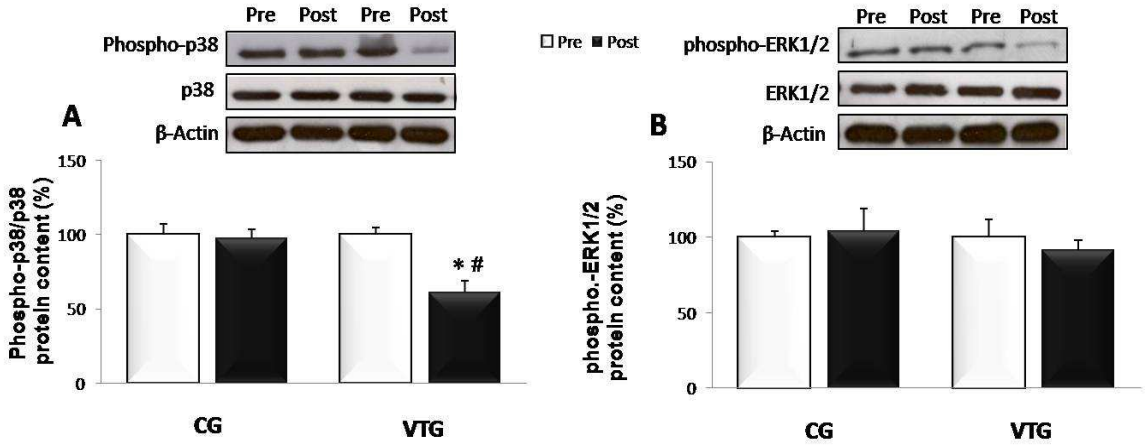

Figure 18. Phospho-p38 / total p38 (A) and phospho-ERK1/2 / total ERK1/2 (B) protein expression, before and after the 8 wks of WBV training for VTG and the same period of normal daily routine for CG, and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.5. EFFECTS OF PHYSICAL TRAINING ON THE TRIF-DEPENDENT PATHWAY EXPRESSION IN PBMC OF THE ELDERLY: IRF3 and IRF7 pathways

Additionally to the MyD88-dependent pathway, some TLRs can also trigger activation through the TRIF-dependent pathway, which leads the phosphorylation and translocation of some IRF transcription factors to induce the expression of IFN genes (Moynagh, 2005).

The same pattern of no change identified in the other proteins previously analyzed for the CG was also shown when the protein expression of TRIF, IKKi/IKKε, phospho-IRF3, IRF3, phospho-IRF7 and IRF7 were evaluated before and after the 8 wks of normal daily routine.

4.5.1. Effects of a resistance training programme

A training effect was identified in TRIF and IKKi/IKKε protein content as Figure 19 illustrated. Despite CG protein levels did not change, the protein expression of TRIF and IKKi/IKKε decreased in response to the resistance exercise protocol ($p < 0.05$ and $p < 0.04$ respectively). These values were statistically significant when they were compared with data elicited from the CG (TRIF, $p < 0.05$; IKKi/IKKε, $p < 0.05$).

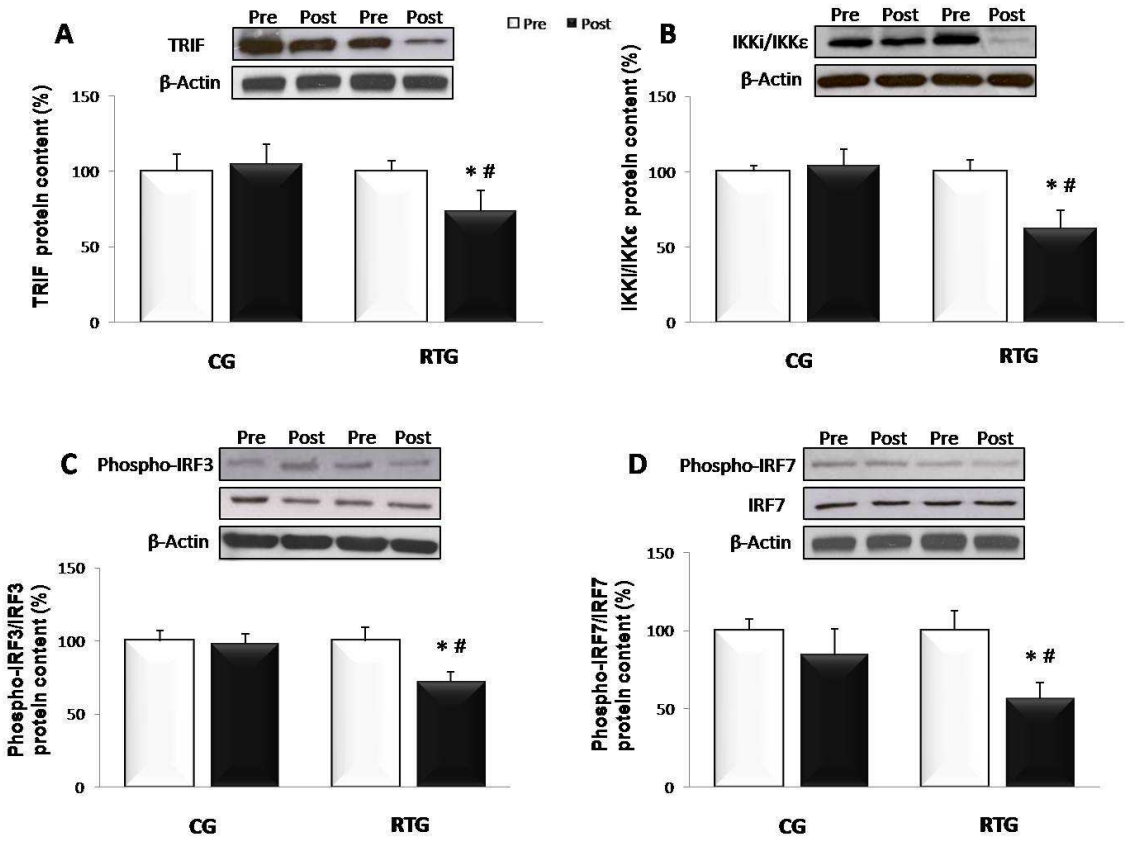

Figure 19. TRIF (A), IKKi/IKKε (B), phospho-IRF3/IRF3 (C) and phospho-IRF7/IRF7 (D) protein expression before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

In the same way, a significant downregulation in the phosphorylated state of IRF3 ($p < 0.04$) and IRF7 ($p < 0.02$) after the 8 wks of resistance training was also observed when these data were contrasted with basal values of the same group. Total values of these two nuclear factors were unchanged after the resistance protocol. Significant decreases were also evident when post values of RTG were compared to data obtained from CG (phosho-IRF3/IRF3, $p < 0.04$; phosho-IRF7/IRF7, $p < 0.03$).

4.5.2. Effects of an aerobic training programme

Figure 20 illustrated how protein expression of both TRIF and IKKi/IKKε in PBMC were unchanged after the 16 sessions of aerobic exercises carried out by ATG.

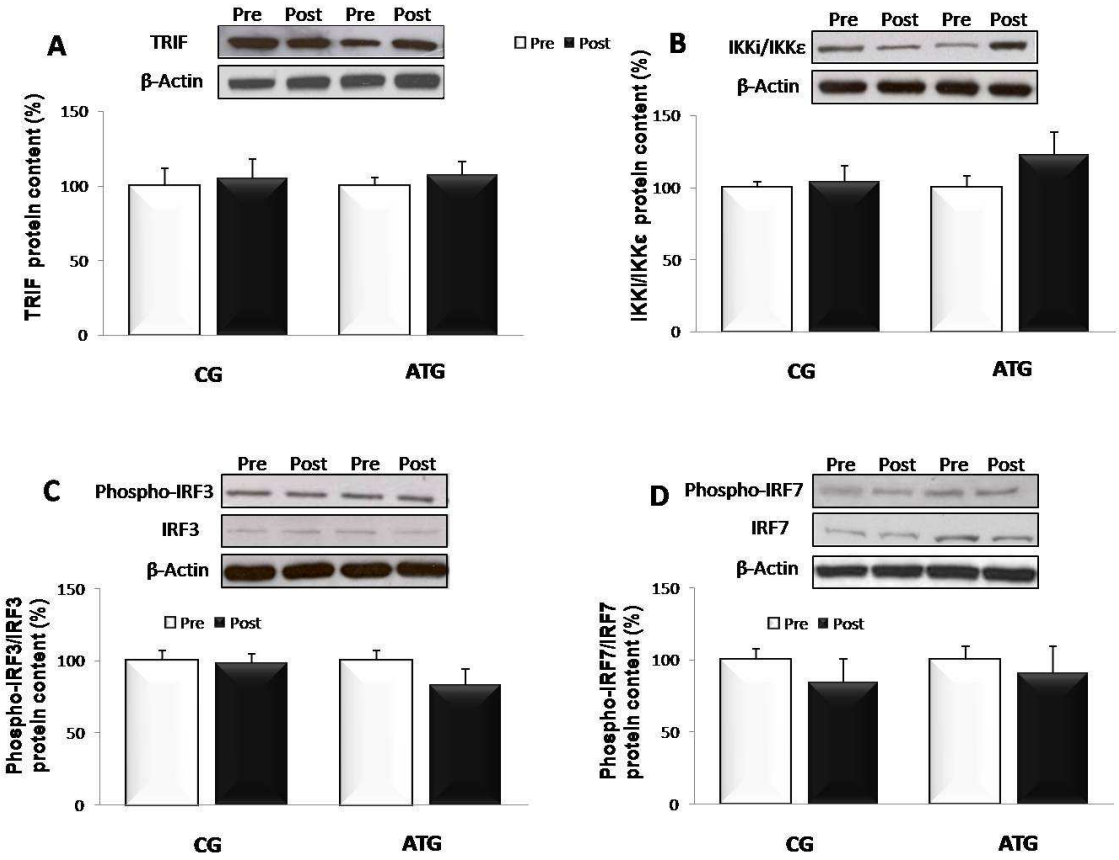

Figure 20. TRIF (A), IKKi/IKKε (B), phospho-IRF3/IRF3 (C) and phospho-IRF7/IRF7 (D) protein expression before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM

According to this pattern, neither phospho-IRF3/IRF3 nor phospho-IRF7/IRF7 showed any change in their protein content expression after contrasted them with pre value same group and basal value CG.

4.5.3. Effects of a WBV training programme

The results of the transcriptional levels of some mediators of the MyD88-independent pathway for the VTG are shown in Figure 21. In this cascade, a similar response to the previously described for the MyD88 -dependent pathway, was also demonstrated.

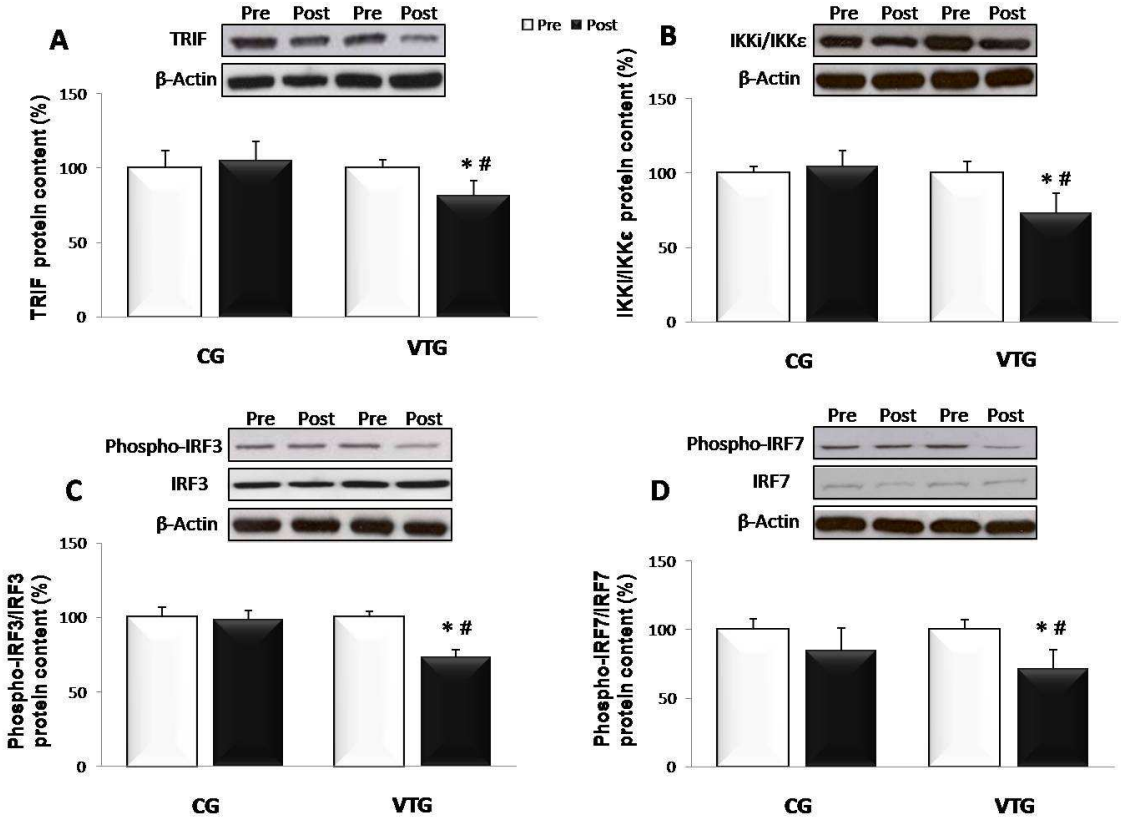

Figure 21. TRIF (A), IKKi/IKKε (B), phospho-IRF3/IRF3 (C) and phospho-IRF7/IRF7 (D) protein expression before and after the 8 wks of WBV training for VTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

Firstly, TRIF and IKKi/IKKε showed a significant downregulation ($p < 0.05$ and $p < 0.04$ respectively) after the 8 wks of WBV training programme accompanied by a

subsequent diminished of the protein expression of both phosphorylated states of IRF3 and IRF7 ($p < 0.04$ and $p < 0.03$ respectively). It is worth highlight that neither of the total levels of those nuclear factors was changed by the physical activity programme conducted by the VTG. After contrasted all the data elicited from these mediators with values of the CG, statistically significant differences appeared (TRIF, $p < 0.05$; IKKi/IKKε, $p < 0.05$; phospho-IRF3/IRF3, $p < 0.05$; phospho-IRF7/IRF7, $p < 0.03$).

4.6. EFFECTS OF PHYSICAL TRAINING ON THE EXPRESSION OF INFLAMMATORY MEDIATORS IN PBMC OF THE ELDERLY

Physical activity has been considered a useful tool to induce a reduction in the low-grade inflammatory state associated with senescence. These benefits have been related with changes in the concentration of pro-inflammatory cytokines towards an increase in the synthesis of anti-inflammatory mediators (Balducci *et al.,* 2010).

Data from the CG showed unchanged values for both TNFα and IL-10 protein content evaluated in PBMC before and after the 8 wks of normal daily routine. Moreover, the protein IL-10/ TNFα ratio, an indicator of the inflammatory status, was almost identical in CG after the evaluated period (1.00±0.11 *vs* 0.97±0.12 arbitrary units).

4.6.1. Effects of a resistance training programme

In response to the resistance exercise protocol execute in this study, RTG showed a significant boost ($p < 0.04$) of IL-10 mRNA level related with the IL-10 protein concentration ($p < 0.02$) (Figure 22) after the training programme when contrasted these values with the data before the 8 wks. Significant differences were also observed after compared IL-10 mRNA ($p < 0.04$) and IL-10 protein expression ($p < 0.02$) with data from CG.

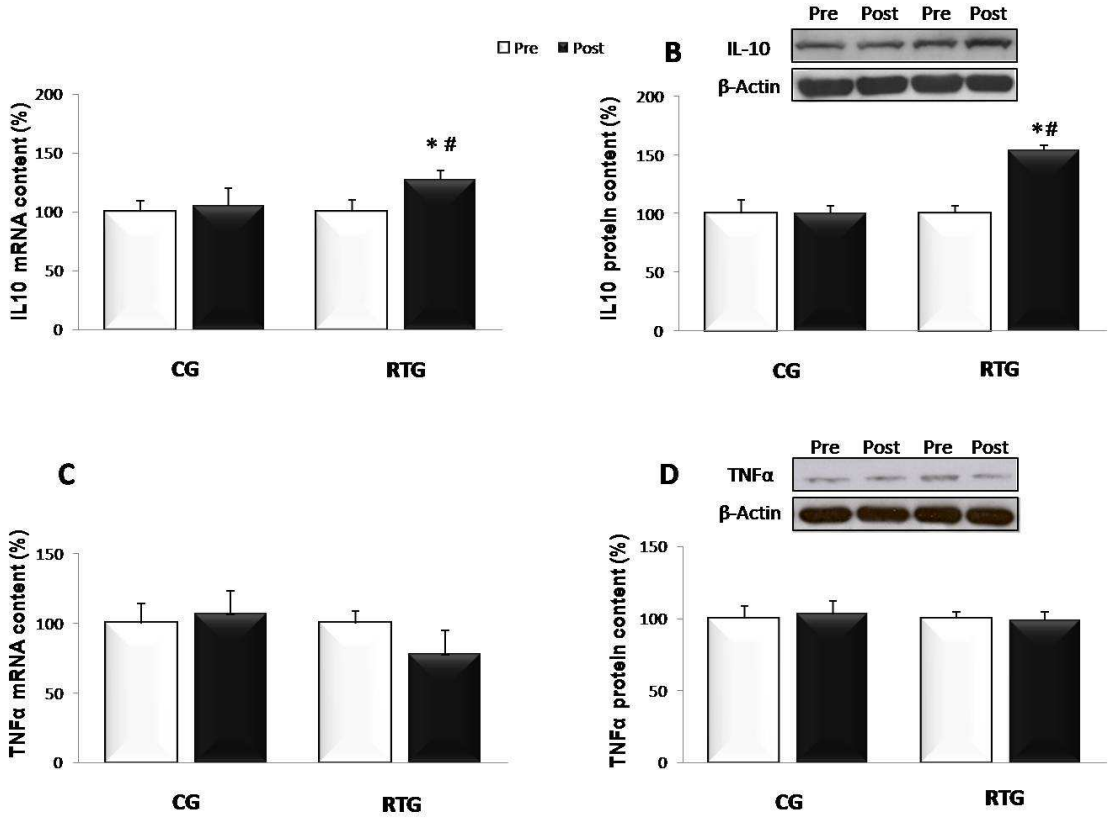

Figure 22. IL-10 mRNA (A) and protein expression (B) and TNFα mRNA (C) and protein expression (D) before and after the 8 wks of resistance training for RTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

Nevertheless, no significant changes were observed in the pro-inflammatory status measured through TNFα mRNA level and protein content (Figure 22) either before or after the resistance training programme for RTG. The same result was noted after contrasted these measures with CG values.

However, analyzing the protein IL-10/ TNFα ratio, a training effect was shown towards anti-inflammatory mediators after the 8 wks of physical activity performed by the RTG (1.05±0.14 *vs* 1.48±0.17 arbitrary units; $p < 0.04$).

4.6.2. Effects of an aerobic training programme

The results of the TNFα gene expression and protein content after the 16 sessions of aerobic exercises are represented in Figure 23. Results from this

inflammatory cytokine at both genetically levels measured were unchanged. Moreover, Figure 23 also indicates the IL-10 mRNA and protein content after the 16 training sessions that ATG carried out. Although in response to this exercise protocol IL-10 showed an upward tendency both at mRNA and protein level, any of these increases were statistically significant after compared with basal values and after contrasted with CG.

Furthermore, analyzing the protein ratio IL-10/ TNFα, any significant change was shown as an effect of the training protocol carried out (1.00±0.11 *vs* 1.18±0.15 arbitrary units).

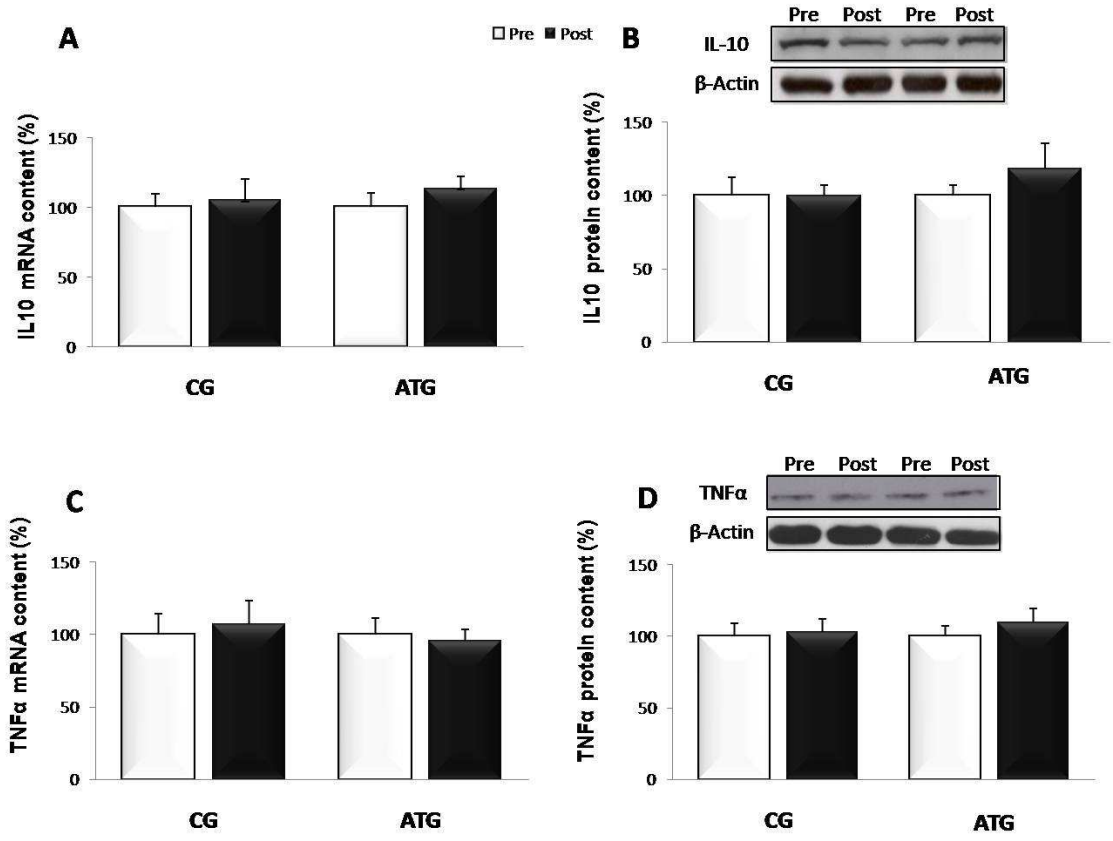

Figure 23. IL-10 mRNA (A) and protein expression (B) and TNFα mRNA (C) and protein expression (D) before and after the 8 wks of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM

4.6.3. Effects of a WBV training programme

Figure 24 illustrates mRNA and protein content of TNFα and anti-inflammatory cytokine IL-10. TNFα mRNA content showed no changes when values before and after the WBV training were contrasted. Same results were obtained after contrasting these values with data from CG. It is worth noting that this expression is in line with protein content since TNFα persist unchanged after the 16 sessions of WVB carried out by VTG.

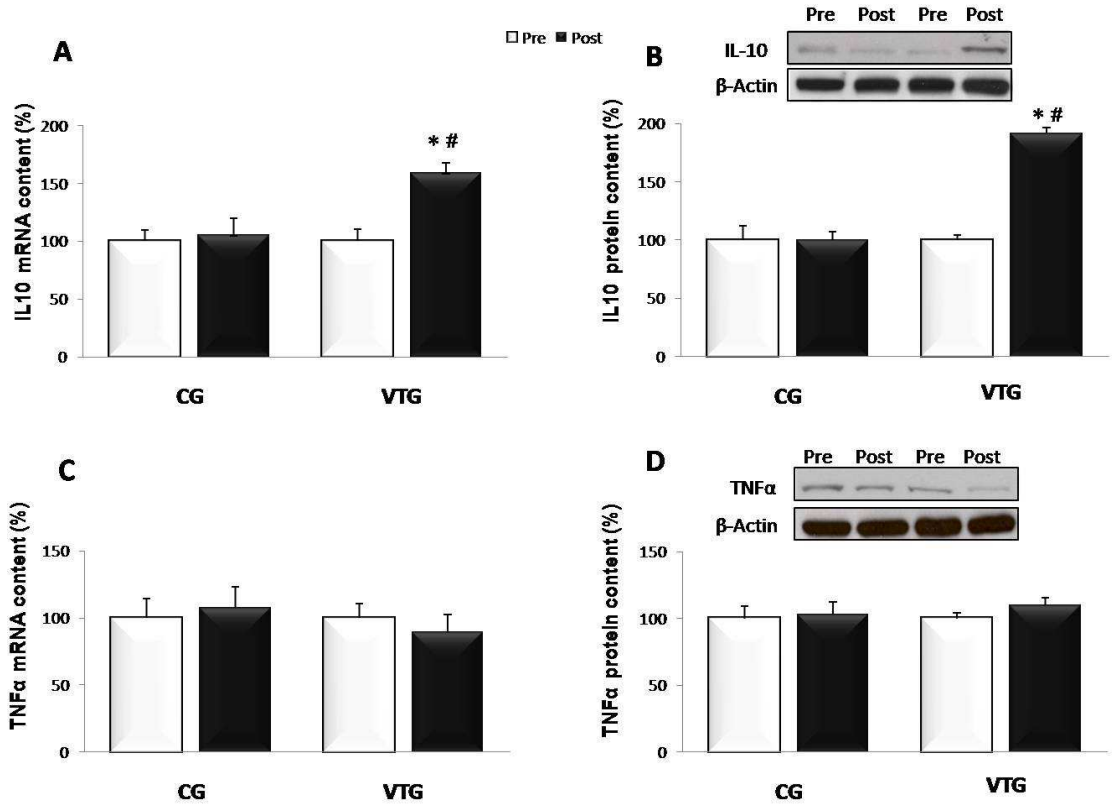

Figure 24. IL-10 mRNA (A) and protein expression (B) and TNFα mRNA (C) and protein expression (D) before and after the 8 wk of WBV training for VTG and the same period of of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM

However, a considerable training effect was observed in IL-10 mRNA content, which boosted significantly ($p < 0.04$) after compared with pre value and with CG ($p < 0.05$). This result is related with IL-10 protein content that underwent a significant increase ($p < 0.02$) after the WVB training programme. Statistically significant changes were also observed after contrast these values with data from CG ($p < 0.03$).

Additionally, analyzing the protein IL-10/ TNFα ratio, there is a significant tendency towards anti-inflammatory mediators in the WTG (1.09±0.09 *vs* 1.54±0.18 arbitrary units; $p < 0.03$).

4.7. EFFECTS OF PHYSICAL TRAINING ON THE EXPRESSION OF HEAT SHOCK PROTEINS IN PBMC OF THE ELDERLY

Multiple external and internal ligands can activated and induce the dimerization of TLRs. Heat shock proteins are some of these potent activators of the innate immune system capable of enhance or inhibit the production of the pro-inflammatory cytokines (Noble and Shen, 2012).

Data obtained from the CG before and after the 8 wk of normal routine showed unchanged results for both Hsp analyzed: Hsp70 and Hsp60.

4.7.1. Effects of a resistance training programme

Figure 25 illustrated results for Hsp70 and Hsp60 for the RTG. As it can be seen, the resistance exercise training practiced in this study triggered a significant increase ($p < 0.03$) in Hsp70 protein concentration in PBMC compared to basal values same group, and a statistically significant difference ($p < 0.03$) was also inferred after contrast with data elicited from CG.

On the other hand, values in RTG showed a marked decreased of Hsp60 ($p < 0.03$) protein level comparing it with data before the 16 sessions of exercise that these participants performed. In addition, these results also indicated a significant reduction ($p < 0.03$) when values are compared with data from CG.

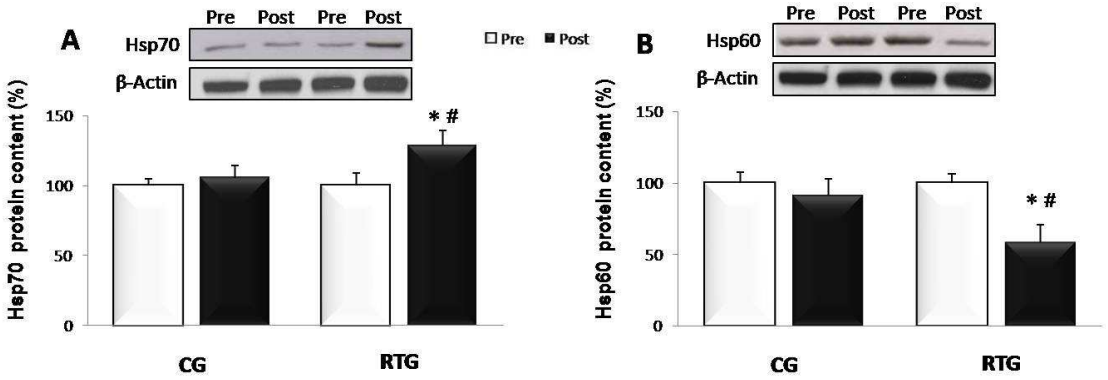

Figure 25. Hsp70 (A) and Hsp60 (B) protein expression before and after the 8 wk of resistance training for RTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

4.7.2. Effects of an aerobic training programme

The results of the Hsp70 and Hsp60 protein content after the 8 wks of aerobic training protocol executed by the ATG are shown in Figure 26. It seems that this physical activity performed along 8 wk showed unchanged values in Hsp70 protein content after contrasted data with basal value same group. Identical results elicited when RTG and CG values were compared. However, the aerobic training programme triggered a downward no statistically significant of Hsp60 protein level when it was compared with values before starting the 16 sessions and with data from the CG.

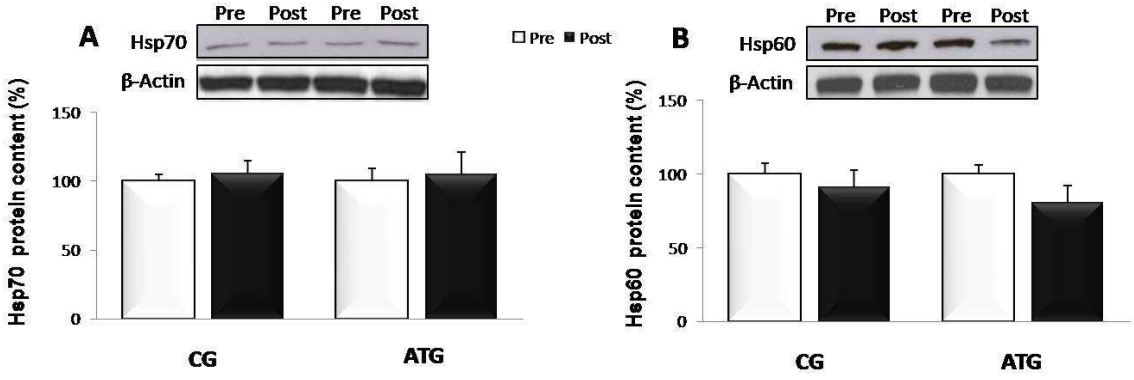

Figure 26. Hsp70 (A) and Hsp60 (B) protein expression before and after the 8 wk of aerobic training for ATG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM

4.7.3. Effects of a WBV training programme

Protein content for Hsp70 and Hsp60 in PBMC of the participants belong to the VTG is shown in Figure 27. Values of Hsp70 analyzed before and after the 8 wks of WBV protocol showed a significant increase ($p < 0.02$) when compared with basal data. In addition, the same pattern was also observed after contrast these values with data obtained from CG ($p < 0.02$).

Conversely, Hsp60 expression was significantly ($p < 0.03$) downregulated in the VTG after completed the 16 sessions of WBV exercise. Accordingly, after compare data from the trained group and the CG, significant difference ($p < 0.04$) also appeared.

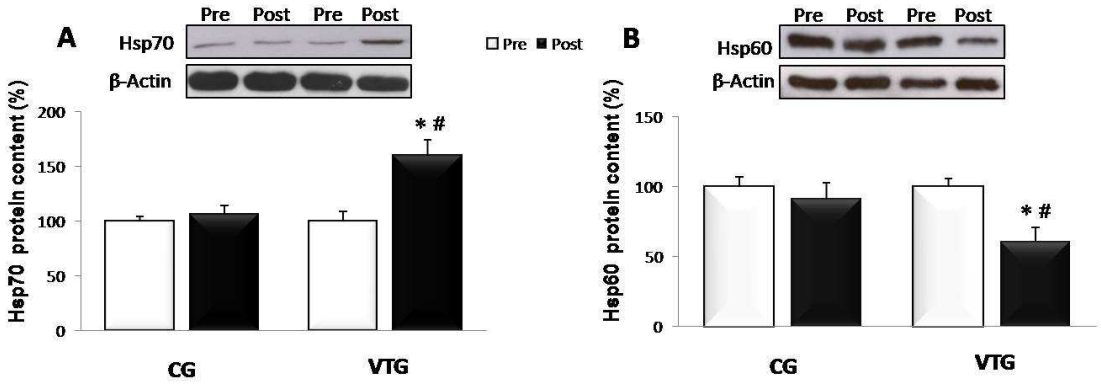

Figure 27. Hsp70 (A) and Hsp60 (B) protein expression before and after the 8 wk of WBV training for VTG and the same period of normal daily routine for CG and representative Western blots. Data are expressed as percent change (%) from pre value and given as mean ± SEM. *Significant differences compared to pre value, same group; #Significant differences compared to CG

Discussion

Ageing is a natural process characterized by a decline in the normal function of the immune system. This inflamm-ageing situation describes a chronic, mostly asymptomatic, low-grade inflammatory state that can lead to a more vulnerable status, increasing the risk of developing chronic illnesses.

Although traditionally, it has been well accepted that ageing is associated with higher levels of pro-inflammatory markers (Bruunsgaard, 2002, Ershler and Keller, 2000), some studies have revealed that younger and older healthy subjects are able to present similar contents of inflammatory biomarkers, establishing the key of this paradigm in the physical activity level (Beharka *et al.,* 2001, Flynn *et al.,* 2003) (Giannopoulou *et al.,* 2005, Oberbach *et al.,* 2006).

As previously mentioned, regular moderate-intensity physical activity is recommended to have a healthy lifestyle for all ages. In the elderly, exercise has not only been considered as a potential method to struggle functional problems, it has been also proposed as an immunosenescence therapy (Simpson *et al.,* 2012). In fact, whole body chronic inflammation appears to be highest in individuals who are sedentary (Pedersen and Saltin, 2006). Hence, exercise could be an efficient countermeasure to either prevent or delay the onset of some chronic diseases associated with this low-grade inflammation status (Pedersen and Saltin, 2006, Barlow *et al.,* 2006, Aoyagi and Shephard, 2010, Simpson *et al.,* 2012).

Literature describes how exercise can trigger important adaptations in the inflammatory system (Mathur and Pedersen, 2008), which vary depending on the type and duration of the exercise intervention (Pedersen and Hoffman-Goetz, 2000). Specifically, individuals who have been very inactive for most of their life can benefit from the incorporation of light to moderate exercise programmes (Lee *et al.,* 2001). Nevertheless, specialists have not yet established the optimal training modality or the intensity that should be employed. In particular, resistance or endurance training has been the most study procedures to elicit positive effects in elderly (Giannopoulou *et al.,* 2005, Oberbach *et al.,* 2006, Stewart *et al.,* 2005), however it has been observed that around 20% of older individuals are not able to execute a classical treadmill

exercise test (Bautmans *et al.,* 2004). Hence, it is beginning to appreciate the possible use of WBV as a modality to enhance physical senior's fitness and low-grade chronic inflammation.

In this regard, the three exercise protocols designed to this study concord with general prescriptions of exercise suggested by senior's health guidelines. Numerous reports assess the effectiveness of similar protocols to achieve functional benefits in this sector of the population (García-López *et al.,* 2007b, Hakkinen *et al.,* 1998, Machado *et al.,* 2010), but there is a lack of knowledge in the possible implications of these routines in low-grade chronic inflammation related with age.

Considerable experimental evidence supports the correlation between physical fitness and anti-inflammatory effects through the decrease of classical inflammatory mediators (Keller *et al.,* 2004, Starkie *et al.,* 2003). Indeed systemic low-level inflammation has been correlated inversely with muscle mass, muscle strength and functional capacity in elderly populations (Cesari *et al.,* 2004, Pedersen *et al.,* 2003, Visser *et al.,* 2002). Moreover, it has been inferred a correlation between such immune effects of physical activity and the pivotal role that TLRs have in the control of the inflammatory response (Gleeson *et al.,* 2006), especially TLR2 and TLR4 which to date, seem to be the most implicated in this response (Flynn *et al.,* 2003, Gleeson *et al.,* 2006, Ma *et al.,* 2013, Stewart *et al.,* 2005). In fact, Lancaster and colleagues demonstrated how physical activity reduced the expression of TLR2 and TLR4 (Lancaster *et al.,* 2005) and McFarlin and co-workers associated similar decreases with improvements in the inflammatory state of a body (McFarlin *et al.,* 2004).

Based on these assumptions, Flynn and co-workers demonstrated how 10 wk of resistance exercise training was effected to decrease the mRNA content of TLR4 in older woman compared with untrained participants (Flynn *et al.,* 2003). Then, from this result, the same research group conducted another similar study to assess the effect of a single bout of strength exercise in TLR4 expression of trained and sedentary old female participants (McFarlin *et al.,* 2004). Again, they confirmed the influence of the exercise training status in the cell-surface TLR4 expression. In

accordance with this and, bearing in mind the cooperation between TLR2 and TLR4 largely demonstrated in immune cells (Zbinden-Foncea *et al.,* 2012), the results elicit from the RTG are agree with these previous findings showing that sedentary older volunteers presented higher levels of TLR2 and TLR4 protein concentration than the resistance trained subjects.

Recently, a similar effect was also observed after an endurance protocol carried out along 8 wks in obese rats (Oliveira *et al.,* 2011). Their outcome indicated that TLR4 expression was blunted after the workout similarly in the liver, muscle and the adipose tissue. Nevertheless, data from the current study infer that the 16 sessions of aerobic exercise that the ATG carried out were not enough to modify the concentration of neither TLR2 nor TLR4 in the trained older individuals. According to these results, a recent investigation performed by Reyna and co-workers showed how the expression of these two receptors were unchanged after a 15-day endurance programme in diabetic patients (Reyna *et al.,* 2013). Apparently, although participants enhanced their physical fitness level, the duration of the aerobic training or the intense employed in this study could be not enough to trigger effects trough these receptors.

Notwithstanding, general recommendations of public health guidelines propose the combination of these two modalities of exercise to achieve better results (Nelson *et al.,* 2007, Pate *et al.,* 1995). Thus, it has convinced researchers to examine the TLR response also after aerobic and resistance exercise training protocols. Hence, groups of older and younger inactive participants were endurance and resistance exercise trained for 12 wks, showed significant decreases in the cell-surface TLR4 expression and therefore, in the inflammatory cytokine production in both populations analyzed (Stewart *et al.,* 2005). Nevertheless, the study performed by Shimizu and co-workers showed how another combine protocol of aerobic and resistance exercises was not able to influence TLR4 protein content (Shimizu *et al.,* 2011). Again, divergences may rely in the different protocols employed.

On the other hand, the third exercise protocol employed in this study based on WBV stimulus shows how the VTG participants were able to reduce their TLR2 and TLR4 expression after complete the physical activity programme. It is worth noting that these results cannot be contrast with previous data in the literature since, as far it is known, there is no preceding investigation evaluating the effect of WVB training in these receptors and therefore, in the response associated to them.

Upon stimulation, the early activation of TLR2 and TLR4 culminate in the induction of the NF-κB pathway and the MAPK cascade (Hofmann *et al.,* 2012) leading to the induction of inflammatory cytokines (Kawai and Akira, 2006). Focusing in the NF-κB pathway, the oligomerization of these TLRs induce the recruitment of the main downstream adaptor MyD88 (Jiang *et al.,* 2010). Indeed, there is a general consensus about the essential role that this protein has in the stimulation of pro-inflammatory cytokines (Kawai and Akira, 2006, Lu *et al.,* 2008). However, to date there are only a handful of studies that connect MyD88 and physical activity (Fernández-Gonzalo *et al.,* 2012, Funk *et al.,* 2011, Ma *et al.,* 2013, Nickel *et al.,* 2011). In view of that, it has been proved how an eccentric exercise protocol carried out after an acute eccentric exercise could diminish MyD88 protein content in young subjects (Fernández-Gonzalo *et al.,* 2012). In this line, while sedentary participants showed unchanged values of MyD88 in the current study, the data elicit from the RTG after complete the strength training protocol demonstrated a decrease of the protein content of this adaptor, showing a similar response that the aforementioned study.

More recently, cerebral ischemia rats also educed a similar observation after complete an endurance exercise programme (Ma *et al.,* 2013). Howbeit, recent research has proven that obese athletes showed unchanged MyD88 protein expression in PBMC after 10 wks of endurance training program (Nickel *et al.,* 2011). This study demonstrates similar results after the 16 aerobic sessions that the ATG carried out, not showing any difference between the training and the sedentary volunteers.

Conversely, the decreased of the expression of TLR2 and TLR4 in the VTG is related with a reduction in the MyD88 protein content after the 16 vibratory sessions. It is worth highlight that physiological changes induced by WBV activities seem to be similar to those after several weeks of resistance training (Bosco *et al.,* 1999).

Afterward, the next step in the early phase activation of NF-κB though TLR2 and TLR4 signalling is mediated by a subsequent cascade of TRAF6, TAK1 and other mediators (Nickel *et al.,* 2011). In turn, they phosphorylate downstream components allowing the recruitment of p50 in the cytoplasm and the translocation to the nucleus of p65 (Kawai and Akira, 2006, Konner and Bruning, 2011) activating the κB sites (McFarlin *et al.,* 2006).

NF-κB plays a key role in the exercise inflammatory response since resistance exercise transiently activates this transcription factor during the first few hours after the stimuli. Jiménez and co-workers supported this theory showing how an eccentric exercise programme carried out in older subjects were able to attenuate the NF-κB activation after an acute eccentric exercise (Jiménez-Jiménez *et al.,* 2008). This premise is in agreement with the data elicit from the present study which indicate that the resistance training protocol employed here has been able to diminish the activation of p65 in the active seniors, asserting the anti-inflammatory effect attributed to regular exercise.

Howbeit, other results available in the literature regarding the activation of NF-κB owing to aerobic exercise are equivocal describing either enhancements (Ho *et al.,* 2005) or reductions (Durham *et al.,* 2004) of the transcription factor content. Recently, Ma and colleagues have also assessed the effectiveness of an endurance exercise therapy in cerebral ischemic injury inflammation by reducing the expression of NF-κB (Ma *et al.,* 2013). However, different endurance exercise protocols have been unable to show any modification of the expression of this nuclear factor in rats (McKenzie and Goldfarb, 2007, Zbinden-Foncea *et al.,* 2012). Our findings are in agreement with the aforementioned results since the ATG volunteers indicated

unchanged values of the p65 protein content, as well as data obtained from the sedentary participants.

Notwithstanding and spite of the large knowledge around this nuclear factor, there is a gap in the literature concerning the capacity of WBV training to affect NF-κB, and therefore the inflammatory response associated to it. In this research work, the result elicited from the p65 protein analysis suggests the attenuation of the NF-κB pathway because of the WBV training protocol employed here. This finding is consistent with previous studies which describe how other types of exercises are well-recognize stimuli for the physiological modification of the NF-κB transcription factor (García-López *et al.,* 2007a). In this way, these results may assess the significant lower TLR2 and TLR4 cascade through the NF-κB pathway.

As already mentioned, MyD88 is also involved in the activation of MAPK signalling which induces the expression of pro-inflammatory genes, adhesion molecules and the activation of adaptive immunity (Akira, 2006). Previous experiments have indicated that specific members of MAPK cascade can be activated by physical activity in an intensity dependent manner (Hayden and Ghosh, 2012).

In addition, although most authors report transient activations following either resistance or endurance activity (Kramer and Goodyear, 2007, Van Ginneken *et al.,* 2006), literature supports a differential and highly specific response of these proteins after exercise (Coffey *et al.,* 2006, Iemitsu *et al.,* 2006, Thompson *et al.,* 2003). Specifically, it has been described, in an animal model, the increase of the phosphorylation state of p38 due to endurance exercise, whereas the activation of the ERK1/2 was not affected (Zbinden-Foncea *et al.,* 2012). On the contrary, several resistance exercise experiments carried out in humans have indicated an upregulation of the ERK1/2 signalling cascade (Creer *et al.,* 2005, Tannerstedt *et al.,* 2009).

Data of the present study are in good agreement with these paradigms, supporting the differential activation state between p38 and ERK1/2. After carrying out the resistance exercise protocol designed for this experiment, the activated form of p38, phospho-p38 diminishes significantly in the trained subjects while the

phosphorylation state of ERK1/2 is significant upregulated in the RTG participants. However and as it was expected, the values of these two MAPK pathway components were unchanged in the sedentary volunteers. The discrepancy between the response of these two mediators is supported by the concept that this signalling is highly exercise specific and contraction form dependent (Lee *et al.,* 2002). This provides enough evidence that, whereas p38 shows the same transitional repression identified in other components of the NF-κB pathway, the over-expression of ERK1/2 is consistent with previous studies carried out in humans that demonstrate an increase after resistance exercise (Taylor *et al.,* 2012). Convincingly, it has been infer that ERK1/2 play a role in the hypertrophic adaptations that used to be linked to resistance exercise (Taylor *et al.,* 2012).

In addition, studies conducted to examine the response of MAPK to different aerobic protocols have report that ERK1/2 is rapidly activated in both animal models of exercise (Dufresne *et al.,* 2001, Nader and Esser, 2001) and in human skeletal muscle (Yu *et al.,* 2001, Yu *et al.,* 2003). Findings from the current study are in agreement with these aforementioned experiments reporting an activation of phopho-ERK1/2 in the volunteers that performed the aerobic exercise training. However, the evaluation of phospho-p38 after the 16 sessions of aerobic exercise that the ATG carried out has inferred any change in the protein content of this mediator. Although numerous studies have suggested an increase of p38 after endurance protocols in rodents (Goodyear *et al.,* 1996, Nader and Esser, 2001) and in humans (Boppart *et al.,* 2000, Widegren *et al.,* 1998, Yu *et al.,* 2001), it is necessarily noted that most of these experiments have examined skeletal muscle where, apart from the ubiquitously expressed isoforms of p38 (α and β), p38γ is almost exclusively expressed in this tissue (Li *et al.,* 1996). There is some evidence to propose an exercise-specific regulation of the different isoforms of p38 since an assay performed by Boppart and co-workers have concluded that only p38γ was activated in marathon runners (Boppart *et al.,* 2000). For this reason, compared results elicited from skeletal muscle with other tissues may not be appropriate.

With regard to the VTG, while p38 is significantly downregulated in the trained group, the phosphorylation of ERK1/2 shows no different between the physical activity participants and the sedentary subjects. It is therefore likely that the reason of such specific deactivation relies in the diverse forms of contraction (Thompson *et al.,* 2003). It should keep in mind that the vibratory training protocol employed here induces concentric, eccentric and isometric contractions of the hip and knee extensor muscle groups and the plantar flexors (Simao *et al.,* 2012), suggesting an explanation to the discrepancy between both proteins. Additionally, several investigations have also associated ERK1/2 upregulation with low-intensity exercises (Turcotte *et al.,* 2005), while heavy activity is more related to the upregulation of p38 (Kramer and Goodyear, 2007). With this, it may conclude that WVB exercise protocol may not induce the appropriate contractile stimulus or the intense were not enough to promote an increase in ERK1/2 protein level.

Additionally to the MyD88-dependent pathway, TLRs can also induce the late activation of NF-κB and MAPK cascades owing to the connection between the TRIF-dependent pathway and TRAF6 (Yamamoto *et al.,* 2002). Indeed, it has been described how mutations in the TRAF6 binding site of TRAF3 fails to activated this late phase of the NF-κB (Jiang *et al.,* 2004, Turcotte *et al.,* 2005).

TRIF has also the ability to stimulate type I IFN production through IRF nuclear factors (Oshiumi *et al.,* 2003). Studies conducted to analyze the role of TRIF have developed that KO mice for this mediator showed no activation of IRF3 and therefore, impairment in IFN inducible genes (Diebold *et al.,* 2003). Besides, Kim and colleagues have reported a functional cooperation between IRF3 and NF-κB to achieve this IFN activation (Kim *et al.,* 2000).

Furthermore, to stimulate this cascade, TRIF interacts through a NAK-associated protein (NAP1) with two structurally related kinases present in the cytoplasm: IKKi/IKKε and TBK1 (Sasai *et al.,* 2005). It has been thought that these kinases lead directly the phosphorylation of IRF3 at its C terminal cluster (McWhirter *et al.,* 2004, Sharma *et al.,* 2003), but three scaffold proteins seem also to be involved

in the IRF3 and IRF7 activation (Gatot *et al.,* 2007, Kawai and Akira, 2007, Ryzhakov and Randow, 2007). Although it is currently not clarified, it seems that different complex assembled between TBK1 and IKKi/IKKε and these key proteins might lead to provide signalling specificity in different situations (Verhelst *et al.,* 2013).

In addition, both TBK1 and IKKi/IKKε seem to present a certain grade of functional redundancy but this might only be relevant in specific cells. This is due to ubiquitous expression of TBK1, whereas IKKi/IKKε production is more restricted to some cells such as pancreas, lymphoid tissues and peripheral blood lymphocytes (Hammaker *et al.,* 2012). However in other cell types, IKKi/IKKε is rapidly upregulated by cytokines such TNFα or IL-1 (Clement *et al.,* 2008).

Recent accumulation evidence has also suggested that TBK1 present an ability to control some inflammatory mediators through the NF-κB pathway (Xie *et al.,* 2012, Yu *et al.,* 2012). This link relies in the direct role that TBK1 has in the phosphorylation of IκBα (Pomerantz and Baltimore, 1999) which induces NF-κB activation and subsequently, affects the inflammatory response. Novel findings have also suggested that TBK1 can phosphorylate IKKβ, p65 and c-Rel broaden its functions (Harris *et al.,* 2006, Kim *et al.,* 2010). However, TBK1 KO mice expressed normally NF-κB target genes, suggesting that this function is not essential in the activation of this pathway (Shen and Hahn, 2011). This novel role has proposed TBK1 as a suitable target for new treatments of inflammatory pathologies since some preliminary results of TBK1 inhibitors, used in inflammatory disease animal models, have elicited good therapeutic effects (Ogasawara *et al.,* 2011).

Additionally, the promoter of the IKKi/IKKε gene contains one functionally active NF-κB binding site (Wang *et al.,* 2005). Hence, this kinase relates with the phosphorylation of the p65 subunit and its translocation, supporting a specific role in the response to pro-inflammatory signals (Clement *et al.,* 2008, Geng *et al.,* 2009, Moreno *et al.,* 2010). In this context, it has been proposed that activated p65 is able to bring IKKi/IKKε to inflammatory gene promoters in the nucleus, serving as a

docking site and leading the phosphorylation of adjacent promoters that exhibit AP-1 and κB sites (Verhelst *et al.,* 2013).

Recently, IKKi/IKKε has acquired an important position in the pathophysiology of several diseases. Its impact on cell proliferation and transformation has linked an abnormal upregulation with tumorigenesis (Guan *et al.,* 2011, Peant *et al.,* 2009). Indeed, high concentrations of this kinase serve as marker for poor prognosis (Guo *et al.,* 2009).

The implication of IKKi/IKKε in the regulation of NF-κB and IFN-inducible genes have been also linked with many inflammatory diseases such as rheumatoid arthritis (Corr *et al.,* 2009), pulmonary inflammation (Gulen *et al.,* 2012) or even obesity (Olefsky, 2009). Therefore, IKKi/IKKε might be a potential target to treat some cancers and inflammatory pathologies related to the ageing process (Li *et al.,* 2012, Unterstab *et al.,* 2005).

As previously mentioned, TBK1 and IKKi/IKKε are also implicated in IRF3 and IRF7 response, stimulating the production of IFN-α and IFN-β (Clement *et al.,* 2008, Kawai and Akira, 2006). The apparent overlap show by these two receptors is explained by the fact that IRF3 is constitutively expressed in many cells while IRF7 needs to be transcriptionally upregulated (Coccia *et al.,* 2004). Some experiments have reported how TBK1 deficient cells showed a reduction in IRF3 activation, whereas IKKi/IKKε deficient cells demonstrated no changes in the phosphorylation of this transcriptional factor (McWhirter *et al.,* 2004, Perry *et al.,* 2004).

Besides, the activation of IRF3 is regulated by redundant phosphorylations of several residues at its carboxy-terminal domain (Fujii *et al.,* 2010) and subsequently, the IFN-I produced can stimulate the induction of IRF7 which is also phosphorylated and activated by TBK1 and IKKi/IKKε (Sharma *et al.,* 2003), enhancing its translocation to the nucleus. Hence, it amplifies IFN production stimulating the transcription of multiple IFN-I subtypes and therefore, establishing a positive feedback loop (Sato *et al.,* 2000).

Taken all together and, as far as it is known, very limited data in the literature link TRIF-dependent pathway with the role that exercise could exert. Yano and colleagues have described the effectiveness of a stressful exercise in immune depression through the reduction of IFN-α production in mice (Yano *et al.,* 2010). Moreover, other report described how an eccentric exercise training after an acute eccentric exercise was able to reduce the expression of the key markers of the TRIF-dependent pathway (Fernández-Gonzalo *et al.,* 2012). Accordingly, in the current study, it is shown how the 16 sessions of resistance exercise protocol employed here were able to diminish the protein values of both TRIF and IKKi/IKKε. Consequently, results obtained from the RTG also elicited a reduction in the phosphorylated downstream nuclear factors IRF3 and IRF7 after complete the strength programme, inferring evidences of the efficacy of this protocol to decline inflammatory pathways.

To date and in contrast to resistance exercises, the combination of MyD88-independent pathway and aerobic or WBV exercise have not yet been explored. In the present study, the ATG showed unchanged values of TRIF and a non-significant trend to increase of IKKi/IKKε protein content after the 8 wk of endurance training.

On the other hand, results from the VTG demonstrate a decrease in the TRIF-dependent cascade since TRIF, IKKi/IKKε and both nuclear factors analyzed, IRF3 and IRF7 were downregulated after complete the 16 sessions of vibratory exercises. These data may propose the protocol of WBV employed in this study as a useful tool to decrease the inflammatory pathway controlled by TRIF.

Overall, two different exercise interventions, resistance and WBV, down-regulate the main pathways induced by TLR2 and TLR4, as well as the content of both receptors but the reason why their expression are reduced after exercise is not fully understood. Researchers have suggested a relationship between TLR expression and endogenous ligands such as cytokines or Hsp (Beg, 2002).

A large number of studies have proposed that high levels of physical activity can be apparently associated with reduced peripheral inflammatory mediators such as TNFα and IL-1β (Nicklas *et al.,* 2008, Phillips *et al.,* 2010). Hence, as previously it

was described, there is a consensus that exercise may exert anti-inflammatory effects (Balducci *et al.,* 2010). This theory agree with previous reports relating low mRNA TLR4 expression with an exercise training-induced reduction of TNFα and IL-1β mRNA (Flynn *et al.,* 2003, Kalis *et al.,* 2003).

Certainly, endurance or resistance chronic exercise have been associated with the elevation of circulating IL-6 concentration (Keller *et al.,* 2001, Steensberg *et al.,* 2001), the production of the anti-inflammatory cytokine IL-10 (Das, 2004, Petersen and Pedersen, 2005) and the shedding of TNFα (Moldoveanu *et al.,* 2001, Steensberg *et al.,* 2003a, Vieira *et al.,* 2007). Indeed, Bruunsgaard has even suggested that only 30 min of moderate physical activity may have the potential to stimulate an anti-inflammatory environment with high levels of IL-10 (Bruunsgaard, 2005).

Results from the current study agree with this assessment since the resistance exercise training employed here induced a marked up-regulation of IL-10 protein expression although TNFα protein level was unchanged. However, although multiple research works have reported decreases in cytokine production in young subjects (Pedersen *et al.,* 2001, Rhind *et al.,* 2001), results from the present study are not at odds with other experiments performed in the elderly that have described no changes in TNFα or IL-1β after exercise protocols (Netea *et al.,* 1996). In addition, McFarlin and colleagues explained how 10 wks of chronic resistance exercise in elderly women could offset TLR4 cell surface expression but not age-associated inflammatory cytokines (McFarlin *et al.,* 2004). Some acute exercises performed in seniors have also described similar responses with no increases in skeletal muscle mRNA and protein levels of inflammatory mediators (Jozsi *et al.,* 2001). These discrepancies between young and older subjects may rely in a different response to exercise stress with age (McFarlin *et al.,* 2004).

Furthermore, it is important to noted that IL-10 acts as a natural antagonist of TNFα, being able to inhibit NF-κB signalling by blocking the release of the IKK and therefore, avoiding the translocation and activation of this nuclear factor (Dhingra *et al.,* 2009). Moreover, ERK1/2 has been documented to be involved in the

phosphorylation of the IL-10 proximal promoter in macrophages (Hofmann *et al.,* 2012), connecting the activation previously explained in the RTG with the increase of IL-10 protein content described.

Data elicited from aerobic training protocols are also equivocal. Goldhammer and co-workers demonstrated how an aerobic exercise training performed along 12 wks were able to reduced plasmatic inflammatory mediators in elderly patients with coronary disease (Goldhammer *et al.,* 2005). A similar response was also observed by Adamopoulos and colleagues after 12 wks of bicycle exercise in chronic heart failure patients (Adamopoulos *et al.,* 2001). However, contradictory results also exist in the literature. This is the case of an 8-wk endurance exercise training in obese adults, which showed any changed in the TNFα analyzed (Ferrier *et al.,* 2004). In the current study, the ATG participants presented unchanged protein content of both IL-10 and TNFα. A part from the large interpersonal variability in the peripheral inflammatory markers, different modes of training intervention, intensity and time are obvious reasons for discrepancies.

Focusing in WBV exercise, studies show still controversial results. Nonetheless Naghii and co-workers have demonstrated in rats the anti-inflammatory effect of 8 wks of a repetitive vibration stimulus in the increased of IL-6 and therefore, in IL-10 (Naghii *et al.,* 2011). Moreover, other recent works have noted how, after different WBV trainings, IL-10 concentration showed a tendency to increase (Cristi *et al.,* 2013) or even a significant increment (Hazell *et al.,* 2013). In the current study, data from the VTG suggest a similar and positive situation since the level of IL-10 in PBMC is significantly increased in the training group compared with the sedentary participants. These values seems to relate the grade of effect more to exercise duration, intensity and muscle mass involved than the type of physical activity, how previously has been already suggested elsewhere (Markovitch *et al.,* 2008).

Besides, it is important to emphasize that the quantification of inflammatory mediators could act as markers of the health status (Bruunsgaard, 2005). Consequently, the IL-10/ TNFα ratio is considered as an indicator of the inflammatory

status associated with the probable course and outcome of a disease (Lira *et al.,* 2009, Petersen and Pedersen, 2005). Data elicited from the RTG assess the efficacy of the resistance exercise protocol to induce a modification of the proportion between these two markers towards a positive balance to anti-inflammatory signals as previoulsy other studies have also supported (Lira *et al.,* 2009, Petersen and Pedersen, 2005). Similar results are also obtained in the VTG after the 8 wks of WBV training that these participants carried out. Therefore, the exercise protocol design for this study may induce a positive effect in the elderly population.

Literature also describes how altered concentration of several cytokines can lead to a decrease of TLR expression (Gleeson *et al.,* 2006). Indeed, this phenomenon is confirmed by the existence of a regulation loop between TLRs and cytokines: while TLR induce cytokine expression via NF-κB pathway, these markers appears to be also involved in the modulation of TLR expression (Miettinen *et al.,* 2001, Seibl *et al.,* 2003). Therefore, low TLR2 and TLR4 expression inhibits pro-inflammatory cytokine production and may explain at least partially, the anti-inflammatory effect attributed to regular exercise (McFarlin *et al.,* 2004).

Moreover, researchers have also suggested an association between exercise and transient alterations of Hsp since these proteins are implicated in the reduction of inflammatory mediators (Noble and Shen, 2012). Hsp are the most phylogenetically conserved proteins present in all prokaryotes and eukaryotes that recognize internal ligands of TLR2 and TLR4, being involved in a number of remodelling processes associated with exercise training (Huey *et al.,* 2010),

Among all the Hsp family, Hsp70 is a potent endogenous activator of the innate immune system (Vabulas *et al.,* 2002), one of many ligands recognized by both TLR2 and TLR4 (Asea *et al.,* 2002) but also, it is able to damp inflammatory pathways (Jones *et al.,* 2011).

Increases in Hsp70 protein levels have been described after different exercise stimulus both in animals (Locke *et al.,* 1990, Samelman, 2000) and humans (Walsh *et al.,* 2001), but contradictory findings also exist (Liu *et al.,* 2004, Ogawa *et al.,* 2010).

Apparently, this controversy may be explained by the fact that Hsp70 production is linked to the intensity and the frequency of the exercise (Harris and Starnes, 2001, Milne and Noble, 2002).

It must be emphasized that Hsp70 can directly stimulated anti-inflammatory cytokines due to an inhibitory effect through the activation of the NF-κB pathway (Schell *et al.,* 2005, Weiss *et al.,* 2007) and therefore, on the expression of pro-inflammatory cytokines (De *et al.,* 2000, Pockley *et al.,* 2009). Thus, the data from the RTG revealed how PBMC Hsp70 enhances its concentration after complete the strength training, being an intense enough stimulus to promote the anti-inflammatory effect attributed to this mediator.

In addition, high circulating levels of another Hsp member, Hsp60 acts as activator of TLR2 and TLR4 (Asea *et al.,* 2002, Kilmartin and Reen, 2004, Ohashi *et al.,* 2000) resulting in the production of inflammatory mediators (Hao *et al.,* 2010). Its effects are complex since depends on the cell type to induce a pro-inflammatory or anti-inflammatory status (Cohen-Sfady *et al.,* 2009). Macrophages and dendritic cells respond to Hsp60 by activation and secretion of pro-inflammatory cytokines such as IL-12 and TNFα (Flohe *et al.,* 2003). Hence, its local expression seems to serve as an immune biomarker for the state of the tissue in infections and body maintenance (Cohen, 2007). Consequently, literature attributed the excess of intracellular Hsp60 production to abnormal cell states closely related to pathologies situations (Hao *et al.,* 2010). However, the exercise is also able to diminish the expression of this Hsp, how previously Marini and colleagues have reported (Marini *et al.,* 2007). Thus, values obtained from the group which carried out the resistance protocol may suggest the capability of this intervention to diminished the protein content of Hsp60, confirming the anti-inflammatory situation.

Conversely, levels of these two Hsp in the ATG showed unchanged values after the training period and in a similar way than the protein content observed in the sedentary participants. It should be noted that protein content of Hsp60 trend to show a non-significant reduction. Overall, these results corroborate the theory that

the aerobic exercise intervention designed and performed in this experiment was not enough to create an anti-inflammatory environment and therefore, induce a diminished in the low-grade chronic inflammation related to senescence.

On the other hand, high circulating levels of Hsp70 has been detected in the PBMC of the VTG participants after the 16 sessions of vibratory stimulus, while Hsp60 protein content were blunted after the training protocol. Bearing in mind all the data elicited from this group and previously associations between excess Hsp60 and autoimmune disorders (Hao *et al.,* 2010), it can be proposed that the WBV exercise intervention carried out in this study may be linked with a healthier status of the whole body.

Conclusions

First conclusion

The 8-week resistance exercise training programme down-regulated significantly TLR2 and TLR4 basal expression, inducing an anti-inflammatory status in elderly subjects. In this context, resistance training impacted both MyD88-dependent and -independent pathways.

Second conclusion

The anti-inflammatory effects induced by resistance training on the TLR2 and TLR4 receptor signalling pathways seems to be associated with changes in the expression of Hsp70 and Hsp60, which may also confer further protection against other age-related disorders.

Third conclusion

The current study suggests that the endurance exercise protocol employed here represents a useful tool to achieve positive functional adaptations and induces no alterations in inflammatory biomarkers in the elderly.

Fourth conclusion

Considering the numerous possible combinations of intensities and duration for endurance training, further studies are needed in order to develop the most effective training protocol to reduce the chronic low-grade inflammation related to age and, at the same time, to study their effects on TLRs pathways.

Fifth conclusion

This study provides novel understanding of the molecular mechanisms behind the inflammatory response controlled by the TLR2 and TLR4 signalling pathway after whole-body vibration training in old women and men. It is suggested that different

signalling pathways involving NF-κB, MAPK, TRIF and the transcription factors IRF3 and IRF7 could be modulated by TLR2 and TLR4 in response to this exercise.

Sixth conclusion

High levels of Hsp70 and blunted content of Hsp60 in PBMC were detected after sixteen sessions of WBV training, which may be associated with improvements in the inflammatory state of the whole body in the elderly population

General conclusion

Data obtained confirm the usefulness of resistance and WBV training for counteracting the chronic low-grade inflammation related to age. Both types of training programmes, in contrast to the aerobic intervention, are purported as a valuable tool to decrease age-induced inflammation through a down-regulation of the TLR2 and TLR4 downstream signalling in the elderly. This may have implications in the prevention and rehabilitation programs currently employed for autoimmune and inflammatory diseases in this population.

Resumen en español

universidad de león

Departamento de Ciencias Biomédicas

Efecto de la realización de diferentes programas de entrenamiento sobre la inflamación asociada al envejecimiento: papel de la vía de señalización de los receptores tipo Toll 2 y 4

Memoria presentada por la Licenciada en Biotecnología Paula Rodríguez Miguélez,

para la obtención del grado de Doctor por la Universidad de León

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Abreviaturas y símbolos

1RM	Una repetición máxima
ADNc	ADN complementario
ANOVA	Analisis de la varianza
AP-1	Proteína activadora 1
ATG	Grupo de entrenamiento aeróbico
CG	Grupo control
EDTA	Ácido etilendiaminotetraacético
EEM	Error estándar de la media
ERK	Quinasa regulada por señales extracelulares
FMI	Fuerza máxima isométrica
GADPH	Gliceraldehído-3-fosfato deshidrogenasa
HRP	Peroxidasa de rábano picante
Hsp	Proteína de estrés térmico
IFN	Interferón
IKK	Quinasa inhidora de κB
IKKi/IKKε	Quinasa inducible inhidora de κB o epsilon
IL	Interleucina
IMC	Índice de masa corporal
IRAK	Quinasa asociada al receptor de la IL-1
IRF	Factor de regulación de IFN
IκB	Inhibidor κB
LPS	Lipopolisacárido
MAPKs	Quinasas activadas por mitógenos
MyD88	Factor de diferenciación mieloide 88
NF-κB	Factor nuclear kappa B
PAR-Q	Cuestionario de aptitud física
PBS	Tampón fosfato salino
PBS-t	PBS con tween
PVDF	Polifluoruro de vinilideno
RTG	Grupo de entrenamiento de fuerza
RT-qPCR	Retrotranscriptasa y reacción de la polimerasa en cadena cuantitativa
SDS	Dodecil sulfato sódico
TBK1	Quinasa de unión a TANK1
TIR	Dominio intercelular tipo toll-IL-1
TLR	Receptor tipo toll
TNFα	Factor de necrosis tumoral alfa
TRAF	Factor asociado al receptor TNF
TRIF	Adaptador con dominio TIR inductor de IFN beta
VCC	Vibración de cuerpo completo
VO_2	Consumo de oxígeno
VTG	Grupo de entrenamiento de vibración

Índice

INTRODUCCIÓN

Envejecimiento e inflamación

De acuerdo con Naciones Unidas, la población mundial envejece a un ritmo alarmante. En concreto se calcula que para el año 2050 habrá más de dos billones de personas mayores de 60 años, representando el 22% de la población mundial (United Nations, 2009). Esta evolución demográfica provocará importantes consecuencias en la sociedad, la cual deberá hacer frente a una gran población de personas mayores que paulatinamente es menos independiente y por lo tanto, requiere más cuidados.

El envejecimiento es un proceso complejo y multifactorial caracterizado por un deterioro progresivo e inevitable, parcialmente atribuido al acúmulo progresivo de sustancias dañinas, que provocan pérdida de la funcionalidad celular (Goto, 2008).

Además de todas las alteraciones que sufre la población anciana en el sistema muscular, óseo, cardiovascular, respiratorio, nervioso o endocrino, entre otros, el envejecimiento induce cambios muy significativos en el sistema inmune (Gruver *et al.,* 2007) aumentando la susceptibilidad de las personas mayores a las infecciones, o favoreciendo incluso la posibilidad de desarrollar enfermedades autoinmunes (Murasko y Jiang, 2005; Pawelec *et al.;* 2006, Prelog, 2006).

Estos cambios en el sistema inmune, junto con el incremento de la esperanza de vida, hacen que las personas estén expuestas durante mucho más tiempo a agentes endógenos o exógenos capaces de activar la respuesta inmunitaria, generando así un estado inflamatorio denominado inmunosenescencia (Franceschi *et al.,* 2000; Franceschi, 2003). Se trata de una situación crónica y no patológica caracterizada por un bajo grado de inflamación asociada directamente al proceso de envejecimiento (Giunta, 2006; Goto, 2008).

La inflamación constituye la primera respuesta del sistema inmune a una infección causada por un agente físico, químico o biológico que desencadena una serie de reacciones como: cambios citológicos, infiltraciones celulares y/o el

incremento de medidores para poder concluir rápidamente con el estado anómalo y reparar el tejido dañado. Dentro de los múltiples cambios que se suceden, el más destacado es el relacionado con la producción de citoquinas proinflamatorias (Boehmer *et al.,* 2004; Renshaw *et al.,* 2002).

Las citoquinas (del griego *cyto-*, célula y *-kinos*, movimiento) son un grupo de péptidos señalizadores de bajo peso molecular que tienen un papel fundamental en una multitud de respuestas incluidas las del sistema inmune, la hematopoyesis, la neurogenesis o los procesos oncológicos. La mayoría de estas proteínas actúan de forma autocrina o paracrina mediante receptores específicos en la superficie celular, aunque algunas pueden desempeñar también funciones de tipo hormonal (Dinarello, 2007).

Estos mediadores se han clasificado tradicionalmente según su acción a favor o en contra del desarrollo de los procesos inflamatorios. Dentro de las citoquinas proinflamatorias cabe destacar el factor de necrosis tumoral α (TNFα), un péptido clave en el desarrollo temprano de los procesos locales inflamatorios, cuya función principal es el reclutamiento de los leucocitos hacia el foco de infección (Bruunsgaard, 2005). Su producción está muy relacionada con el tejido adiposo (Hotamisligil, 2006; Krogh-Madsen *et al.,* 2006) y con las poblaciones de monocitos y macrófagos (Brotas *et al.,* 2012).

Por otra parte se encuentra la interleucina (IL) 6, una citoquina pleiotrópica con un doble papel en los procesos inflamatorios (Pedersen, 2006). Su producción se induce en respuesta a múltiples estímulos incluyendo la acción de otras citoquinas, como el TNFα (Kishimoto, 2005). Se sabe que la IL-6 juega un papel clave en el desarrollo de algunas enfermedades autoinmunes y en múltiples procesos inflamatorios (Kishimoto, 2005) lo que la ha convertido en un indicador de mortalidad y morbilidad en las personas mayores (Franceschi *et al.,* 2000).

Como ejemplo de mediador antiinflamatorio merece especial atención la IL-10. Se trata de una proteína producida por la mayoría de células del sistema inmune innato y adaptativo (Yanaba *et al.,* 2009) que presenta múltiples actividades, donde

destacan la inhibición de la síntesis de citoquinas proinflamatorias en macrófagos y monocitos (Iyer y Cheng, 2012). Esta acción la lleva a cabo a través de la unión a la quinasa inhibidora de kappa B (κB) (IKK) enmascarando la señal de localización nuclear del factor de transcripción κB (NF-κB) y manteniéndolo en el citoplasma en un estado inactivo (Hayden y Ghosh, 2012). Con ello se genera un bucle entre el NF-κB, controlador principal de la expresión de citoquinas de tipo proinflamatorio, y la IL-10 involucrada a su vez en la inhibición de este factor de transcripción (Moore *et al.,* 2001).

Receptores tipo Toll

La reacción inflamatoria se desencadena como una parte de la primera línea de defensa del sistema inmune, en respuesta a múltiples factores internos, o frente a agresiones mediadas por agentes patógenos externos. Para ello, existen numerosas proteínas que reconocen patrones estructurales específicos localizados en la superficie de los microorganismos, como las defensinas, los péptidos antimicrobianos o los recetores de transducción de señales (Kappagoda y Amsterdam, 2012).

Una de las cuatro familias principales de receptores que reconocen patrones específicos, y que inician la señalización intracelular del proceso inflamatorio, es la conocida como receptores tipo Toll (TLR) (Takeuchi y Akira, 2010); proteínas altamente conservadas que presentan un papel fundamental en la respuesta inflamatoria y en la regulación inmunológica (Kawai y Akira, 2010). Hasta ahora se han identificado trece miembros (TLR1-TLR13). Los primeros nueve receptores se localizan tanto en humanos como en roedores, el TLR10 es exclusivo de humanos, y los TLR11-TLR13 están presentes sólo en roedores (Kawai y Akira, 2010).

La mayoría de estos receptores se localizan en la superficie celular de monocitos, neutrófilos o células dendríticas, aunque algunos se encuentran casi de manera exclusiva en membranas de compartimentos endosomales (Pietrocola *et al.,* 2011). Estructuralmente se trata de glicoproteínas con un dominio extracelular rico en leucina y un dominio intercelular tipo Toll-IL-1 (TIR) (Pietrocola *et al.,* 2011).

Dichos receptores poseen un papel fundamental en el reconocimiento de una amplia variedad de componentes estructurales de la pared celular, material genético vírico o señales de estrés como las proteínas de estrés térmico (Hsp) (Cristofaro y Opal, 2006). Diferentes TLRs reconocen distintos ligandos. Este es el caso del TLR2, involucrado en la detección de lipoproteínas, peptidoglicanos y el ácido lipoteitoico de las bacterias Gram positivas (Takeuchi y Akira, 2010), u otros elementos no microbianos entre los que podemos citar las proteínas de shock térmico Hsp60 y Hsp70 (Campos *et al.,* 2001).

El TLR4 es el receptor fundamental para la detección y transducción de la señal desde LPS, uno de los mayores inmunoestimuladores (Hoshino *et al.,* 1999). Además también está involucrado en la identificación de moléculas endógenas como Hsp60 y Hsp70 (Ohashi *et al.,* 2000) o el ácido hialurónico (Termeer *et al.,* 2002).

Tras el reconocimiento de los ligandos por los correspondientes TLRs, se inicia la ruta para desencadenar la respuesta inmunológica (Takeda y Akira, 2005) promoviendo la oligomerización de los receptores y el posterior reclutamiento de mediadores a través de su dominio TIR (Takeuchi y Akira, 2010).

Existen cinco tipos de adaptadores que también presentan dominio TIR, donde destacan el factor de diferenciación mieloide 88 (MyD88) y el adaptador con dominio TIR inductor de interferón (IFN) β (TRIF) (Takeuchi y Akira, 2010). La utilización de uno u otro dividirá a las rutas de los TLRs en MyD88 dependiente, común a todos los TLRs y responsable de la expresión de citoquinas proinflamatorias, y MyD88 independiente o TRIF dependiente, específica sólo de TLR3 y TLR4 y directamente involucrada en el control de los genes relacionados con el IFN (Lu *et al.,* 2008; Takeuchi y Akira, 2010).

Ruta de señalización dependiente de MyD88

El adaptador MyD88 es una proteína clave para numerosos mecanismos fisiológicos, tanto del sistema inmune como de otros procesos (Wu y Arron, 2003). Su papel consiste en transducir la señal desde los TLRs hacia mediadores presentes en el citoplasma celular, como son la familia de quinasas asociadas al receptor IL-1 (IRAK)

(Kawai y Akira, 2010). En concreto, IRAK-4 es la proteína que establece la interacción inicial con el adaptador TIR y tras ello activa a IRAK-1 e IRAK-2 (Takeuchi y Akira, 2010). A su vez, este complejo inicia el reclutamiento del factor asociado al receptor TNF (TRAF) 6, un mediador que actúa como una ubiquitín ligasa (Takeuchi y Akira, 2010) dando lugar a la activación de dos cascadas de señalización diferentes: la ruta del NF-κB y la de las quinasas activadas por mitógenos (MAPK) (Lu *et al.,* 2008).

La primera de ellas, la vía de señalización del NF-κB, comienza con la acción de TRAF6, que mediante diversas fosforilaciones, estimulará la degradación del inhibidor κB (IκB), permitiendo así el desenmascaramiento de la secuencia de localización nuclear del NF-κB y su posterior translocación al núcleo.

El NF-κB es un factor de trascripción clave en múltiples procesos celulares como la supervivencia celular o la diferenciación y proliferación entre otros, además de controlar la expresión de las citoquinas proinflamatorias (Hayden y Ghosh, 2012). En mamíferos existen cinco miembros de la familia NF-κB, siendo p65 y p50 los componentes más ampliamente estudiados (Hayden y Ghosh, 2012).

El complejo formado a partir de TRAF6 también induce la activación de las proteínas MAPK. Se trata de una familia de serín treonín quinasas que conectan los receptores de la superficie celular con factores de transcripción reguladores de numerosos eventos como la inflamación, la proliferación y la supervivencia celular. Además, están implicadas en la activación del factor de trascripción de la proteína activadora 1 (AP-1), íntimamente relacionado con la expresión de citoquinas proinflamatorias (Chang y Karin, 2001).

Hasta la fecha se han descrito cuatro rutas principales dentro de las MAPK donde destacan la proteína quinasa regulada por señales extracelulares (ERK) y p38, (Li *et al.,* 2010). La subfamilia ERK fue la primera en ser identificada (Boulton y Cobb, 1991) y aunque existen diferentes subtipos, las principales funciones se ha atribuido a ERK1 y ERK2 (ERK1/2). Se trata de proteínas que tras su fosforilación y activación, se translocan al núcleo donde pueden activar un amplio número de mediadores que estimularán al factor de transcripción AP-1 (Shaulian, 2010).

Por su parte, p38 está directamente relacionado con la producción de citoquinas proinflamatorias como IL-1, IL-6 o TNFα (Huang *et al.,* 2010). Se han descrito cuatro isoformas (α, β, γ y δ) que muestran diferentes expresiones en función del tejido del que se trate (Roux y Blenis, 2004). En relación a lo comentado anteriormente hay que destacar que, debido a la expresión ubicua de p38α, la mayoría de estudios publicados se refieren a esta isoforma de manera general como p38.

Ambos mediadores activan al factor de trascripción AP-1, involucrado en el control de una amplia variedad de genes de la respuesta celular temprana a estímulos fisiológicos y patológicos, como son las citoquinas proinflamatorias o algunas señales de estrés (Vaiopoulos *et al.,* 2010). Es importante señalar que debido a ese gran abanico de intermediarios que activan AP-1, existen múltiples puntos de regulación que dan lugar a la activación de una ruta determinada de las MAPK (Sakurai, 2012).

Ruta de señalización dependiente de TRIF

Como se describió con anterioridad, el mediador MyD88 es fundamental para la expresión de citoquinas proinflamatorias. Sin embargo se ha demostrado que la estimulación de TLRs en macrófagos deficientes de MyD88 también da lugar a la activación de las rutas del NF-κB y MAPK (Kawai *et al.,* 1999). Esto es debido al mediador TRIF, una proteína de 712 aminoácidos con un papel fundamental en la expresión de genes inducibles por IFN y en la fase tardía de la síntesis de citoquinas (Moynagh, 2005; Tseng *et al.,* 2010).

A partir de TRIF la cascada continúa con la interacción entre la porción N terminal, la quinasa de unión a TANK 1 (TBK1) y la quinasa inducible inhibidora de κB (IKKi/IKKε), un importante activador de la familia IKK (Guo y Cheng, 2007; Hacker *et al.,* 2006; Oganesyan *et al.,* 2006). Este complejo es fundamental para promover la dimerización y posterior translocación de algunos factores de transcripción de la familia IRF como son IRF3 e IRF7 (Lu *et al.,* 2008).

Los factores nucleares IRF3 e IRF7 son proteínas relacionadas estructuralmente que se localizan en el citoplasma en condiciones inactivas (Kawai y Akira, 2006). Inicialmente se lleva a cabo la activación de IRF3 mediante fosforilación directa de su extremo C terminal por la acción de TBK1 e IKKi/IKKε, permitiendo así la formación de homodímeros que se translocarán al núcleo para activar la transcripción de los genes que codifican para IFN β (Chau *et al.,* 2008). Tras ello, IRF3 activa un complejo denominado factor de estimulación de los genes de IFN (ISGF3) que facilitará la síntesis de IRF7 y su posterior activación de la misma forma que IRF3 (Moynagh, 2005).

Actividad física y envejecimiento

Durante la última década, innumerables evidencias científicas han demostrado los beneficios de la práctica regular de actividad física en la salud y por lo tanto, en la calidad de vida (De Lemos *et al.,* 2012; Viña *et al.,* 2012). La actividad física se define como cualquier movimiento producido por músculos esqueléticos que requiere un gasto de energía (Caspersen *et al.,* 1985). Aunque ejercicio y actividad física no son exactamente lo mismo, ya que se ha descrito ejercicio como un tipo de actividad física planeada, estructurada y repetida que mejora o mantiene uno o más componentes de la forma física (Caspersen *et al.,* 1985), se han considerado tradicionalmente como términos intercambiables.

Es por todos conocido que la práctica regular de ejercicio a todas las edades, proporciona múltiples beneficios en la salud mejorando el sistema cardiorrespiratorio, previniendo la aparición de un exceso de peso, conservando la masa muscular e incrementando el nivel del metabolismo basal (Strasser *et al.,* 2012). También es importante destacar, que la actividad física realizada de manera frecuente, ayuda a mantener la fuerza y el tono muscular y a potenciar la densidad mineral ósea (Crocker *et al.,* 2013).

Además de estos efectos positivos, múltiples estudios apoyan que la práctica regular de ejercicio es capaz de contrarrestar las enfermedades inflamatorias

asociadas al envejecimiento (Greiwe *et al.,* 2001; Pedersen *et al.,* 2000). Por ello, se ha propuesto al ejercicio físico como una posible terapia para prevenir o retrasar el desarrollo de ciertas patologías relacionadas con la inmunosenescencia (Baltgalvis *et al.,* 2008; Pawelec *et al.,* 2006).

Aunque tradicionalmente se han utilizado ejercicios de fuerza o ejercicios aeróbicos para contrarrestar los efectos del envejecimiento (American College of Sports Medicine *et al.,* 2009; Haskell *et al.,* 2007), aún no se ha establecido ni una modalidad, ni unos protocolos óptimos para la obtención de los máximos beneficios inmunológicos.

El ejercicio de fuerza se ha descrito como la activación voluntaria de los músculos esqueléticos en contra de alguna forma externa de resistencia (American College of Sports Medicine *et al.,* 2009). Esta práctica infiere numerosos beneficios sobre el sistema músculo-esquelético, ayudando a mantener la masa muscular y a evitar la pérdida de fuerza característica del procesos de senescencia (Krebs *et al.,* 2007; Reeves *et al.,* 2006). Se trata de un método seguro, simple y que requiere muy poco tiempo para obtener beneficios evidentes (American College of Sports Medicine *et al.,* 2009).

Algunos estudios han atribuido a la práctica frecuente de ejercicios de esta modalidad la propiedad de estimular efectos positivos sobre el sistema inmune. De hecho, se ha relacionado la práctica en ancianos de ejercicios agudos o crónicos de este tipo, con disminuciones en los niveles de citoquinas proinflamatorias (Phillips *et al.,* 2010) y del contenido de TLR4 (Flynn *et al.,* 2003; McFarlin *et al.,* 2004).

El ejercicio aeróbico es aquel que tiene la capacidad de mantener un determinado nivel de actividad física a lo largo del tiempo, aumentando para ello la frecuencia cardiaca y respiratoria (Frankel *et al.,* 2006). La práctica frecuente de esta modalidad induce innumerables beneficios, como la disminución de la presión arterial, la reducción de los niveles de colesterol o el incremento de las lipoproteínas de alta densidad (Bean *et al.,* 2004; Kokkinos *et al.,* 2001; Mazzeo y Tanaka, 2001).

A nivel inmunológico, se ha descrito que la realización frecuente de actividades de tipo aeróbico de baja intensidad, puede dar lugar a la reducción de la concentración de citoquinas proinflamatorias, y a su vez, a un aumento de los mediadores antiinflamatorios (Gano *et al.,* 2011; Goldhammer *et al.,* 2005; Kadoglou *et al.,* 2007). Estos efectos también se han relacionado con disminuciones en la expresión de TLR2 y TLR4 (Oliveira *et al.,* 2011) o de NF-κB (Durham *et al.,* 2004; Ma *et al.,* 2013). Sin embargo, hay que poner de manifiesto que la aplicación de otros protocolos diferentes en intensidad o duración, no han producido estos cambios (Ferrier *et al.,* 2004; Ho *et al.,* 2005; McKenzie y Goldfarb, 2007; Nickel *et al.,* 2011; Reyna *et al.,* 2013; Zbinden-Foncea *et al.,* 2012).

Además de estas dos modalidades de ejercicio, hoy en día se está empezando a considerar el uso de la vibración de cuerpo completo (VCC) como una forma de actividad física fácil y segura, capaz de inducir ciertos beneficios en algunas alteraciones de tipo óseo y/o muscular, asociadas al proceso de envejecimiento (Abercromby *et al.,* 2007; Ronnestad, 2004; Verschueren *et al.,* 2004).

Sin embargo, los estudios que relacionan esta modalidad de ejercicio físico con un estado inflamatorio son hasta el momento limitados y muestran resultados controvertidos y contrapuestos. Algunos investigadores han descrito incrementos en la concentración de IL-6 (Naghii *et al.,* 2011) o reducciones en los niveles de IL-10 (Nowak *et al.,* 2012). Sin embargo, Hazell y colaboradores han demostrado muy recientemente como al realizar una sesión de VCC, de intensidad moderada, no se inducen daño muscular por lo que no observaron incrementos en los marcadores musculares de inflamación (Hazell *et al.,* 2013). Al igual que en el resto de modalidades anteriormente descritas, hay que destacar que la duración del protocolo de vibración puede ser crucial para alcanzar dichos beneficios inmunológicos (Pawlak *et al.,* 2013).

OBJETIVOS

Teniendo en cuenta todo lo anteriormente expuesto, los objetivos específicos planteados en este estudio fueron los siguientes:

-Analizar, en un grupo de edad avanzada, la expresión de los receptores Toll 2 y 4 antes y después de los programas de actividad física propuestos.

-Profundizar en el conocimiento de la activación y regulación de diversas rutas de señalización dependientes de MyD88 en los diferentes grupos experimentales.

-Estudiar el efecto de distintos protocolos de entrenamiento sobre la ruta dependiente de TRIF en una población anciana.

-Evaluar la producción de diversos marcadores de inflamación tras ocho semanas de entrenamiento en células mononucleares de ancianos.

-Determinar, en la población de estudio, el papel de las proteínas de estrés térmico Hsp60 y Hsp70 sobre la ruta de los receptores tipo Toll en los tres tipos de actividad física realizados.

MATERIAL Y MÉTODOS

Diseño general

El diseño del estudio fue planeado para ser completado en 10 semanas. En la primera semana se llevó a cabo la recogida de los datos personales, la valoración basal antes del entrenamiento, así como la extracción de muestras sanguíneas. Antes de comenzar con los diferentes protocolos de entrenamiento, los grupos que iban a realizar alguna actividad física relacionada con el estudio, realizaron una sesión de familiarización con la metodología y los aparatos que iban a ser utilizados posteriormente en los entrenamientos. Tras ello, los participantes del grupo control continuaron con su rutina diaria, mientras que los voluntarios que habían sido designados a los grupos de entrenamientos, llevaron a cabo las 8 semanas de los protocolos de ejercicio correspondientes. Al finalizar, se repitió para todos los participantes la recogida de datos descriptivos, los valores post-entrenamiento y una nueva obtención de muestras sanguíneas (Figura 4).

Sujetos y procedimientos

Cincuenta y nueve participantes sanos, con edades comprendidas entre los 65 y los 87 años, se ofrecieron voluntarios para la realización del estudio. Sólo se permitió participar a aquellos sujetos sin contraindicación médica para la realización regular de ejercicio, y que no hubieran tomado medicación hormonal o de tipo antiinflamatorio al menos un mes antes del inicio del estudio. Sólo, y como excepción, se permitieron tratamientos de tipo antilipemiante. Posteriormente y tras informar detalladamente a todos los voluntarios seleccionados de los propósitos, y de los riesgos y/o beneficios posibles asociados con su participación en el estudio, se les solicitó que facilitaran su consentimiento por escrito (investigación revisada y aprobada por el Comité de Ética de la Universidad de León y que sigue la Declaración de Helsinki) (World Medical Association General Assembly, 2001).

Seguidamente, los sujetos fueron asignados aleatoriamente a uno de los cuatro grupos: 13 al grupo control (CG, del inglés *control group*), 15 al grupo de entrenamiento de fuerza (RTG, del inglés *resistance training group*), 16 al grupo de entrenamiento aeróbico (ATG, del inglés *aerobic training group*) y 15 al grupo de entrenamiento de vibración (VTG, del inglés *whole body vibration training group*), no existiendo diferencias significativas entre los grupos ni en edad, ni en composición corporal.

Recogida de datos descriptivos y basales

Previo a la realización de cualquier actividad relacionada con este trabajo, todos los participantes cumplimentaron un cuestionario de salud (*Physical Activity Readiness Questionnaire*, PAR-Q). Tras ello, y en estado de reposo, se realizó un electrocardiograma seguido de una prueba submáxima espirométrica en cicloergómetro para evaluar el ritmo cardiaco y su relación con el consumo de oxígeno (VO_2). A continuación se llevó a cabo una valoración antropométrica donde se midió la altura (estadiómetro Detecto D52, Webb City, MO, EE.UU.) y el peso corporal (báscula Cobol 20, Barcelona, España) (Tabla 1). Además se procedió a determinar el índice de masa corporal (IMC) mediante la relación entre la masa por el cuadrado de la estatura.

Valoración de la fuerza máxima

Aproximadamente una semana antes y una semana después del periodo de entrenamiento, se llevó a cabo la valoración de la fuerza máxima en los voluntarios mediante un test de fuerza máxima isométrica (FMI) en una prensa de piernas inclinada 45° (Gervasport, Madrid, España). A continuación, se procedió a la evaluación de la fuerza máxima en los flexores del codo en un banco para bíceps (Gervasport). Tras aproximadamente 30 min de descanso, se realizó una segunda valoración de la fuerza máxima mediante un test de una repetición máxima (1RM) en la misma prensa de piernas y el mismo banco de bíceps anteriormente descritos. A

continuación también se llevó a cabo dicho test para la evaluación de la fuerza máxima de pectorales en una máquina de pectoral contractor (BH Fitness Nevada Pro, BH, Vitoria, España). Todos los sujetos consiguieron su estimado 1RM entre 3 y 5 intentos.

Protocolos de entrenamiento

Entrenamiento de fuerza

Los participantes pertenecientes al RTG completaron 16 sesiones de entrenamiento a lo largo de 8 semanas, con al menos 48 horas de recuperación entre cada una de las sesiones. El protocolo comenzaba con un calentamiento estandarizado de 10 min en cicloergómetro seguido de tres ejercicios tanto de tren superior como de inferior: *press* de piernas, *curl* de bíceps y pectoral contractor (realizados en los mismos aparatos descritos anteriormente). Las repeticiones y cargas fueron aumentando progresivamente, siguiendo el protocolo que se detalla en la Tabla 2. La recuperación entre ejercicios fue siempre de 5 min y entre repeticiones de 2 min.

Entrenamiento aeróbico

El entrenamiento aeróbico fue diseñado para que los participantes del estudio (ATG) pedalearan en una bicicleta estática, aumentándose progresivamente la intensidad y la carga. Para ello se realizaron dos sesiones de entrenamiento a la semana para completar un total de 16 sesiones. Todas ellas seguían un patrón común comenzando siempre con un calentamiento de 5 min hasta alcanzar el 70% de su frecuencia cardiaca máxima, seguido del tiempo estimado de pedaleo con intervalos donde se les solicitaba que ejercieran el esfuerzo máximo posible. La sesión terminaba con un enfriamiento gradual de 5 min. Las características del protocolo empleado se pueden ver en la Tabla 3 y el perfil detallado de una sesión en la Figura 5.

Entrenamiento de vibración

Los voluntarios que habían sido designados al grupo VTG llevaron a cabo un entrenamiento en plataforma de vibración (Fitvibe, Gymna Uniphy NV, Bilzen, Bélgica) durante las 8 semanas que duró la fase experimental. Para ello, en cada una de las sesiones se realizó un calentamiento estandarizado de 10 min en cicloergómetro, seguido de ejercicios de tipo estático o dinámico en función del diseño de cada sesión. Los ejercicios podían ser media sentadilla (Figura 6a) entre 120° y 130° de flexión de rodilla, sentadilla completa (Figura 6b) con flexión de rodilla de 90°, sentadilla amplia en cuclillas (Figura 6c) o gemelos (Figura 6d) con flexión de rodilla entre 120° y 130°. El volumen de entrenamiento (repeticiones y tiempo por ejercicio) y la frecuencia se fue incrementado semanalmente de manera progresiva. Las características del protocolo seguido están reflejadas en la Tabla 4.

Procesado de las muestras sanguíneas

Se recogió un volumen de 30 ml de sangre de la vena braquiocefálica, entre 5 y 6 días antes y después del período de entrenamiento, usando el sistema Vacutainer (BD, Franklin Lakes, NJ, EE.UU.) con ácido etilendiaminotetraacético (EDTA) y heparina como anticoagulantes.

Las muestras recogidas en heparina se usaron para el recuento leucocitario que se realizó mediante recuento en una cámara de contaje estándar.

La sangre con EDTA, se centrifugó a 1500 xg durante 10 min (4°C) para separar el plasma. Con el resto de la muestra, reconstituida con PBS, se llevó a cabo un gradiente por densidad con solución separadora Ficoll (Biochrom AG, Berlin, Alemania) para proceder al aislamiento de las células mononucleares de sangre periférica (PBMC, del inglés *peripheral blood mononuclear cells*) (Cuevas *et al.,* 2005). Una vez aspirada la interfase correspondiente a dichas células, se transfirieron a un nuevo tubo donde se añadió PBS hasta un volumen final de 10 ml y se centrifugó a 890 xg (a temperatura ambiente) durante 10 min. Tras ello, se decantó el PBS y se

resuspendió el pellet en 1 ml de PBS seguido de una última centrifugación de 5 min a 2630 xg para finalmente extraer cuidadosamente el sobrenadante y congelar el precipitado de PBMC a -80 °C.

Cuantificación del ARNm y PCR cuantitativa

El ARN total de las PBMC se aisló utilizando el kit RiboPure-Blood (Ambion, Paisley, Reino Unido) y se cuantificó por espectrofotometría (Nanodrop 1000, Thermo Scientific, Waltham, MA, EE.UU.). Tras ello se procedió a la síntesis de ADN complementario (ADNc) utilizando la enzima transcriptasa inversa del kit High-Capacity cDNA Archive (Applied Biosystems, Paisley, Reino Unido). El ADNc se amplificó utilizando TaqMan Universal PCR Master Mix (Applied Biosystems) en un equipo StepOnePlus Real-Time PCR System (Applied Biosystems). Las sondas e iniciadores para IL-10 (Genbank M57627.1 y Hs00961622_m1), TNFα (Genbank M10988.1 y Hs00174128_m1) y GADPH como gen control (Genbank M33197.1 y Hs99999905_m1) se obtuvieron comercialmente de TaqMan Assays-on-Demand Gene (Applied Biosystems). Los cambios relativos en los niveles de expresión génica se determinaron usando el método del $2^{-\Delta\Delta CT}$ como se describió previamente (Livak y Schmittgen, 2001). El número de ciclos al cual los transcriptos fueron detectables (CT) se normalizó respecto al número de ciclos para la detección del GAPDH, refiriéndose como ΔCT.

Cuantificación proteica por Western blot

Las PBMC se homogeneizaron en 150 µl de tampón de sonicación al que previamente se le había añadido una mezcla comercial de inhibidores de proteasas y fosfatasas (Sigma-Aldrich, St. Louis, MO, EE.UU.) Tras el análisis de la concentración de proteína por el método Bradford (Bradford, 1976), se tomó una cantidad de muestra equivalente a 50 µg de proteína, para su separación mediante electroforesis en geles de diferentes porcentajes de poliacrilamida con SDS. Para el análisis de Hsp60, Hsp70, TLR2, TLR4, TRIF e IKKi/IKKε se usaron geles al 9%, para MyD88, p65,

ERK1/2, fosfo-ERK1/2, p38, fosfo-p38, IRF3, fosfo-IRF3, IRF7 y fosfo-IRF7 se usaron geles al 12% y para el análisis de TNFα e IL-10, geles al 15%. Además, en cada uno de los geles también se analizó la proteína β actina con el fin de verificar que se había cargado la misma cantidad de proteína.

Una vez separadas las muestras, fueron transferidas a una membrana de PVDF Immobilon-P (Millipore, Billerica, MA, EE.UU.) mediante el sistema Trans Blot Turbo Transfer (Biorad, Hercules, CA, EE.UU.). La membrana se incubó a continuación, durante media hora, en solución de bloqueo (2,5% de leche en polvo desnatada en PBS-t) a 37°C, para proceder posteriormente a su exposición, a 4°C durante toda la noche, a un anticuerpo específico para cada proteína (Tabla 5).

A la mañana siguiente, la membrana se lavó 6 veces con PBS-t y posteriormente se incubó durante una hora con un anticuerpo secundario conjugado con peroxidasa de rábano picante (HRP) (Dako, Glostrup, Dinamarca) adecuado al origen del primer anticuerpo. Tras ello, se incubó la membrana con una mezcla comercial de HRP y quimioluminiscencia (Luminol Reagent, Santa Cruz Biotechnology) y se expuso a películas fotográficas (Amersham Hyperfilm ECL, Amersham, Little Chalfont, Reino Unido), para su revelado y posterior cuantificación mediante densitometría, utilizando para ello el programa Image J (Bethesda, MD, EE.UU.).

Análisis estadístico

Los valores se presentan como media ± error estándar de la media (EEM). Todos los datos se analizaron estadísticamente usando un análisis de varianza (ANOVA) con medidas repetidas para grupo (CG, RTG, ATG y VTG) y tiempo (pre y post). Para ajustar el valor de *p* en relación al número de contrastes, se utilizó un análisis *post hoc* con corrección Bonferroni. Se consideraron diferencias estadísticamente significativas cuando $p < 0.05$. Todos los análisis se llevaron a cabo usando el programa SPSS, versión 18 (SPSS Inc., Chicago, IL, EE.UU.).

RESULTADOS

Fuerza máxima

Los resultados obtenidos tras la evaluación dinámica (1RM) de la fuerza máxima para *press d*e piernas, *curl* de biceps y pectoral contractor se muestran en la Tabla 6, donde también se representan los datos derivados de la evaluación isométrica (FMI) de la fuerza.

Los valores del grupo CG muestran como no existieron cambios significativos en ninguno de los test de fuerza llevados a cabo, ni en los grupos musculares analizados, antes y después del programa de entrenamiento.

Efecto de un programa de entrenamiento de fuerza

Los valores obtenidos para el RTG muestran como este grupo presentó un incremento significativo entre los resultados iniciales y tras el entrenamiento para los test de 1RM de *press* de pierna ($p < 0,03$), *curl* de biceps ($p < 0,04$) y pectoral contractor ($p < 0,04$) y para el test de FMI de *press* de pierna ($p < 0,05$) y *curl* de biceps ($p < 0,04$). También se observaron diferencias significativas al contrastar estos resultados con los valores anteriormente descritos para CG (1RM de *press* de pierna, $p < 0,05$; 1RM de *curl* de biceps, $p < 0,05$; 1RM de pectoral contractor, $p < 0,05$; FMI de *press* de pierna, $p < 0,04$; FMI de *curl* de biceps, $p < 0,05$).

Efecto de un programa de entrenamiento aeróbico

Los resultados obtenidos tras realizar la valoración de la fuerza máxima para el ATG, no muestran incrementos significativos en la mayoría de los test realizados, salvo en el de FMI de *press* de piernas donde se observaron diferencias estadísticamente significativas ($p < 0,05$). Al comparar estos valores con los anteriormente descritos para CG, tampoco se evidenciaron diferencias significativas salvo en el ya citado caso de FMI de *press* de pierna ($p < 0,04$).

Efecto de un programa de entrenamiento de vibración

Los test para la evaluación de la fuerza máxima realizados en el VTG muestran como los participantes de este grupo aumentaron significativamente algunos de los valores analizados, al comparar los resultados obtenidos antes y después de llevar a cabo las 8 semanas de entrenamiento (1RM de *press* de pierna, $p < 0,04$; 1RM de pectoral contractor, $p < 0,05$; FMI de *press* de pierna, $p < 0,04$; FMI de *curl* de biceps, $p < 0,05$). Se evidenciaron también diferencias significativas entre algunos de los valores de VTG y los descritos anteriormente para CG (1RM de *press* de pierna, $p < 0,05$; 1RM de pectoral contractor, $p < 0,05$; FMI de *press* de pierna, $p < 0,03$; FMI de *curl* de biceps, $p < 0,05$).

Consumo de oxígeno

Los resultados obtenidos tras la evaluación del VO_2 en el test submáximo en cicloergómetro se muestran en la Tabla 7. Los valores del grupo CG muestran como no existieron cambios significativos entre el antes y el después a las 8 semanas de rutina diaria de este grupo.

Efecto de un programa de entrenamiento de fuerza

Los resultados obtenidos tras evaluar el consumo pico de oxígeno mostraron que no hubo diferencias estadísticamente significativas en el grupo RTG al comparar los valores previos y posteriores a las 16 sesiones de ejercicios de fuerza que realizó este grupo. Tampoco se observaron diferencias al contrastar estos datos con los obtenidos por CG.

Efecto de un programa de entrenamiento aeróbico

El test submáximo realizado en el ATG muestra valores significativamente elevados ($p < 0,03$) entre los valores previos y posteriores a las 8 semanas de ejercicios aeróbicos que este grupo llevó a cabo. A su vez, también se muestran

cambios significativos ($p < 0.02$) al comparar estos resultados con los obtenidos por CG.

Efecto de un programa de entrenamiento de vibración

Los datos obtenidos por VTG tras la realización del test submáximo antes y después de las 8 semanas de ejercicio, ponen de manifiesto como no existieron cambios significativos entre ambos puntos, ni tampoco al compararlos con los datos obtenidos del CG.

Efecto de un programa de entrenamiento en la expresión de TLR2 y TLR4 en PBMC de ancianos

La literatura existente describe como el ejercicio físico puede inducir adaptaciones en la respuesta inflamatoria a través de los TLRs, debido al papel fundamental que éstos tienen en la iniciación de la respuesta inmune. Por ello, su desregulación puede inducir el desarrollo de enfermedades inflamatorias crónicas (Mookherjee *et al.,* 2006).

El análisis de los valores proteicos de CG para TLR2 y TLR4 no muestra cambios significativos en los valores obtenidos en ninguno de dichos receptores entre las muestras tomadas inicialmente y tras las 8 semanas.

Hay que destacar que dado que el recuento de PBMC no mostró ninguna variación entre los puntos de muestreo antes y después de los diferentes protocolos de ejercicio llevados a cabo, los resultados no se corrigieron en función de dichos valores.

Efecto de un programa de entrenamiento de fuerza

Los resultados de la expresión proteica de TLR2 y TLR4 tras las 8 semanas de entrenamiento del RTG (Figura 7) disminuyeron significativamente (TLR2, $p < 0,03$;

TLR4, $p < 0,04$) tras el protocolo de fuerza llevado a cabo, cuando se comparan con los valores basales del mismo grupo y con los del CG (TLR2, $p < 0,03$; TLR4, $p < 0,04$).

Efecto de un programa de entrenamiento aeróbico

Los datos obtenidos tras el análisis del contenido proteico de TLR2 y TLR4 transcurridas las 16 sesiones de ejercicio aeróbico (Figura 8), muestran que el protocolo de actividad física que el ATG llevó a cabo, no modificó los valores de estos receptores, tras compararlos con los valores basales del propio grupo. Tampoco se pusieron de manifiesto cambios en relación a los valores del grupo CG.

Efecto de un programa de entrenamiento de vibración

Los valores de la expresión proteica de TLR2 y TLR4 tras completar el protocolo de entrenamiento de vibración (Figura 9), muestran como TLR2 disminuye significativamente ($p < 0,03$) tras las 16 sesiones de ejercicio. Al analizar los contenidos proteicos de TLR4 se observaron cambios similares a los obtenidos para TLR2 ($p < 0,03$). También se observan diferencias estadísticamente significativas tras contrastar los valores de ambos receptores con los valores correspondientes de CG (TLR2, $p < 0,04$; TLR4, $p < 0,04$).

Efecto de un programa de entrenamiento en la vía de señalización dependiente de MyD88 en PBMC de ancianos

Expresión de MyD88

Tras la estimulación de TLR2 y TLR4, se inicia el reclutamiento de diferentes adaptadores que presentan una secuencia TIR. Uno de los principales, MyD88, lidera la activación de múltiples mediadores que concluyen en la activación de factores nucleares de transcripción cruciales en los procesos inflamatorios, como es el caso del NF-κB y de las MAPKs (Lu *et al.,* 2008).

Los datos obtenidos tras la evaluación de los valores de MyD88 para CG revelan que no hubo cambios significativos en la expresión de esta proteína, cuando se comparan los valores iniciales con los obtenidos tras el tiempo de estudio.

Efecto de un programa de entrenamiento de fuerza

El contenido proteico de MyD88 para RTG (Figura 10) muestra un patrón similar al descrito para los receptores anteriormente analizados. Los datos de este grupo muestran una disminución significativa en la expresión proteica de este adaptador ($p < 0,05$) al comparar los valores pre y post entrenamiento. El análisis estadístico revela también disminuciones significativas (MyD88, $p < 0,04$) tras contrastar los resultados con los obtenidos por el grupo CG.

Efecto de un programa de entrenamiento aeróbico

La Figura 11 muestra la concentración de proteína MyD88 para el ATG, donde se observa que no existieron diferencias significativas entre los valores iniciales y finales tras completar las 8 semanas del programa de ejercicio aeróbico. Tampoco se manifestaron diferencias al contrastarlos con los resultados obtenidos de CG.

Efecto de un programa de entrenamiento de vibración

El contenido proteico de MyD88 tras completar la intervención que el VTG realizó (Figura 12) indica que esta proteína sigue una tendencia idéntica a la previamente observada para los datos de TLR2 y TLR4, mostrando una disminución significativa ($p < 0,04$) al comparar los valores iniciales y finales de dicho grupo. También se observaron diferencias estadísticamente significativas al contrastar estos datos con los resultados obtenidos por CG (MyD88, $p < 0,05$).

Ruta del NF-κB

El adaptador MyD88 recluta diferentes proteínas en el citoplasma que consecutivamente darán lugar a la formación de un complejo encargado de la

fosforilación de IκB, permitiendo así la activación y translocación del NF-κB al núcleo (Hayden y Ghosh, 2012).

El análisis de p65, uno de los componentes claves en la familia NF-κB, muestra como su contenido proteico en el grupo CG no se modificó entre los valores iniciales y finales.

Efecto de un programa de entrenamiento de fuerza

La expresión proteica de p65 para RTG (Figura 13) pone de manifiesto, al igual que los resultados anteriormente descritos para este grupo, una disminución estadísticamente significativa ($p < 0,03$) al comparar los valores previos y posteriores a completar el protocolo de entrenamiento. Además, también se observa una disminución significativa ($p < 0,04$) al comparar estos resultados con los del CG.

Efecto de un programa de entrenamiento aeróbico

La Figura 14 muestra el contenido proteico de p65 para el ATG. Como en casos anteriores, la expresión de esta proteína no se vio modificada por el programa de actividad física que este grupo llevó a cabo. Al comparar los datos con los del grupo que siguió con su rutina diaria, tampoco se observaron cambios estadísticamente significativos.

Efecto de un programa de entrenamiento de vibración

El contenido proteico de p65 tras completar el programa de ejercicio que el VTG realizó (Figura 15) indica que esta proteínas disminuyó significativamente ($p < 0,05$) al comparar los valores iniciales y finales de dicho grupo. También se observaron diferencias significativas al contrastar estos datos con los resultados obtenidos por CG ($p < 0,05$).

Ruta MAPK

La activación del adaptador MyD88 también estimula otros mediadores como los involucrados en la ruta de señalización MAPK en la que a través de diferentes rutas, como ERK1/2 y p38, se estimula la acción del factor de trascripción AP-1 (Sakurai, 2012).

Los valores para ERK1/2 fosforilado, ERK1/2 total, p38 fosforilado y p38 total en el grupo CG no mostraron diferencias significativas entre los datos previos y posteriores a las 8 semanas de rutina normal.

Efecto de un programa de entrenamiento de fuerza

El análisis de la relación entre la forma fosforilada de p38 y su contenido total en el grupo RTG (Figura 16) indica que el contenido proteico de la forma activa disminuyó significativamente ($p < 0,03$) en respuesta al ejercicio. También se observaron diferencias significativas ($p < 0,03$) al contrastar estos valores con los obtenidos en el grupo CG.

Para otra de las proteínas de la vía MAPK, ERK1/2, el contenido proteico de su forma fosforilada experimenta un incremento estadísticamente significativo ($p < 0,02$) en el grupo entrenado tras compararlo con los valores totales de ERK1/2. De nuevo, el mismo resultado se observa cuando se realiza la comparación con los datos obtenidos de CG ($p < 0,03$).

Efecto de un programa de entrenamiento aeróbico

Los resultados de la expresión proteica de la forma activa de p38 y de su contenido total para el ATG (Figura 17) indican que no existieron cambios significativos ente los valores previos y posteriores al entrenamiento aeróbico.

Sin embargo, los resultados de los análisis de la forma fosforilada de ERK1/2 (Figura 17) mostraron un aumento significativo ($p < 0,03$) en respuesta al ejercicio al contrastarlos con los resultados totales de este mediador. También se identificaron

diferencias estadísticamente significativas (p < 0,03) al comparar los resultados de la relación ERK1/2 fosforilado / ERK1/2 total con los obtenidos para el grupo sedentario.

Efecto de un programa de entrenamiento de vibración

La fosforilación de p38 tras un entrenamiento de vibración de cuerpo completo (Figura 18) sufre una disminución significativa ($p < 0,01$) como resultado de las 8 semanas de ejercicio. También se observan diferencias estadísticamente significativas ($p < 0,03$) al contrastar estos valores con los correspondientes obtenidos en el grupo CG.

Por otra parte, el resultado de la expresión proteica de la forma fosforilada de ERK1/2 para el grupo VTG (Figura 18) muestra como el contenido de dicha proteína no se ve modificado por el ejercicio, al igual que sucede con el valor total de ERK1/2.

Efecto de un programa de entrenamiento en la vía de señalización dependiente de TRIF en PBMC de ancianos: Rutas del IRF3 e IRF7

Además de la cascada de señalización dependiente del mediador MyD88, algunos TLRs también pueden activar una ruta dependiente de TRIF, induciendo la fosforilación, dimerización y posterior translocación al núcleo de factores de transcripción como IRF3 y IRF7 (Moynagh, 2005).

El mismo patrón identificado en el grupo no sometido a entrenamiento para las proteínas previamente analizadas, se observa en la expresión proteica de TRIF, IKKi/IKKε, IRF3 fosforilado, IRF3 total, IRF7 fosforilado e IRF7 total; es decir, no existieron cambios estadísticamente significativos.

Efecto de un programa de entrenamiento de fuerza

El contenido proteico de TRIF e IKKi/IKKε para el grupo RTG (Figura 19) muestra como la expresión de dichos mediadores disminuye significativamente (TRIF, $p < 0,05$; IKKi/IKKε, $p < 0,04$) en respuesta al entrenamiento de fuerza que este grupo

llevó a cabo. También se observó una disminución estadísticamente significativa al contrastar estos datos con los obtenidos por el grupo CG (TRIF, $p < 0,05$; IKKi/IKKε, $p < 0,05$).

De la misma manera, la expresión de la forma fosforilada de IRF3 e IRF7 y sus correspondientes contenidos totales (Figura 19) manifestó una respuesta similar a la descrita para los anteriores mediadores, con disminuciones estadísticamente significativas (IRF3 fosforilado/IRF3 total, $p < 0.04$; IRF7 fosforilado/IRF7 total, $p < 0.02$) tras completar la intervención. También se evidenciaron reducciones significativas al comparar estos resultados con los valores obtenidos por el grupo CG (IRF3 fosforilado/IRF3 total, $p < 0,04$; IRF7 fosforilado/IRF7 total, $p < 0,03$).

Efecto de un programa de entrenamiento aeróbico

Los resultados obtenidos tras el análisis proteico de TRIF, IKKi/IKKε, IRF3 fosforilado, IRF3 total, IRF7 fosforilado e IRF7 total para ATG (Figura 20), no muestran cambios significativos entre los valores iniciales y finales tras completar el programa de entrenamiento aeróbico. Tampoco se identificaron diferencias al comparar estos resultados con los valores obtenidos por el grupo CG.

Efecto de un programa de entrenamiento de vibración

El contenido proteico de TRIF e IKKi/IKKε en el grupo VTG (Figura 21) manifiesta un patrón muy similar al anteriormente descrito para las proteínas pertenecientes a la ruta dependiente de MyD88. Inicialmente, TRIF e IKKi/IKKε muestran una disminución significativa ($p < 0,05$ y $p < 0,04$ respectivamente) entre los valores iniciales y finales tras completar las 8 semanas de entrenamiento de vibración. Esta reducción se vio acompañada con la disminución de los estados fosforilados de IRF3 e IRF7 ($p < 0,04$ y $p < 0,03$ respectivamente), si bien ninguno de los contenidos totales de estos factores de trascripción se modificaron tras la realización del entrenamiento. Tras contrastar todos estos datos con los obtenidos por el grupo CG, también se observaron diferencias estadísticamente significativas

(TRIF, $p < 0,05$; IKKi/IKKε, $p < 0,05$; IRF3 fosforilado/IRF3 total, $p < 0,05$; IRF7 fosforilado/IRF7 total, $p < 0,03$).

Efecto de un programa de entrenamiento en la expresión de mediadores inflamatorios en PBMC de ancianos

La actividad física se ha considerado como una herramienta útil para reducir la inflamación crónica de bajo grado asociada al envejecimiento. De hecho, se han relacionado estos beneficios con cambios en la concentración de citoquinas proinflamatorias, que inducen la síntesis de mediadores de tipo antiinflamatorio (Balducci *et al.,* 2010).

Los datos obtenidos en el grupo CG antes y después de las 8 semanas de rutina normal, muestras valores parecidos entre ambos puntos analizados, tanto para el contenido de ARNm de TNFα e IL-10, como para los niveles de proteína de dichas citoquinas. Además, el ratio proteico entre IL-10/TNFα, utilizado como indicador del estado inflamatorio, muestra que no existieron diferencias estadísticamente significativas (1,00 ± 0,11 *vs* 0,97 ± 0,12 unidades arbitrarias).

Efecto de un programa de entrenamiento de fuerza

Los valores de ARNm y proteína de IL-10 del RTG (Figura 22) muestran un aumento significativo ($p < 0,04$ y $p < 0,02$ respectivamente) al comparar los datos previos y posteriores a las 16 sesiones de entrenamiento de fuerza. Tras el análisis estadístico, se identificaron diferencias significativas al contrastar los valores de ARNm ($p < 0,04$) y proteína ($p < 0,02$) con los valores de IL-10 del grupo CG.

Por otra parte, el contenido de ARNm y proteína de TNFα del grupo RTG (Figura 19) no mostró cambios significativos en la estimación del estado proinflamatorio, ni al compararlos con los valores iniciales del propio grupo, ni con los resultados correspondientes al grupo CG.

Analizando el ratio proteico IL-10/TNFα, se observa un balance hacia un estado antiinflamatorio tras completar el programa de ejercicios de fuerza que el grupo RTG llevó a cabo (1,05 ± 0,14 *vs* 1,48 ± 0,17 unidades arbitrarias; p<0,04).

Efecto de un programa de entrenamiento aeróbico

Los resultados obtenidos tras el análisis de la expresión génica y proteica de TNFα e IL-10 para el grupo ATG (Figura 23) no muestran cambios significativos entre los valores previos y posteriores a la realización del entrenamiento aeróbico. Al contrastar estos datos con los descritos anteriormente en el grupo CG, tampoco se identificaron diferencias significativas. En relación al ratio proteico IL-10/TNFα, tampoco observó un efecto del entrenamiento ni hacia un estado proinflamatorio, ni hacia un estado más antiinflamatorio (1,00 ± 0,11 *vs* 1,18 ± 0,15 unidades arbitrarias).

Efecto de un programa de entrenamiento de vibración

Los resultados del contenido de ARNm y proteico del TNFα para el grupo VTG no mostraron cambios, al contrastar los resultados previos y posteriores al entrenamiento de vibración (Figura 24). También se observaron los mismos resultados al comparar dichos valores con los anteriormente descritos para el grupo CG.

Sin embargo, los valores de ARNm y proteína de IL-10 (Figura 24) ponen de manifiesto como el entrenamiento realizado por este grupo estimuló un aumento significativo (p < 0,04 y p < 0,02 respectivamente), si tenemos en cuenta los valores iniciales del propio grupo. También se observó el mismo efecto, estadísticamente significativo, al contrastar estos valores con los obtenidos para el grupo CG (ARNm IL-10, p < 0,05; proteína IL-10, p < 0,03).

Cabe destacar que el ratio IL-10/TNFα muestra una tendencia significativa hacia los mediadores antiinflamatorios en el grupo que desarrolló en entrenamiento de vibración (VTG) (1,09 ± 0,09 *vs* 1,54 ± 0 ,18 unidades arbitrarias; p < 0,03).

Efecto de un programa de entrenamiento en la expresión de proteínas de estrés térmico en PBMC de ancianos

Existen múltiples ligandos externos e internos que pueden activar e inducir la dimerización de TLR2 y TLR4, entre los que podemos destacar algunas Hsp. Se trata de potentes activadores del sistema inmune innato capaces de estimular o bloquear la síntesis de citoquinas proinflamatorias (Noble y Shen, 2012).

Los datos obtenidos en el grupo CG antes y después de las 8 semanas de vida normal, muestran que para el contenido proteico de Hsp70 y Hsp60 no existieron cambios significativos.

Efecto de un programa de entrenamiento de fuerza

El contenido proteico de Hsp70 y Hsp60 para el grupo RTG (Figura 25) indica un efecto diferenciado del entrenamiento. Mientras que la concentración de Hsp70 aumentó significativamente ($p < 0,03$) tras finalizar el programa de entrenamiento, los valores de Hsp60 manifestaron una reducción significativa ($p < 0,03$) como causa de las 8 semanas de ejercicios realizados. Al contrastar ambos valores con los obtenidos para el grupo CG, también se evidencio un aumento del contenido proteico de Hsp70 ($p < 0,03$) y una disminución del mismo en Hsp60 ($p < 0,03$).

Efecto de un programa de entrenamiento aeróbico

Los resultados de la expresión proteica de Hsp70 y Hsp60 tras completar las 8 semanas de ejercicios aeróbicos que el grupo ATG llevó a cabo (Figura 26) muestran como la actividad física realizada a lo largo de las 16 sesiones no modificó los valores proteicos iniciales ni de Hsp70 ni de Hsp60. Sin embargo hay que destacar que Hsp60 manifestó una tendencia, aunque el estudio estadístico de la misma no resulto significativo, a disminuir su contenido proteico tras el período de entrenamiento.

Efecto de un programa de entrenamiento de vibración

El contenido proteico de Hsp70 y Hsp60 de los participantes del grupo VTG (Figura 27) muestra como los resultados del análisis del mediador Hsp70 revelan un marcado incremento ($p < 0,02$) como efecto del entrenamiento realizado. Sin embargo, el contenido proteico de Hsp60 disminuyó significativamente ($p< 0,03$) cuando VTG completó las 16 sesiones de ejercicios de vibración. Estos resultados muestran el mismo patrón al contrastarlos con los valores anteriormente estudiados para el grupo CG (Hsp70, $p < 0,02$; Hsp60, $p< 0,04$).

DISCUSIÓN

El envejecimiento es un proceso natural caracterizado, entre otros, por una disminución de las funciones normales del sistema inmune innato, que conduce a un estado de inmunosenescencia caracterizado por una inflamación de bajo grado, y que aumenta la probabilidad de desarrollar algunas enfermedades de tipo crónico (Goto, 2008).

Tradicionalmente se ha aceptado que el envejecimiento estaba relacionado con altas concentraciones de marcadores proinflamatorios (Bruunsgaard, 2002; Ershler y Keller, 2000). Sin embargo hoy en día se sabe que tanto individuos jóvenes como mayores pueden presentar contenidos muy similares de estos marcadores, estableciéndose la principal diferencia en el nivel de actividad física desarrollada (Beharka *et al.,* 2001; Flynn *et al.,* 2003; Giannopoulou *et al.,* 2005; Oberbach *et al.,* 2006). Por ello, además de los beneficios a nivel funcional que puede ofrecer la práctica frecuente de ejercicio, se sabe que también puede ser utilizada como una potente herramienta para aminorar los estados inflamatorios asociados a los procesos de senescencia (Simpson *et al.,* 2012). Sin embargo los especialistas aún no han establecido ni la modalidad óptima ni la intensidad que debe ser empleada para obtener beneficios de tipo inmunológico.

Principalmente se han utilizado entrenamientos basados en ejercicios de fuerza o de tipo aeróbico, en los que se ha demostrado ese papel antiinflamatorio (Giannopoulou *et al.,* 2005; Oberbach *et al.,* 2006; Stewart *et al.,* 2005). Además de estos protocolos, y como una nueva alternativa, se está empezado a considerar la práctica de ejercicios de vibración con el objetivo de mejorar las habilidades funcionales de los mayores y como inductor de posibles, si bien aun no suficientemente contrastados, beneficios inmunológicos.

Con esta finalidad, en este estudio se llevó a cabo el diseño de tres protocolos de ejercicio, siguiendo las recomendaciones generales realizadas por las guías de salud para personas mayores. De hecho, estudios previos han evaluado la efectividad

de protocolos similares a éstos en la mejora de las características funcionales de las personas mayores (García-López *et al.,* 2007b; Hakkinen *et al.,* 1998, Machado *et al.,* 2010), pero hasta la actualidad, no existen resultados publicados de como puedan influir estas rutinas de ejercicios, sobre la inflamación crónica de bajo grado asociada al envejecimiento.

Existen considerables evidencias científicas que describen una relación entre la forma física y la disminución de marcadores proinflamatorios (Keller *et al.,* 2004; Starkie *et al.,* 2003). De hecho, se ha establecido una relación entre los beneficios que induce la actividad física y el papel crucial que tienen los TLRs en el control de la respuesta inflamatoria (Gleeson *et al.,* 2006), especialmente TLR2 y TLR4 (Flynn *et al.,* 2003; Gleeson *et al.,* 2006; Ma *et al.,* 2013, Stewart *et al.,* 2005).

En este sentido, diversos estudios han demostrado como la realización de diferentes actividades físicas han sido capaces de reducir la expresión de TLR2 y TLR4 (Flynn *et al.,* 2003; Lancaster *et al.,* 2005), relacionándose esta disminución con mejoras en el estado inflamatorio (McFarlin *et al.,* 2004). Así, en el presente estudio, los resultados que muestra el grupo RTG concuerdan con la bibliografía revisada, demostrando que los ancianos que completaron las 8 semanas de ejercicios de fuerza mostraron contenidos de TLR2 y TLR4 más reducidos que los sujetos pertenecientes al grupo CG.

Este mismo efecto se ha observado también recientemente, tras la realización de un protocolo de entrenamiento aeróbico en ratas de 8 semanas de duración, demostrándose que la expresión de TLR4 disminuyó a causa del ejercicio realizado (Oliveira *et al.,* 2011). Sin embargo, los datos del presente estudio contrastan con estos resultados ya que, tras completar el protocolo de ejercicio aeróbico que los participantes del ATG realizaron, no se observaron modificaciones en las concentraciones ni de TLR2 ni de TLR4. En concordancia con estos datos, una publicación reciente de Reyna y colaboradores ha demostrado como la expresión de estos dos receptores tampoco variaba tras 15 días de entrenamiento aeróbico en pacientes diabéticos (Reyna *et al.,* 2013). Por lo tanto, aunque los participantes

mejoraron su nivel físico, parece ser que la duración del entrenamiento aeróbico o la intensidad aplicada no fue suficiente para inducir cambios en la concentración de estos receptores.

Si tenemos en cuenta el tercer ejercicio analizado en este estudio, el entrenamiento de vibración, observamos como sus participantes manifestaron una disminución significativa tanto de TLR2 como de TLR4 tras completar la intervención. Si bien hay que señalar, que no existen resultados previos en la literatura que analicen el efecto que puede tener un estímulo de vibración sobre la expresión de estos receptores.

Tras la estimulación de los receptores, se induce la activación de diversos mediadores que inducirán la activación de las cascadas del NF-κB y de las MAPK (Hofmann *et al.,* 2012), y con ello, la expresión de citoquinas proinflamatorias (Kawai y Akira, 2006).

Centrándonos en la ruta del NF-κB, la activación de los receptores y su posterior oligomerización inducen el reclutamiento de uno de los adaptadores TIR principales: MyD88 (Jiang *et al.,* 2010). A pesar del papel clave que esta proteína tiene en la estimulación de las citoquinas proinflamatorias (Kawai y Akira; 2006, Lu *et al.,* 2008), a día de hoy aparecen pocos estudios que relacionen este adaptador con la práctica de actividad física (Fernández-Gonzalo *et al.,* 2012; Funk *et al.,* 2011; Ma *et al.,* 2013; Nickel *et al.,* 2011).

Uno de los pocos trabajos llevados a cabo en humanos ha demostrado como un entrenamiento previo, basado en ejercicios de tipo excéntrico realizado en sujetos jóvenes, fue capaz de reducir el contenido proteico de MyD88 tras una sesión de ejercicio excéntrico agudo (Fernández-Gonzalo *et al.,* 2012). De manera similar y mostrando un resultado parecido a dicho estudio, los participantes que realizaron el ejercicio de fuerza en el presente estudio, también han mostrado una disminución del contenido de este adaptador tras completar las 16 sesiones del protocolo diseñado.

De forma análoga, un programa de ejercicio aeróbico realizado en ratas con isquemia cerebral puso de manifiesto una disminución del contenido de MyD88 (Ma *et al.,* 2013). Sin embargo, estos datos contrastan con los resultados obtenidos por Nickel y colaboradores, quienes no observaron cambios en la expresión de MyD88 en atletas obesos que habían realizado un entrenamiento aeróbico de 10 semanas de duración (Nickel *et al.,* 2011). Estos datos se relacionan con los resultados del presente estudio donde tampoco se identificaron diferencias significativas entre los valores de MyD88 de los participantes que completaron la intervención de ejercicios aeróbicos y el grupo de sujetos sedentarios.

Por otra parte y en relación a la disminución de la expresión de TLR2 y TLR4 descrita en el grupo VTG, también se observa una reducción en el contenido proteico de MyD88 tras la finalización del protocolo de entrenamiento. Cabe destacar que los cambios fisiológicos inducidos por las actividades de vibración parecen correr en paralelo a los observados tras la realización de varias semanas de ejercicios de fuerza (Bosco *et al.,* 1999).

El siguiente paso en la activación temprana de NF-κB está mediada por una cascada de señalización formada por TRAF6 y TAK1, entre otros mediadores (Nickel *et al.,* 2011), los cuales fosforilan a su vez diversos componentes, permitiendo así el reclutamiento posterior de p50 en el citoplasma y la translocación al núcleo del p65 (Kawai y Akira, 2006; Konner y Bruning, 2011), y con ello la activación de los sitios κB (McFarlin *et al.,* 2006).

El NF-κB tiene un papel fundamental en la respuesta inflamatoria y por lo tanto, en los beneficios que induce el ejercicio sobre el sistema inmune. De hecho se ha descrito en la bibliografía como un ejercicio agudo de fuerza induce la activación de este factor de trascripción, mientras que la realización de un entrenamiento excéntrico puede atenuarla (Jiménez-Jiménez *et al.,* 2008). Estos datos concuerdan con los obtenidos para el grupo RTG en el cuál se observa una disminución de la activación de p65, lo que apoyaría la teoría del efecto antiinflamatorio que se atribuye al ejercicio.

No obstante, en la literatura existen resultados contradictorios en relación a la activación de NF-κB inducida por el ejercicio aeróbico. Recientemente Ma y sus colaboradores han comprobado la eficacia de un entrenamiento aeróbico como terapia frente a la inflamación, relacionada con la isquemia cerebral, observándose una reducción de la expresión de este factor de trascripción (Ma *et al.,* 2013). Sin embargo otros protocolos de ejercicio aeróbico no han puesto de manifiesto cambios en la expresión de NF-κB (McKenzie y Goldfarb, 2007; Zbinden-Foncea *et al.,* 2012) o inclusive han descrito disminuciones del mismo (Durham *et al.,* 2004). En relación a ello, los datos del presente estudio tampoco describen ningún cambio en la activación de p65 tras las 16 sesiones de ejercicio aeróbico, obteniéndose valores proteicos de esta subunidad del NF-κB similares a los de los participantes del grupo CG.

Hay que destacar también que, a pesar del gran conocimiento que hay en torno a este factor nuclear, no hay estudios previos que relacionen el NF-κB y el ejercicio de vibración. En este trabajo los resultados obtenidos por el grupo VTG muestran como tras completar las 8 semanas de intervención, el contenido proteico de p65 disminuyó significativamente en comparación con los valores iniciales del grupo. Este resultado está en concordancia con lo anteriormente discutido para el grupo, sugiriéndose la aparición de una atenuación de la vía del NF-κB, y por ende, la disminución del desarrollo del proceso inflamatorio asociado a esta vía.

Como se mencionó anteriormente, el adaptador MyD88 controla también la activación de la ruta de las MAPK, involucradas por una parte en la expresión de genes proinflamatorios y por otra en la activación del sistema inmune adaptativo (Akira, 2006). Datos previos existentes en la literatura indican que, en función de la intensidad del ejercicio realizado, la actividad física puede estimular miembros específicos de la cascada MAPK (Hayden y Ghosh, 2012). Además la bibliografía existente describe una respuesta diferencial y altamente específica de las distintas proteínas que componen esta ruta (Coffey *et al.,* 2006; Iemitsu *et al.,* 2006; Thompson *et al.,* 2003).

Los datos obtenidos en este estudio concuerdan con esta última idea, ya que existe una activación diferencial entre p38 y ERK1/2. De hecho, tras la realización del entrenamiento de fuerza diseñado para este experimento, la forma fosforilada de p38 disminuye significativamente en el RTG, mientras que la forma activa de ERK1/2, ERK1/2 fosforilado, aumenta en los sujetos que completaron este entrenamiento de fuerza. Esta respuesta diferencial de la activación de ambos mediadores podría ser consecuencia de la alta especificidad que presentan en función del tipo de contracción (Lee *et al.,* 2002). Estudios previos existentes han descrito incrementos en la expresión de ERK1/2 tras la realización de ejercicios de fuerza debido a la relación que existe entre esta proteína y la aparición de adaptaciones hipertróficas (Taylor *et al.,* 2012).

En cuanto a la respuesta de las diferentes MAPK a ejercicios de tipo aeróbico, la bibliografía describe como ERK1/2 es rápidamente activada tanto en modelos animales (Dufresne *et al.,* 2001; Nader y Esser, 2001), como en humanos (Yu *et al.,* 2001; Yu *et al.,* 2003). Estos resultados concuerdan con el aumento de ERK1/2 fosforilado identificado en el ATG. Sin embargo, tras completar el protocolo de 16 semanas de ejercicio aeróbico no se observaron modificaciones en el contenido proteico de p38 fosforilado. Aunque estudios previos si han descrito incrementos en esta última proteína tras la realización de un ejercicio aeróbico (Boppart *et al.,* 2000; Widegren *et al.,* 1998; Yu *et al.,* 2001), es importante destacar que la mayoría de estos experimentos analizan músculo esquelético donde, además de las isoformas α y β expresadas de forma ubicua, se expresa p38γ de manera casi exclusiva (Li *et al.,* 1996). Además, se ha descrito una regulación dependiente del ejercicio en cada una de las isoformas de p38. De hecho, Boppart y colaboradores han explicado como en corredores de maratones sólo se activa p38γ (Boppart *et al.,* 2000). Por esta razón, consideramos que no es apropiado comparar los resultados de estudios que analizan músculo esquelético con los de otros tejidos.

Si bien el contenido de p38 disminuye significativamente en el grupo VTG, ERK1/2 no muestra diferencias cuando se analiza el grupo que llevo a cabo la actividad física frente al grupo sedentario. Quizá la razón de esta "no activación"

pueda venir explicada por las diferentes modalidades de contracción provocadas (Thompson *et al.,* 2003), ya que el ejercicio vibratorio induce contracciones concéntricas, excéntricas e isométricas de la cadera y del grupo muscular de los extensores de rodilla y los flexores plantares (Simao *et al.,* 2012), lo que podría explicar las discrepancias en activación de estas dos proteínas de la familia MAPK.

Además de la ruta del MyD88 dependiente, los TLRs también pueden activar de forma tardía las cascadas del NF-κB y de MAPK por la vía dependiente de TRIF (Yamamoto *et al.,* 2002); aunque el papel principal de esta ruta es estimular la producción de IFN tipo I a través de los factores nucleares IRF3 e IRF7 (Oshiumi *et al.,* 2003a). Para estimular esta cascada, TRIF interacciona, como ya hemos comentado, con IKKi/IKKε y TBK1 (Sasai *et al.,* 2005).

A pesar del posible grado de redundancia entre la actividad de estas dos quinasas, parece ser que la primera está expresada de forma ubicua mientras que la síntesis de IKKi/IKKε es inducible por citoquinas como TNFα o IL-1 (Clement *et al.,* 2008). Esto es debido a que el gen que codifica para IKKi/IKKε posee una zona de unión para el NF-κB (Wang *et al.,* 2005). También es importante mencionar que, más recientemente, se ha relacionado esta quinasa con la fosforilación y activación de la subunidad p65 (Clement *et al.,* 2008; Geng *et al.,* 2009; Moreno *et al.,* 2010).

Nuevamente, se debe poner de manifiesto la escasa literatura existente que relaciona la ruta dependiente de TRIF con la actividad física. Dentro de esos estudios está el realizado por Yano y colaboradores que describe la eficacia de un ejercicio aeróbico extenuante en la reducción de la producción de IFN α en ratones (Yano *et al.,* 2010). Otro estudio posterior describió como la realización de un entrenamiento excéntrico era capaz de disminuir la ruta dependiente de TRIF tras inducir su activación mediante un ejercicio excéntrico agudo (Fernández-Gonzalo *et al.,* 2012). De acuerdo con esto, en la presente memoria, y tras completar las 16 sesiones de ejercicios de fuerza que el RTG llevó a cabo, se muestra como el protocolo empleado disminuye los valores proteicos de TRIF y de IKKi/IKKε. En relación con estos

resultados, también disminuyeron los valores de los factores de trascripción IRF3 e IRF7 en su forma fosforilada, en los sujetos que completaron el protocolo de fuerza.

Por el contrario, y en concordancia con lo anteriormente descrito para las rutas del NF-κB y de las MAPK, el grupo ATG mostró valores para el adaptador TRIF y para IKKi/IKKε similares a los que presentaban antes del comienzo del protocolo de ejercicio, y parecidos a su vez, a los mostrados por el grupo CG.

Además, en la bibliografía no parece haber investigaciones previas que combinen la ruta independiente de MyD88 con el ejercicio de vibración. Los resultados recogidos en este estudio, muestran un marcado descenso de la cascada dependiente de TRIF, ya que tanto este adaptador como IKKi/IKKε, disminuyeron significativamente en respuesta al ejercicio realizado a lo largo de las 8 semanas de duración. En relación a los dos factores nucleares analizados, IRF3 e IRF7, también se pudo constatar una reducción en la expresión proteica de sus formas fosforiladas.

En resumen, dos ejercicios diferentes inducen una disminución de la expresión de TLR2 y TLR4, y a su vez, de las rutas principales que estos receptores gobiernan, pero los motivos de esa disminución no están aún del todo explicados. Si bien se ha descrito previamente que altas concentraciones de diversas citoquinas pueden modular la expresión de los TLRs creando un bucle entre estos receptores y la síntesis de mediadores inflamatorios (Gleeson *et al.,* 2006; Miettinen *et al.,* 2001; Seibl *et al.,* 2003).

De hecho, y como ya se ha mencionado anteriormente, la actividad física es capaz de disminuir los niveles de algunos mediadores proinflamatorios (Nicklas *et al.,* 2008; Phillips *et al.,* 2010) y a su vez aumentar las proteínas con efecto antiinflamatorio (Balducci *et al.,* 2010). En concreto se ha descrito como ejercicios crónicos aeróbicos o de fuerza están asociados con concentraciones elevadas de IL-6 (Keller *et al.,* 2001; Steensberg *et al.,* 2001) y de IL-10 (Das, 2004; Petersen y Pedersen, 2005), al mismo tiempo que se manifiesta una disminución de los niveles de TNFα (Moldoveanu *et al.,* 2001; Steensberg *et al.,* 2003; Vieira *et al.,* 2007).

Los resultados del presente estudio corroboran estos resultados, ya que el entrenamiento de fuerza llevado a cabo induce un marcado incremento de la IL-10, a pesar de que los valores de TNFα no variaron. Aunque múltiples trabajos han descrito disminuciones en la producción de citoquinas proinflamatorias en sujetos jóvenes (Pedersen *et al.,* 2001; Rhind *et al.,* 2001), otros estudios han descrito como un entrenamiento de fuerza de 10 semanas de duración, llevado a cabo en personas mayores, disminuyó la expresión de TLR4 pero no los niveles de citoquinas inflamatorias (McFarlin *et al.,* 2004). Estas diferencias en la respuesta registrada entre los individuos jóvenes y mayores, podrían explicarse debido a la existencia de una respuesta diferencial al ejercicio en función de la edad (McFarlin *et al.,* 2004),

Hay que destacar además que, la IL-10 actúa como un antagonista natural del TNFα gracias a la capacidad de bloqueo que tiene sobre IKK, evitándose así la activación, y posterior translocación al núcleo del NF-κB (Dhingra *et al.,* 2009). La bibliografía también ha descrito como ERK1/2 está involucrada en la fosforilación de un promotor para IL-10 en macrófagos (Hofmann *et al.,* 2012), conectando con ello la activación previamente descrita en el RTG con el aumento de IL-10 en los participantes.

En relación al ejercicio aeróbico, algunos autores han explicado como un entrenamiento prolongado es capaz de reducir la concentración plasmática de algunos mediadores inflamatorios en pacientes con problemas cardiacos (Adamopoulos *et al.,* 2001; Goldhammer *et al.,* 2005). Sin embargo, existen también resultados contrarios a éstos en la literatura, como el publicado por Ferrier y colaboradores que describen como tras un entrenamiento aeróbico de 8 semanas de duración, realizado en obesos adultos, no se observaron variaciones en los niveles de TNFα (Ferrier *et al.,* 2004). Este último resultado es similar al obtenido en los participantes que completaron las 16 sesiones de entrenamiento aeróbico, en los cuales no se encontraron cambios significativos ni en los niveles de IL-10 ni de TNFα. Teniendo en cuenta la gran variabilidad interpersonal que puede existir en el contenido de los marcadores inflamatorios, hay que señalar que las diferentes formas

de entrenamiento, intensidades o duraciones de los protocolos son razones obvias que podrían explicar las discrepancias existentes en la literatura.

En cuanto al entrenamiento de vibración, los estudios existentes muestran resultados controvertidos. Naghii y sus colaboradores han descrito como un estímulo vibratorio en ratas repetido a lo largo de 8 semanas, puede inducir un efecto antiinflamatorio (Naghii *et al.,* 2011). De manera similar, otro estudio aún más reciente describe como las concentraciones de IL-10 aumentaron significativamente tras completar un entrenamiento de vibración de cuerpo completo, de 5 semanas de duración (Hazell *et al.,* 2013). Los datos obtenidos en el VTG sugieren un efecto positivo similar a los estudios anteriormente mencionados ya que los niveles de IL-10 en las PBMC aumentaron significativamente tras completar el programa de entrenamiento. En realidad, parece ser que este efecto está más relacionado con la duración, la intensidad y la masa muscular involucrada en el ejercicio que con la propia modalidad de la actividad física realizada (Markovitch *et al.,* 2008).

Por otra parte, hay que destacar la importancia que tiene la cuantificación del ratio IL-10/TNFα, considerado un importante indicador asociado al desarrollo de enfermedades (Lira *et al.,* 2009; Petersen y Pedersen, 2005). Los valores de estas citoquinas analizados en el RTG muestran como el entrenamiento de fuerza promovió una modificación de las proporciones entre estos dos marcadores, induciendo un balance positivo hacia señales antiinflamatorias, tal y como se había descrito anteriormente (Lira *et al.,* 2009; Petersen y Pedersen, 2005). De manera similar, las modificaciones obtenidas por el VTG demuestran el efecto positivo provocado por el programa de entrenamiento llevado a cabo en este estudio, pudiéndose así considerar los efectos beneficiosos que la intervención propuesta podría inducir en las personas mayores.

Por otra parte, diversos estudios han relacionado también la reducción de estos mediadores inflamatorios, con las alteraciones que induce el ejercicio sobre algunas Hsp (Noble y Shen, 2012). En concreto sobre Hsp70, un potente activador endógeno del sistema inmune (Vabulas *et al.,* 2002), que actúa como ligando tanto de

TLR2 como de TLR4 (Asea *et al.,* 2002) siendo capaz con ello de inhibir algunas rutas inflamatorias (Jones *et al.,* 2011).

La literatura ha descrito aumentos en los niveles de Hsp70 tras la realización de diferentes ejercicios tanto en animales (Locke *et al.,* 1990; Samelman, 2000), como en humanos (Walsh *et al.,* 2001); aunque como casi siempre existen resultados contradictorios (Liu *et al.,* 2004; Ogawa *et al.,* 2010). Estas divergencias pueden deberse al hecho de que la producción de Hsp70 está directamente ligada a la intensidad y a la frecuencia del ejercicio realizado (Harris y Starnes, 2001; Milne y Noble, 2002). En este sentido, hay que destacar que esta Hsp media directamente en la inhibición de las citoquinas proinflamatorias debido a un efecto represor sobre NF-κB (Schell *et al.,* 2005; Weiss *et al.,* 2007).

Altas concentraciones de otra Hsp, como es la Hsp60, pueden actuar en el sentido opuesto induciendo la síntesis de mediadores proinflamatorios (Hao *et al.,* 2010). Todo ello ha hecho que su expresión esté directamente relacionada con la producción de IL-12 y TNFα y sirva, por lo tanto, como marcador proinflamatorio para el sistema inmune (Flohe *et al.,* 2003). De hecho la literatura atribuye un exceso intracelular de Hsp60 a estados anómalos en la célula, cercanos a situaciones de tipo patológico, como son las enfermedades autoinmunes (Hao *et al.,* 2010).

Teniendo en cuenta lo anteriormente expuesto y los datos obtenidos en el grupo RTG, el aumento de los niveles de Hsp70 y la disminución de los valores intracelulares de Hsp60 demuestran que el ejercicio de fuerza diseñado para este estudio, estimuló un estado antiinflamatorio beneficioso para los participantes.

Por el contrario, los niveles de estas dos Hsp en el grupo ATG no mostraron cambios significativos tras completar el período de entrenamiento al que fueron sometidos, siendo estos similares a los observados en los participantes pertenecientes al CG. Con todos los datos analizados, estos resultados corroboran la teoría de que el entrenamiento aeróbico diseñado y realizado en este experimento no fue el adecuado para aminorar la propia inflamación crónica asociada a los procesos de envejecimiento.

En último término y en cuanto al grupo que completo las 16 sesiones del entrenamiento de vibración, se detectaron altas concentraciones de Hsp70 en las PBMC de los participantes. Además, los valores proteicos de Hsp60 se vieron significativamente reducidos tras la realización del protocolo de actividad física. Con esto, y teniendo en cuenta todos los resultados previamente descritos para este grupo, parece ser que el entrenamiento de vibración de cuerpo completo llevado a cabo en este estudio fue capaz de reducir el estado inflamatorio asociado a los procesos de senescencia.

CONCLUSIONES

Conclusión primera

La realización de un programa de entrenamiento de fuerza, de ocho semanas de duración, provocó una disminución significativa en la expresión basal de los receptores TLR2 y TLR4, induciendo un estatus antiinflamatorio en los ancianos. En este contexto, el entrenamiento de resistencia parece tener efecto tanto sobre la ruta dependiente como independiente de la proteína adaptadora MyD88.

Conclusión segunda

Los efectos antiinflamatorios inducidos por el entrenamiento de fuerza sobre las rutas de señalización de los receptores TLR2 y TLR4, parecen estar asociados con cambios en la expresión de las proteínas de estrés térmico Hsp70 y Hsp60. Dichas modificaciones podrían conferir protección frente a otros daños asociados con el envejecimiento.

Conclusión tercera

Los resultados de la presente memoria sugieren que el protocolo de ejercicio aeróbico empleado representa una herramienta útil para lograr adaptaciones funcionales beneficiosas, sin inducir alteraciones en los biomarcadores inflamatorios en ancianos.

Conclusión cuarta

Considerando las múltiples combinaciones de intensidad y duración existentes para la realización de un entrenamiento aeróbico, es necesario profundizar en el estudio de alternativas con objeto de establecer el protocolo aeróbico más adecuado para reducir la inflamación crónica de bajo grado asociada al envejecimiento y, al mismo tiempo, estudiar sus efectos sobre la ruta de los TLRs.

Conclusión quinta

Este estudio proporciona resultados novedosos sobre los mecanismos moleculares implicados en la respuesta inflamatoria controlada por las rutas de los receptores TLR2 y TLR4, en mujeres y hombres ancianos que completaron un programa de vibración de cuerpo completo. Así, los datos obtenidos ponen de manifiesto que diferentes rutas de señalización, tales como NF-κB, MAPK, TRIF y diversos factores de transcripción de la familia IRF, parecen estar moduladas en respuesta al ejercicio a través de TLR2 y TLR4.

Conclusión sexta

El incremento de los contenidos de Hsp70 y la disminución de Hsp60 en células mononucleares de sangre periférica, tras completar las dieciséis sesiones del entrenamiento de vibración, parecen estar asociados con una mejoría en el estado inflamatorio de la población anciana.

Conclusión general

Los datos recogidos confirman la utilidad de los entrenamientos de fuerza y vibración en la disminución de la inflamación crónica de bajo grado asociada al envejecimiento. De esta manera, ambos tipos de entrenamientos, a diferencia del protocolo aeróbico empleado, podrían ser considerados idóneos para contrarrestar dicha inflamación a través de la desregulación de las cascadas de señalización del TLR2 y TLR4, en ancianos. Todo ello puede tener importantes implicaciones en los programas de prevención y rehabilitación actualmente empleados para combatir las enfermedades inflamatorias y autoinmunes en este sector de la población.

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