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Review

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

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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

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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