Mechanisms of Ageing and Development 150 (2015) 12–19

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

Whole-body vibration improves the anti-inflammatory status in elderly subjects through toll-like receptor 2 and 4 signaling pathways

Paula Rodriguez-Miguelez[a], Rodrigo Fernandez-Gonzalo[b], Pilar S. Collado[c], Mar Almar[c], Susana Martinez-Florez[c], José A. de Paz[c], Javier González-Gallego[c], María J. Cuevas[c,*]

[a] Division of Clinical Translational Science, Georgia Prevention Institute, Department of Pediatrics, Georgia Regents University, USA
[b] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden
[c] Institute of Biomedicine (IBIOMED), University of León, León, Spain

ARTICLE INFO

Article history:
Received 14 April 2015
Received in revised form 21 July 2015
Accepted 2 August 2015
Available online 4 August 2015

Keywords:
Whole body vibration
Elderly
Inflammation
Toll-like receptors
Heat shock proteins

ABSTRACT

Regular physical exercise has anti-inflammatory effects in elderly subjects. Yet, the inflammatory responses after whole body vibration (WBV) training, a popular exercise paradigm for the elderly, remain to be elucidated. This study assessed the effects of WBV training on the inflammatory response associated with toll-like receptors (TLRs) signaling pathways. Twenty-eight subjects were randomized to a training group (TG) or a control group (CG). TG followed an 8-week WBV training program. Blood samples were obtained before and after the training period in both groups. Peripheral blood mononuclear cells were isolated, and mRNA and protein levels of makers involved in the TLR2/TLR4 myeloid differentiation primary response gen 88 (MyD88) and TIR domain-containing adaptor inducing interferon (TRIF)-dependent pathways were analyzed. Plasma TNFα and C-reactive protein levels were also assessed. The WBV program reduced protein expression of TLR2, TLR4, MyD88, p65, TRIF and heat shock protein (HSP) 60, while HSP70 content increased. IL-10 mRNA level and protein concentration were upregulated, and TNFα protein content decreased, after WBV training. Plasma concentration of C-reactive protein and TNFα decreased in the TG. The current data suggest WBV may improve the anti-inflammatory status of elderly subjects through an attenuation of MyD88- and TRIF-dependent TLRs signaling pathways.

1. Introduction

Regular moderate-intensity physical activity is recommended for a healthy lifestyle at all ages, but more so in the elderly. Indeed, exercise has been proposed as an effective tool to combat some negative effects of aging, and to ameliorate the risk of developing numerous chronic diseases (Aoyagi and Shephard, 2010; Barlow et al., 2006), including a potential role to counteract the age-related pro-inflammatory status, known as immunosenescence (Simpson et al., 2012). Exercise can trigger important adaptations in the inflammatory system (Mathur and Pedersen, 2008), which vary depending on the type and/or intensity of the exercise intervention (Walsh et al., 2011). There is general consensus that single, prolonged or vigorous bouts of exercise can impair the immune response, whereas regular moderate-intensity exercise (i.e., training) may have positive effects on the immune system (Simpson and Bosch, 2014). Thus, aerobic or resistance exercise training are effective in reducing the pro-inflammatory profile in the elderly, decreasing levels of pro-inflammatory cytokines (Gano et al., 2011; Phillips et al., 2010; Tiainen et al., 2010).

Whole body vibration (WBV) is an innovative physical activity paradigm that 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). WBV has been proposed as an alternative method to other conventional modalities for enhancing muscle activity, force, and power (Kemmler et al., 2010; Machado et al., 2010; Tapp and Signorile, 2014). However, in spite of some reported changes in immune parameters following vibration exercise (Pawlak et al., 2013; Broadbent et al., 2010), little is known about the possible effects that WBV training could exert in the age-related inflammatory state.

The immune effects of physical activity are often related with the pivotal role that toll-like receptors (TLRs), mainly TLR2 and TLR4, have in controlling the inflammatory response (Gleeson et al., 2006; Ma et al., 2013). TLRs are germline-encoded pattern recognition receptors highly expressed by cells of the innate

* Corresponding author at: Institute of Biomedicine (IBIOMED), Campus Vegazana s/n, University of León, 24071 León, Spain. Fax: +34 987291267.
E-mail address: mj.cuevas@unileon.es (M.J. Cuevas).

http://dx.doi.org/10.1016/j.mad.2015.08.002
0047-6374/

immune system but also found in other cell types. They recognize pathogen-associated molecular patterns, triggering—an inflammatory response through the action of several transcription factors (Akira et al., 2006; Brown et al., 2011) which stimulate the synthesis of inflammatory cytokines, type I interferon (IFN) and chemokines (Kawai and Akira, 2010). In an early activation, TLRs undergo oligomerization followed by the recruitment of the downstream mediator myeloid differentiation primary response gene 88 (MyD88), an essential protein in the production of pro-inflammatory cytokines (Lu et al., 2008). This adaptor prompts the MyD88-dependent pathway with the phosphorylation of the inhibitor κB (IκB), allowing the translocation of the nuclear factor kappa B (NF-κB) from the cytoplasm to the nucleus, where it binds to DNA and promotes gene expression (Konner and Bruning, 2011). TLRs stimulation also involves the activation of another proximal protein adaptor, named TIR (Toll/interleukin-1 receptor)-domain-containing adapter-inducing IFNβ (TRIF) that may trigger a late synthesis of cytokines (Kawai and Akira, 2010). The mechanism(s) by which the TRIF-dependent pathway lead to the activation of those pro-inflammatory mediators is currently unknown, although has been proposed that both N and C terminal portions of TRIF are involved in NF-κB activation (Tseng et al., 2010).

Several investigations have reported the effect of aerobic and resistance exercise training modalities in TLR2 and TLR4 signaling pathways (Gleeson et al., 2006). Many potential factors have been speculated to control the reduction of TLRs activity as a result of exercise (McFarlin et al., 2006). Accumulated evidences indicate that one putative mechanism is mediated by heat shock proteins (HSP), endogenous ligands for TLRs that may induce cross-tolerance or tolerance in the cell, resulting in a receptor down-regulation, in an intracellular signaling reduction or in an anti-inflammatory synthesis promotion (Kilmartin and Reen, 2004). Previous studies from our group have confirmed the anti-inflammatory effects of regular resistance exercise on the TLR2 and TLR4 pathways in peripheral blood mononuclear cells (PBMC) in both young and old subjects (Fernandez-Gonzalo et al., 2012, 2014; Rodriguez-Miguelez et al., 2014). Interestingly, these effects seem to be associated, at least in part, with changes in the expression of HSP70, and possibly HSP60, in the elderly (Rodriguez-Miguelez et al., 2014).

To this background, the aim of the current study was to assess the effect of an 8-week WBV training program on the inflammatory response in PBMC of elderly volunteers, by analyzing the expression of TLR4 and TLR2, MyD88 and TRIF-dependent pathways, and their relationship with the TLR-endogenous ligands HSP60 and HSP70. It was hypothesized that the vibration exercise protocol could promote an anti-inflammatory status in healthy aged individuals through changes in the toll-like receptor 2 and 4 signaling pathways.

2. Material and methods

2.1. Participants

Twenty-eight seniors (eight men, twenty women), were recruited for the study. All subjects completed the study in 10 weeks, including the pre- and post-training baseline data collection (i.e., anthropometric measurements, strength tests and blood samples) and the 8-week training period. Inclusion criteria were no contraindications to exercise, no hormonal or inflammatory medication one month previous to the study, and no experience in WBV. Procedures, risks and discomforts associated with the study were explained, and written informed consent was obtained from all participants after explaining them the procedures, risks and premises associated with the study. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki.

Participants were randomly divided into a control group (CG; $n = 12$; age, 70.0 ± 0.9 yr; height, 158.9 ± 1.9 cm; weight, 68.1 ± 2.5 kg; body mass index, 27.08 ± 0.8 kg/m2) or a training group (TG; $n = 16$; age, 71.04 ± 1.5 yr; height,: 156.2 ± 1.7 cm; weight, 65.7 ± 3.1 kg; body mass index, 26.8 ± 1.1 kg/m2). TG performed an 8-week WBV exercise training program, whereas the CG maintained their normal daily routine.

2.2. Baseline data collection

Baseline data were collected during a laboratory session carried out one week before and one week after the training period. After a standardized 10-min warm up on a cycle ergometer (Tunturi F35, Tunturi®, Turku, Finland), subjects performed a leg-press maximal isometric voluntary contraction (MVIC) test of the leg extensors to register the maximal strength using a 45°-inclined leg-press (Gervasport, Madrid, Spain) at 110° knee flexion. Isometric force was measured with a load cell (Globus Ergometer, Globus, Codogne, Italy). After ~30 min of rest and in the same leg-press device, one repetition maximum (1RM) test was performed.

2.3. Exercise program

Participants from the TG performed an 8-week WBV training program on a vibration platform (Fitvibe, Gymna Uniphy NV, Bilzen, Belgium). Each training session (2/week) consisted of static or dynamic exercises including half-squat between 120° and 130° knee angle, deep squat with 90° knee angle, wide-stance squat and calves with a knee angle between 120° and 130°. Following a standardized cycling warm-up, subjects performed two sets per exercise mode. Training volume (number and duration of repetitions) and vibration frequency were increased weekly. A resting period of 2.5–3 and 5 min was allowed between exercises modes and sets, respectively. This protocol was adapted from (Machado et al., 2010), and is described in detailed in Table 1.

2.4. Venus blood sampling

Using Vacutainer™ system (BD, Franklin Lakes, NJ) with EDTA as anticoagulant, blood samples (30 ml) were obtained in the early morning in the fasted state from the brachiocephalic vein, 5–6 days before and after the training period. Peripheral blood mononuclear cells were isolated from the whole blood by density gradient centrifugation on Ficoll separating solution (Biochrom AG, Berlin, Germany) (Cuevas et al., 2005).

2.5. RNA isolation, reverse transcription, and real-time PCR

Total RNA was isolated from PBMC using a RiboPure™-Blood Kit (Ambion®, Paisley, UK) and then, quantified by spectrophotometry (NanoDrop 1000, Thermo Scientific, Waltham, MA, USA). Using High-Capacity cDNA Archive Kit (Applied Biosystems®, Paisley, UK), 2 μg of total RNA of each sample were reverse transcribed to cDNA and then, amplified using TaqMan® Universal PCR Master Mix (Applied Biosystems®) through StepOnePlus™ Real-Time PCR System (Applied Biosystems®). TaqMan® primers and probes for tumor necrosis factor α (TNFα) (Genbank M10988.1 and Hs00174128_m1), IL-10 (Genbank M57627.1 and Hs00961622_m1) and GAPDH, as endogenous control (Genbank M33197.1 and Hs99999905_m1), were derived from the commercially available TaqMan® Assays-on-Demand Gene (Applied Biosystems®). Relative changes in target genes in relation to the endogenous control

Table 1
Characteristics of the whole-body vibration training program used. Exercises included half-squat (a), deep squat (b), wide-stance squat (c) and calves (d).

Week	Repetitions per set				Exercise duration	Rest between exercises	Rest between sets	Amplitude	Frequency	Modality
	a	b	c	d	s	Minute	Minute	mm	Hz	
1	1	1	1	–	30	3	5	4	20	Static
2	1	1	1	1	30	3	5	4	25	Static
3	2	2	1	1	30	3	5	4	30	Static
4	1	1	2	2	30	3	5	4	30	Dynamic
5	2	2	1	1	45	2.5	5	4	35	Dynamic
6	1	1	2	2	45	2.5	5	4	35	Dynamic
7	2	1	2	2	60	2.5	5	4	35	Dynamic
8	1	2	2	2	60	2.5	5	4	35	Dynamic

were determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.6. Western blot analysis

PBMC were homogenized in 150 μl buffer containing 0.25 mM sucrose, 1 mM EDTA, 10 mM Tris and a protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA) with an ultrasonic processor (UP100H, Hielscher, Teltow, Germany). Samples containing 50 μg of protein were separated by SDS-PAGE on 9% (HSP60, HSP70, TLR2, TLR4 and TRIF), 12% (MyD88, p65 and β-actin) or 15% (TNFα and IL-10) SDS-polyacrylamide gels. Separated proteins were transferred to PVDF membranes and then, non-specific binding was blocked by pre-incubation of the membranes in 2.5% non-fat milk-PBS for 30 min at 37 °C. After that, incubation with specific primary antibodies was performed overnight at 4 °C. Antibodies against HSP70 (70 kDa) and TRIF (66 kDa) were purchased from Abcam® (Cambridge, UK); antibodies against HSP60 (60 kDa), TLR2 (90–100 kDa), TLR4 (95 kDa), MyD88 (33 kDa), p65 (65 kDa), TNFα (17 kDa) and IL-10 (20 kDa) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); β-actin (42 kDa), which served as control protein, was purchased from Sigma–Aldrich (St Louis, MO, USA). Bound primary antibody was detected using a peroxidase-conjugated secondary antibody (Dako, Glostrup, Denmark) and an enhanced chemiluminiscence-HRP kit (Luminol Reagent Santa Cruz Biotechnology). The density of the specific bands was quantified with an imaging densitometer (ImageJ, Bethesda, MD, USA).

2.7. Serum markers of inflammation

Serum high sensitive C-reactive protein (hsCRP) was measured by a particle-enhanced immunoturbidimetric assay on a Hitachi 917 analyzer (Roche Diagnostics, Mannheim, Germany). Serum TNFα was measured using enzyme-linked immunoabsorbent assay (Quantikine High Sensitivity kit: R&D Systems, Minneapolis, MN).

2.8. Statistical analysis

Values are presented as mean ± standard error of means (SEM). Post-training values were normalized to pre-training values. Saphiro–Wilk test was used to verify normal data distribution; when data were skewed, log transformation was used. All data were analyzed using a two-way analysis of variance (ANOVA) with repeated measures for group (GG and TG) and time (pre and post). Bonferroni analysis was used to compensate for multiple post hoc comparisons. Pearson's correlation analyses were used to assess the relationship between changes in HSP70 and HSP60 expression and those in the expression of TLR2 and TLR4. Differences were considered significant when $p < 0.05$. All statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL, USA).

3. Results

At baseline, there were no differences between TG and CG in age, height, body weight, and body mass index. All participants from TG showed a 100% compliance with the training protocol. While MVIC (106.6 ± 15.8 *vs.* 142.4 ± 16.7 kg; $p < 0.05$) and 1RM (158.7 ± 12.4 *vs.* 193.9 ± 18.35 kg; $p < 0.05$) increased in TG from pre to post, CG showed no changes (MVIC; 97.8 ± 12.7 *vs.* 99.75 ± 16.6 kg, 1RM; 160.0 ± 12.9 *vs.* 171.63 ± 18.1 kg).

Although TNFα mRNA expression (Fig. 1A) did not change significantly between or within groups, TNFα protein content (Fig. 1C) decreased ($p < 0.05$) after the 8-week training period in TG. Thus, at post TNFα protein content was lower in TG than CG ($p < 0.05$). Both IL-10 mRNA content and protein concentration increased (Fig. 1B and D) in TG after the WBV program ($p < 0.02$ and $p < 0.01$, respectively). IL-10 mRNA expression and protein content were greater in TG than CG at post ($p < 0.03$ and $p < 0.02$, respectively). Additionally, the IL-10/TNFα ratio increased after training in TG (1.11 ± 0.09 *vs.* 1.44 ± 0.18 arbitrary units; $p < 0.04$) whereas it did not change in CG (1.09 ± 0.10 *vs.* 0.98 ± 0.13 arbitrary units).

Plasma levels of hsCRP (1.29 ± 0.23 *vs.* 0.69 ± 0.12 mg/L; $p < 0.03$) and TNFα (3.14 ± 0.09 *vs.* 2.68 ± 0.08 mg/L; $p < 0.05$) decreased in TG from pre to post. No significant differences were observed in CG for these markers (hsCRP, 0.95 ± 0.13 *vs.* 0.90 ± 0.15 mg/L, TNFα, 3.02 ± 0.11 *vs.* 3.19 ± 0.21 mg/L).

The protein content of HSP70 (Fig. 2A) increased significantly ($p < 0.02$) in TG after training, while conversely, HSP60 (Fig. 2B) protein content was significantly ($p < 0.03$) down-regulated. HSP70 and HSP60 values did not change from pre to post in CG.

TLR2 (Fig. 3A) and TLR4 (Fig. 3B) protein expression decreased significantly ($p < 0.03$ and $p < 0.04$, respectively) after the intervention in the TG. TLR2 and TLR4 post values were lower in TG compared with CG ($p < 0.02$ and $p < 0.04$, respectively).

As for TLR2 and TLR4, MyD88 (Fig. 4A) and TRIF (Fig. 4B) protein concentration showed a significant ($p < 0.04$ and $p < 0.03$, respectively) down-regulation from pre to post in TG. The WBV protocol also triggered a significant ($p < 0.05$) reduction of p65 protein expression (Fig. 4C) in TG. Thus, MyD88, TRIF and p65 values at post were lower in TG compared with CG ($p < 0.04$, $p < 0.05$ and $p < 0.05$, respectively).

Significant correlations were identified between protein concentrations of HSP60 and both TLRs (TLR2, $r = 0.665$, $p < 0.02$; TLR4, $r = 0.891$; $p < 0.001$), and between those of HSP70 and TLR4 ($r = -0.789$; $p < 0.04$). In addition, a significant negative relationship existed between HSP70 and HSP60 ($r = -0.783$, $p < 0.04$).

4. Discussion

The current study assessed the efficacy of 8 weeks of WBV training to induce anti-inflammatory adaptations in the elderly. A major finding of this investigation was the increased mRNA and protein levels in PBMC of the anti-inflammatory cytokine IL-10, whereas the plasma concentration of pro-inflammatory markers,

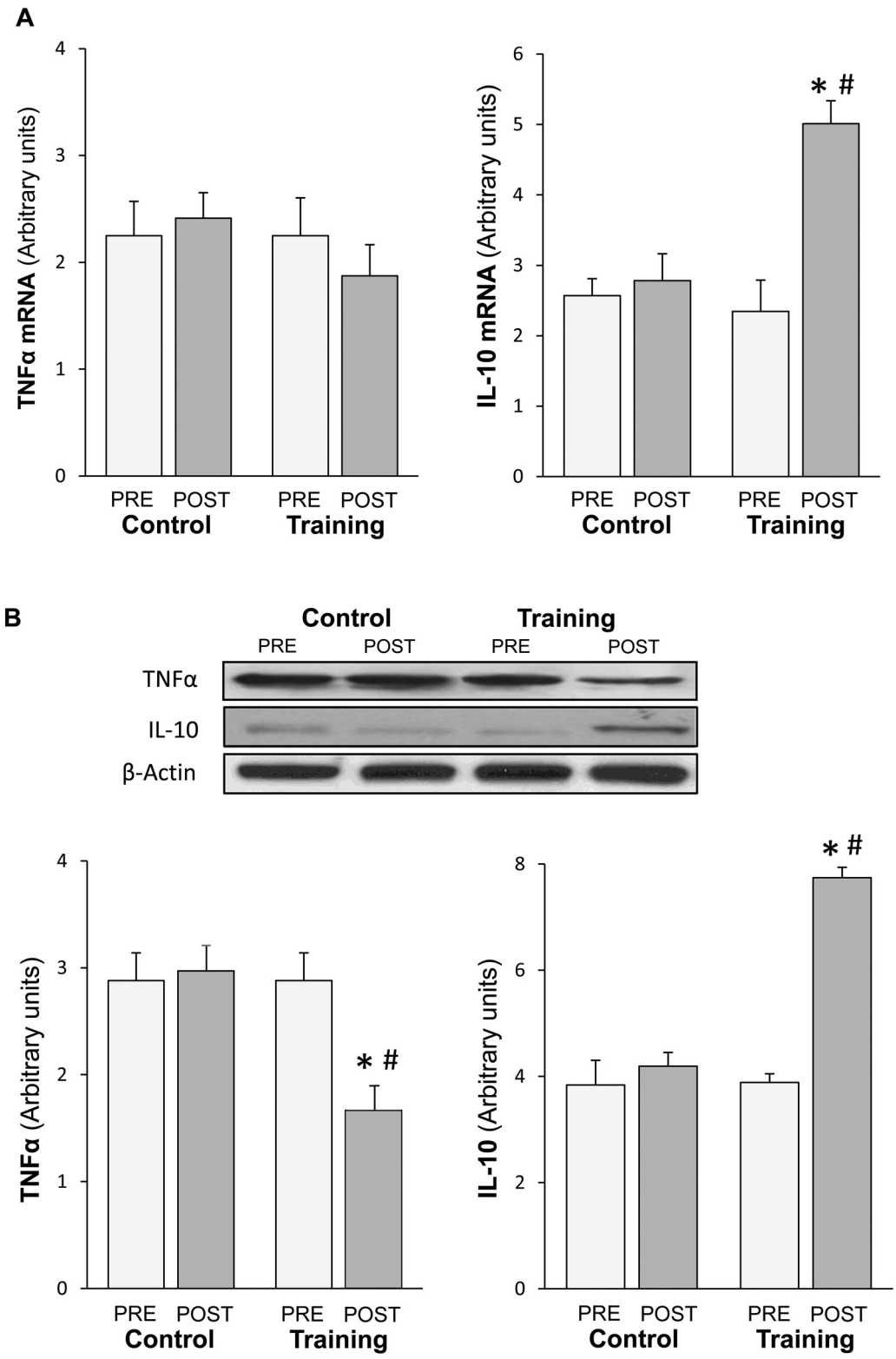

Fig. 1. mRNA levels of TNFα (1A) and IL-10 (1B) and protein content of TNFα (1C) and IL-10 (1D) in PBMC after 8 weeks of WBV for TG and the same period of normal daily routines for CG. Values are mean ± SEM in percentage of change compared Pre value in the same group. $^{*}p < 0.05$ *vs.* CG; $^{\#}p < 0.05$ *vs.* Pre within a group.

CRP and TNFα, decreased. Further, TLR2 and TLR4 proteins were significantly reduced by the current exercise paradigm. A potential explanation for the beneficial adaptations triggered by the WBV in the inflammatory status may include the endogenous ligand HSP70, since its protein content increased after the intervention in TG. To our knowledge, this is the first investigation reporting positive adaptations on TLR2 and TLR4 signaling pathways in response to WBV training in humans. These results resemble previous data employing other exercise training modalities, i.e., resistance exercise (Rodriguez-Miguelez et al., 2014). However, special attention should be paid when comparing inflammatory adaptations after different exercise regimes, since the load, intensity and exercise

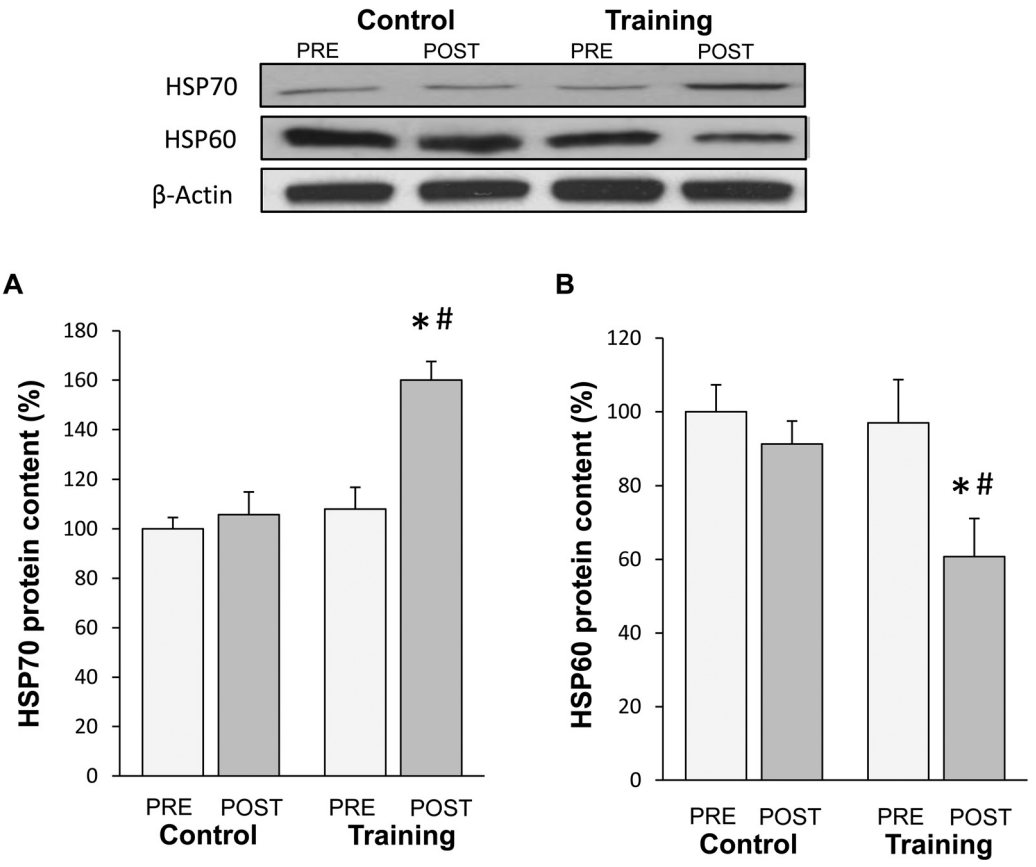

Fig. 2. HSP70 (2A) and HSP60 (2B) protein content in PBMC after 8 weeks of WBV for TG and the same period of normal daily routines for CG. Values are mean ± SEM in percentage of change compared with Pre value in the same group. $^{*}p < 0.05$ *vs.* CG; $^{\#}p < 0.05$ *vs.* Pre within a group.

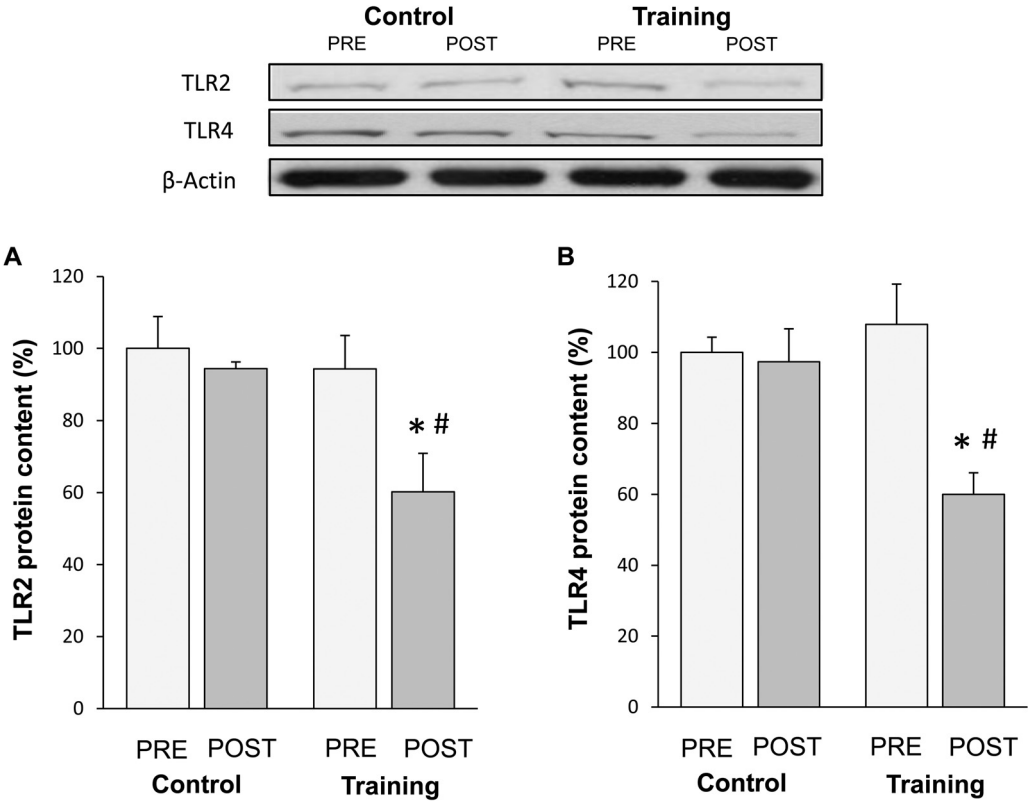

Fig. 3. TLR2 (3A) and TLR4 (3B) protein content in PBMC after 8 weeks of WBV for TG and the same period of normal daily routines for CG. Values are mean ± SEM in percentage of change compared with Pre value in the same group. $^{*}p < 0.05$ *vs.* CG; $^{\#}p < 0.05$ *vs.* Pre within a group.

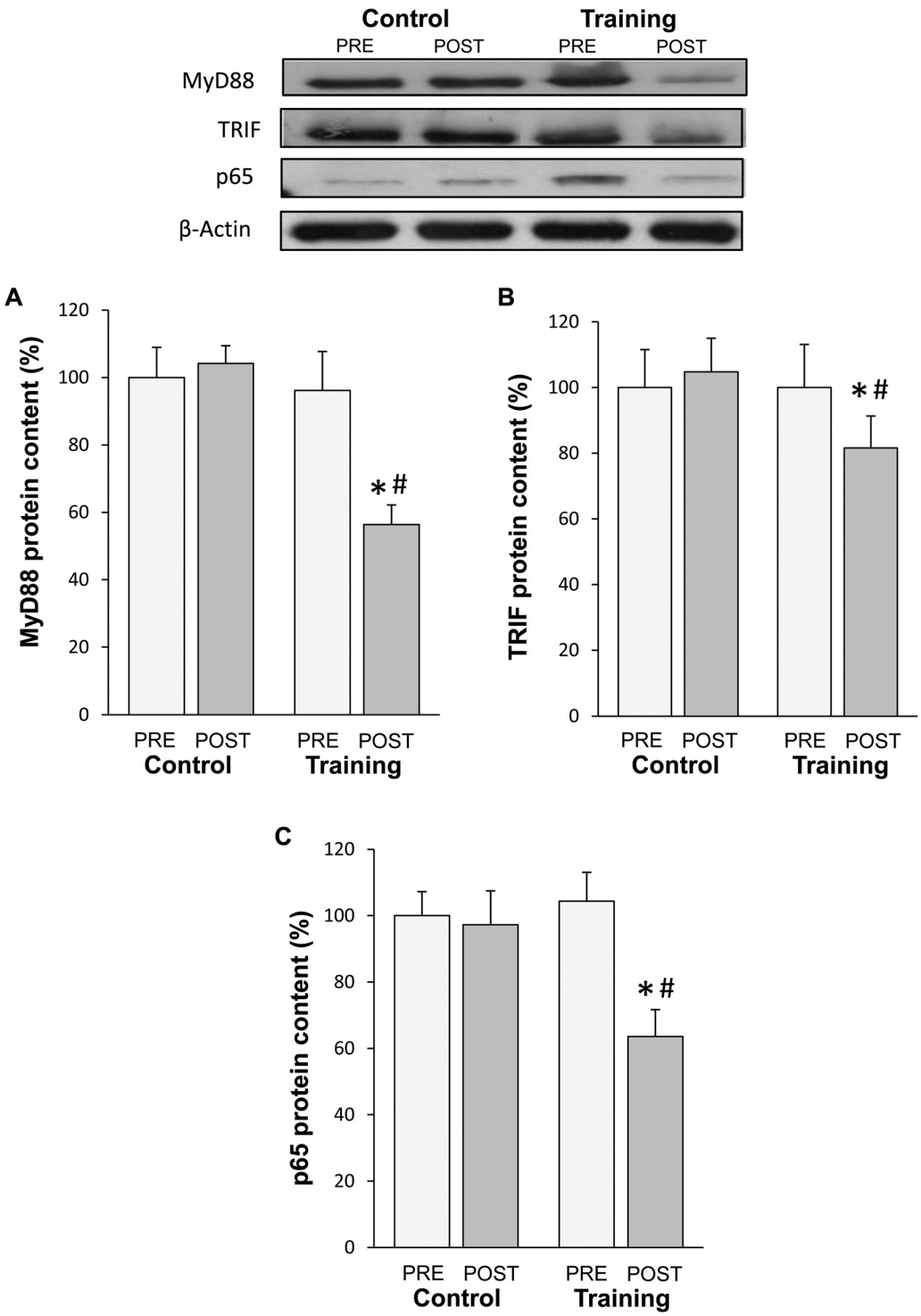

Fig. 4. MyD88 (4A), TRIF (4B) and p65 (4C) protein content in PBMC after 8 weeks of WBV for TG and the same period of normal daily routines for CG. Values are mean ± SEM in percentage of change compared with Pre value in the same group. $^{*}p < 0.05$ *vs.* CG; $^{\#}p < 0.05$ *vs.* Pre within a group.

duration between different types of exercise training may greatly differ.

Exercise can trigger important adaptations in the inflammatory response (Mathur and Pedersen, 2008), with reductions in the circulating levels of pro-inflammatory cytokines (Jiménez-Jiménezet al., 2008; Nicklas et al., 2008; Phillips et al., 2010; Rodriguez-Miguelez et al., 2014) and in the over-expression of mRNA and protein content of IL-10 (Jiménez-Jiménez et al., 2008; Petersen and Pedersen, 2005; Rodriguez-Miguelez et al., 2014). Literature has mainly focused on the analysis of those effects after either resistance or aerobic exercise, with the scarce studies on WBV suggesting a tendency to increase or even a significant elevation in the production of IL-10 (Cristi et al., 2014; Hazell et al., 2014). Our results confirm such notion, since the expression of IL-10 in PBMC significantly increased in the TG after the current 8-week WBV protocol. The anti-inflammatory role of IL-10 has been related with its involvement in the inhibition of several inflammatory cytokines by blocking the translocation and activation of NF-κB (Dhingra

et al., 2009). Indeed, TNFα protein content, a pro-inflammatory cytokine controlled by NF-κB, was reduced by the WBV intervention. Supporting these anti-inflammatory effects, hsCRP and TNFα plasma levels were also reduced in the TG. In addition, literature has reported the use of the IL-10/TNFα ratio as a marker of health status associated with the probable course and outcome of a disease (Lira et al., 2009; Petersen and Pedersen, 2005). The current exercise intervention modified the IL-10/TNFα ratio towards a positive balance to anti-inflammatory signals, as previously suggested by other studies employing different exercise models (Lira et al., 2009; Petersen and Pedersen, 2005; Rodriguez-Miguelez et al., 2014). Although the WBV training employed in the current study induced an anti-inflammatory status in elderly subjects, considering the numerous combinations of amplitudes and frequencies available, and the potential risks of WBV previously reported (McCann et al., 2015; Robbins et al., 2012), the possibility that other training protocols could be potentially harmful should not be excluded.

The exercise adaptations in HSPs levels (Huey et al., 2010; Rodriguez-Miguelez et al., 2014) have been suggested to play a significant role in controlling the reduction of pro-inflammatory mediators (Noble and Shen, 2012). Among all the HSP family, HSP70 is a potent endogenous activator of the innate immune system and a TLR ligand, able to counteract inflammation (Jones et al., 2011) by exerting an inhibitory effect through the NF-κB (Schell et al., 2005). In fact, in the present study we have shown that there is a negative correlation between of HSP70 and TLR4. Since exercise induces increments in HSP70 expression in an intensity- and frequency-dependent manner (Milne and Noble 2002), our data indicate that WBV, as conducted here, is an intense enough stimulus to trigger positive HSP70 adaptations in PBMC of elderly individuals. This accretion in the expression of HSP70 could be directly implicated in the down-regulation of the inflammatory response owing to its action in the synthesis of IL-10. Contrary to HSP70, high circulating levels of HSP60 stimulate some TLRs resulting in the initiation of a pro-inflammatory response within the immune system. Indeed, intracellular HSP60 up-regulation has often been related with abnormal cell state during pathologic situations (Hao et al., 2010). Exercise seems to effectively reduce the expression of this HSP, counteracting its pro-inflammatory role (Marini et al., 2007; Rodriguez-Miguelez et al., 2014). Current data support a similar effect on the expression of HSP60 after the WBV training protocol, emphasizing the contribution that this exercise training protocol to the anti-inflammatory effects of physical activity.

Lower TLR2 and TLR4 cell surface expression is frequently associated with the anti-inflammatory situation induced by a physically active lifestyle (Ma et al., 2013). The current novel finding of decreased protein content of TLR2 and TLR4 in PBMC after WBV training in elderly subjects concords with previous results using different exercise paradigms, such as resistance (Fernandez-Gonzalo et al., 2012, 2014; Rodriguez-Miguelez et al., 2014) or endurance exercise (Oliveira and Gleeson, 2010; Simpson et al., 2009) in young and senior subjects. The fact that TLR2 and TLR4 activation is influenced by different exercise modalities seems to indicate that such activation is more related to the overall intensity of the stimulus, rather than to the exercise modality *per se* (Booth et al., 2010).

In an early activation, TLRs lead the activation of NF-κB via the adaptor MyD88. Following the current down-regulated pattern described for TLR2 and TLR4, MyD88 protein expression was significantly ameliorated by the WBV training protocol. These results concord with other studies showing reduced MyD88 protein content in PBMC after 6 weeks of eccentric resistance training in young men and women (Fernandez-Gonzalo et al., 2012, 2014), and 8 weeks of conventional resistance exercise in elderly subjects (Rodriguez-Miguelez et al., 2014). MyD88 recruitment provides a cluster of TIR domains used as a stable platform for its oligomerization with other proteins, triggering sequential rapid phosphorylations of different mediators that, finally, induce translocation of NF-κB to the nucleus. This nuclear factor may comprise five different subunits, but the heterodimer p50–p65 is the most abundant (Kawai and Akira, 2007). As for the upstream components, the current WBV training attenuated p65 protein concentration in PBMC of elderly subjects, indicating a down-regulation of NF-κB pathway due to the exercise intervention. This finding is consistent with the literature, since multiple exercise modalities are a well-recognize stimulus for the physiological modification of the NF-κB transcription factor in different populations (Garcia-Lopez et al., 2007; Fernandez-Gonzalo et al., 2012, 2014; Jiménez-Jiménez et al., 2008; Rodriguez-Miguelez et al., 2014).

After activation of the MyD88-dependent pathway, some TLRs are able to recruit the intracellular adaptor protein TRIF (Lu et al., 2008). The TRIF-dependent pathway initiates a downstream signaling event which results in the activation of the IFN-regulatory factor and in a delayed stimulation of the NF-κB pathway (Takeuchi and Akira, 2010). Hence, TRIF may play a central role in the synthesis of IFN-β and ithas also been proposed to lead to the expression of several cytokines (Moynagh, 2005). Data relating exercise and TLRs' TRIF-dependent pathway in humans are very limited. The few available reports indicate resistance exercise is able to reduce TRIF expression. In particular, eccentric resistance exercise training was able to attenuate the expression of the key markers of the TRIF- dependent pathway in young men and women (Fernandez-Gonzalo et al., 2012, 2014) after an acute bout of eccentric exercise, and 8 weeks of conventional resistance exercise also decreased TRIF concentration in elderly subjects (Rodriguez-Miguelez et al., 2014). Similarly, WBV seems to induce an attenuation of the TRIF-dependent pathway, since TRIF protein concentration was reduced after the current 16-session WBV protocol. Taken together, these data indicate the TLRs-related anti-inflammatory effects of vibration exercise benefit from a down-regulation of both the MyD88- and the TRIF-dependent pathways.

A limitation in our study comes from the fact that flow cytometry was not performed and we do not know the composition of PBMC subsets. It has been previously shown that endurance and resistance exercise training reduce the proportion of inflammatory (CD14+CD16+) monocytes (Markofski et al., 2014; Timmerman et al., 2008). The decrease in plasma TNF and CRP with training would indicate that our results are, at least partially, independent of cell shifts. Nevertheless, the possibility that different cell subsets among PBMCs could be contributing to the observed WBV effects cannot be ruled out.

5. Conclusions

This study provides novel understanding of the molecular mechanisms behind the inflammatory response controlled by the TLR2 and TLR4 signaling pathways after WBV training in old women and men. The current investigation supports the efficacy of WBV training to counteract, at least in part, the age-related low-grade chronic inflammation. This response seems to be mediated by a down-regulation of the TLR2 and TLR4 MyD88- and TRIF-dependent signaling pathways. Although further research is necessary to provide a strong basis for the immunomodulatory effects of WBV, the current results could have implications in the prevention and rehabilitation programs currently employed for autoimmune and inflammatory diseases in elderly population.

Funding

This study was supported by *Plan Nacional I+D+i* DEP2013-47659-R, Spain.

References

Akira, S., Uematsu, S., Takeuchi, O., 2006. Pathogen recognition and innate immunity. Cell 124, 783–801.

Aoyagi, Y., Shephard, R.J., 2010. Habitual physical activity and health in the elderly: the Nakanojo Study. Geriatr Gerontol. Int. 10, S236–S243.

Barlow, C.E., LaMonte, M.J., Fitzgerald, S.J., Kampert, J.B., Perrin, J.L., Blair, S.N., 2006. Cardiorespiratory fitness is an independent predictor of hypertension incidence among initially normotensive healthy women. Am. J. Epidemiol. 163, 142–150.

Booth, S., Florida-James, G.D., McFarlin, B.K., Spielmann, G., O'Connor, D.P., Simpson, R.J., 2010. The impact of acute strenuous exercise on TLR2, TLR4 and HLA. DR expression on human blood monocytes induced by autologous serum. Eur. J. Appl. Physiol. 110, 1259–1268.

Broadbent, S., Rousseau, J.J., Thorp, R.M., Choate, S.L., Jackson, F.S., Rowlands, D.S., 2010. Vibration therapy reduces plasma IL6 and muscle soreness after downhill running. Br. J. Sports Med. 44, 888–894.

Brown, J., Wang, H., Hajishengallis, G.N., Martin, M., 2011. TLR-signaling networks: an integration of adaptor molecules, kinases, and cross-talk. J. Dent. Res. 90, 417–427.

Cristi, C., Collado, P.S., Márquez, S., Garatachea, N., Cuevas, M.J., 2014. Whole-body vibration training increases physical fitness measures without alteration of inflammatory markers in older adults. Eur. J. Sport Sci. 14, 611–619.

Cuevas, M.J., Almar, M., García-Glez, J.C., García-López, D., De Paz, J.A., Alvear-Ordenes, I., et al., 2005. Changes in oxidative stress markers and NF-kappaB activation induced by sprint exercise. Free Radic. Res. 39, 431–439.

Dhingra, S., Sharma, A.K., Arora, R.C., Slezak, J., Singal, P.K., 2009. IL-10 attenuates TNF-alpha-induced NF-kappaB pathway activation and cardiomyocyte apoptosis. Cardiovasc. Res. 82, 59–66.

Fernandez-Gonzalo, R., De Paz, J.A., Rodriguez-Miguelez, P., Cuevas, M.J., González-Gallego, J., 2012. Effects of eccentric exercise on toll-like receptor 4 signaling pathway in peripheral blood mononuclear cells. J. Appl. Physiol. 112, 2011–2018.

Fernandez-Gonzalo, R., De Paz, J.A., Rodriguez-Miguelez, P., Cuevas, M.J., González-Gallego, J., 2014. TLR 4-mediated blunting of inflammatory responses to eccentric exercise in young women. Mediators Inflamm. 2014, 479395, http://dx.doi.org/10.1155/2014/479395

Gano, L.B., Donato, A.J., Pierce, G.L., Hamza, M., Magerko, K.A., Roeca, C., et al., 2011. Increased pro-inflammatory and oxidant gene expression in circulating mononuclear cells in older adults: amelioration by habitual exercise. Physiol. Genomics 43, 895–902.

Garcia-Lopez, D., Cuevas, M.J., Almar, M., Lima, E., De Paz, J.A., González-Gallego, J., 2007. Effects of eccentric exercise on NF-kappaB activation in blood mononuclear cells. Med. Sci. Sports Exerc. 39, 653–664.

Gleeson, M., McFarlin, B., Flynn, M., 2006. Exercise and toll-like receptors. Exerc. Immunol. Rev. 12, 34–53.

Hao, H.N., Zheng, B., Nasser, S., Ren, W., Latteier, M., Wooley, P., et al., 2010. The roles of monocytic heat shock protein 60 and Toll-like receptors in the regional inflammation response to wear debris particles. J. Biomed. Mater. Res. A 92, 1373–1381.

Hazell, T.J., Kenno, K.A., Jakobi, J.M., 2010. Evaluation of muscle activity for loaded and unloaded dynamic squats during vertical whole-body vibration. J. Strength. Cond. Res. 24, 1860–1865.

Hazell, T.J., Olver, T.D., Hamilton, C.D., Lemon, P.W., 2014. Addition of synchronous whole-body vibration to body mass resistive exercise causes little or no effects on muscle damage and inflammation. J. Strength Cond. Res. 228, 53–60.

Huey, K., Vieira, V., Woods, J., 2010. Heat shock proteins, exercise, and aging. In: Asea, A.A.A., Pedersen, B.K. (Eds.), Heat Shock Proteins and Whole Body Physiology. Springer-Verlag, Berlin, pp. 387–400.

Jiménez-Jiménez, R., Cuevas, M.J., Almar, M., Lima, E., García-López, D., De Paz, J.A., et al., 2008. Eccentric training impairs NF-kappaB activation and over-expression of inflammation-related genes induced by acute eccentric exercise in the elderly. Mech. Ageing Dev. 129, 313–321.

Jones, Q., Voegeli, T.S., Li, G., Chen, Y., Currie, R.W., 2011. Heat shock proteins protect against ischemia and inflammation through multiple mechanisms. Inflamm. Allergy Drug Targets 10, 247–259.

Kawai, T., Akira, S., 2007. Signaling to NF-kappaB by toll-like receptors. Trends Mol. Med. 13, 460–469.

Kawai, T., Akira, S., 2010. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat. Immunol. 11, 373–384.

Kemmler, W., Stengel, S., Mayer, S., Niedermayer, M., Hentschke, C., Kalender, W.A., 2010. Effect of whole body vibration on the neuromuscular performance of females 65 years and older. One-year results of the controlled randomized ELVIS study. Z. Gerontol. Geriatr. 43, 125–132.

Kilmartin, B., Reen, D.J., 2004. HSP60 induces self-tolerance to repeated HSP60 stimulation and cross-tolerance to other pro-inflammatory stimuli. Eur J. Immunol. 34, 2041–2051.

Konner, A.C., Bruning, J.C., 2011. Toll-like receptors: linking inflammation to metabolism. Trends Endocrinol. Metab. 22, 16–23.

Lira, F.S., Rosa, J.C., Zanchi, N.E., Yamashita, A.S., Lopes, R.D., Lopes, A.C., et al., 2009. Regulation of inflammation in the adipose tissue in cancer cachexia: effect of exercise. Cell Biochem. Funct. 27, 71–75.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.

Lu, Y.C., Yeh, W.C., Ohashi, P.S., 2008. LPS/TLR4 signal transduction pathway. Cytokine 42, 145–151.

Ma, Y., He, M., Qiang, L., 2013. Exercise therapy down regulates the overexpression of TLR4, TLR2, MyD88 and NF-kappaB after cerebral ischemia in rats. Int. J. Mol. Sci. 14, 3718–3733.

Machado, A., Garcia-Lopez, D., González-Gallego, J., Garatachea, N., 2010. Whole-body vibration training increases muscle strength and mass in older women: a randomized-controlled trial. Scand. J. Med. Sci. Sports 20, 200–207.

Marini, M., Lapalombella, R., Margonato, V., Ronchi, R., Samaja, M., Scapin, C., et al., 2007. Mild exercise training cardioprotection and stress genes profile. Eur. J. Appl. Physiol. 99, 503–510.

Markofski, M.M., Flynn, M.G., Carrillo, A.E., Armstrong, C.L., Campbell, W.W., Sedlock, D.A., 2014. Resistance exercise training-induced decrease in circulating inflammatory CD14+CD16+ monocyte percentage without weight loss in older adults. Eur. J. Appl. Physiol. 114, 1737–1748.

Mathur, N., Pedersen, B.K., 2008. Exercise as a mean to control low-grade systemic inflammation. Mediators Inflamm. 2008, 109502.

McCann, M.R., Patel, P., Pest, M.A., Ratneswaran, A., Lalli, G., Beaucage, K.L., et al., 2015. Repeated exposure to high-frequency low-amplitude vibration induces degeneration of murine intervertebral discs and knee joints. Arthritis Rheumatol. 67, 2164–2175.

McFarlin, B.K., Flynn, M.G., Campbell, W.W., Craig, B.A., Robinson, J.P., Stewart, L.K., et al., 2006. Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. J. Gerontol. A Biol. Sci. Med. Sci. 61, 388–393.

Milne, K.J., Noble, E.G., 2002. Exercise-induced elevation of HSP70 is intensity dependent. J. Appl. Physiol. 93, 561–568.

Moynagh, P.N., 2005. TLR signalling and activation of IRFs: revisiting old friends from the NFkappaB pathway. Trends Immunol. 26, 469–476.

Nicklas, B.J., Hsu, F.C., Brinkley, T.J., Church, T., Goodpaster, B.H., Kritchevsky, S.B., et al., 2008. Exercise training and plasma C-reactive protein and interleukin-6 in elderly people. J. Am. Geriatr. Soc. 56, 2045–2052.

Noble, E.G., Shen, G.X., 2012. Impact of exercise and metabolic disorders on heat shock proteins and vascular inflammation. Autoimmune Dis. 2012, 836519.

Oliveira, M., Gleeson, M., 2010. The influence of prolonged cycling on monocyte toll-like receptor 2 and 4 expression in healthy men. Eur. J. Appl. Physiol. 109, 251–257.

Pawlak, M., Kaczmarek, D., Nowak, A., Krutki, P., 2013. Low-volume whole-body vibration lasting 3 or 6 months does not affect biomarkers in blood serum of rats. Acta Physiol. Hung. 100, 48–53.

Petersen, A.M., Pedersen, B.K., 2005. The anti-inflammatory effect of exercise. J. Appl. Physiol. 98, 1154–1162.

Phillips, M.D., Flynn, M.G., McFarlin, B.K., Stewart, L.K., Timmerman, K.L., 2010. Resistance training at eight-repetition maximum reduces the inflammatory milieu in elderly women. Med. Sci. Sports Exerc. 42, 314–325.

Robbins, D., Zeinstra, E., Jimenez, A., Goss-Sampson, M., 2012. Does whole body vibration have clinically significant neuropsychological and neurovascular implications? Int. J. Prevent Treat. 1, 18–26.

Rodriguez-Miguelez, P., Fernandez-Gonzalo, R., Almar, M., Mejías, Y., Rivas, A., de Paz, J.A., et al., 2014. Role of Toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects. Age (Dordr.) 36, 9734, http://dx.doi.org/10.1007/s11357-014-9734-0

Schell, M.T., Spitzer, A.L., Johnson, J.A., Lee, D., Harris, H.W., 2005. Heat shock inhibits NF-kB activation in a dose- and time-dependent manner. J. Surg. Res. 129, 90–93.

Simpson, R.J., Bosch, J.A., 2014. Special issue on exercise immunology: current perspectives on aging, health and extreme performance. Brain Behav. Immun. 39, 1–7.

Simpson, R.J., Lowder, T.W., Spielmann, G., Bigley, A.B., LaVoy, E.C., Kunz, H., 2012. Exercise and the aging immune system. Ageing Res. Rev. 11, 404–420.

Simpson, R.J., McFarlin, B.K., McSporran, C., Spielmann, G., Hartaigh, B., Guy, K., 2009. Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. Brain Behav. Immun. 23, 232–239.

Takeuchi, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140, 805–820.

Tapp, L.R., Signorile, J.F., 2014. Efficacy of WBV as a modality for inducing changes in body composition, aerobic fitness, and muscular strength: a pilot study. Clin. Interv. Aging 9, 63–72.

Tiainen, K., Hurme, M., Hervonen, A., Luukkaala, T., Jylhä, M., 2010. Inflammatory markers and physical performance among nonagenarians. J. Gerontol. A Biol. Sci. Med. Sci. 65, 658–663.

Timmerman, K.L., Flynn, M.G., Coen, P.M., Markofski, M.M., Pence, B.D., 2008. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J. Leukoc. Biol. 84, 1271–1278.

Tseng, P.H., Matsuzawa, A., Zhang, W., Mino, T., Vignali, D.A., Karin, M., 2010. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. Nat. Immunol. 11, 70–75.

Walsh, N.P., Gleeson, M., Shephard, R.J., Gleeson, M., Woods, J.A., Bishop, N.C., et al., 2011. Position statement. Part one: immune function and exercise. Exerc. Immunol. Rev. 17, 6–63.

Wilcock, I.M., Whatman, C., Harris, N., Keogh, J.W., 2009. Vibration training: could it enhance the strength, power, or speed of athletes? J. Strength Cond. Res. 23, 593–603.