Healthy Male Subjects

Inflammatory Biomarkers to Short-Lasting Exercise Training in Systemic Response of Antioxidants, Heat Shock Proteins, and Research Article

Volume 2021, Article ID 1938492, 15 pages
Oxidative Medicine and Cellular Longevity
https://doi.org/10.1155/2021/1938492
Hindawi

Academic Editor:
Received 27 May 2021; Accepted 29 October 2021; Published 22 November 2021
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Regular physical activity can enhance immune function and eff
ectively prevents the spread of the cytokine response, thus reducing
systemic low-grade inflammation and improving various immune markers. Moreover, regular exercise maintains redox
homeostasis in skeletal muscle and other tissues, including immune cells, but the interconnection between the anti-
flammatory effects of exercise with the redox status of immune cells is still poorly understood. With the aim to verify the
overall beneficial effect of regular training on the immune system, we have examined the acute and short-term effect of a 5-day
exercise program on the modulation of protein and lipid oxidation, antioxidants (i.e., superoxide dismutase-1 (SOD1) and
heat shock protein expression (i.e., heat shock protein-70 (HSP70) and heat shock protein-27 (HSP27)), at both mRNA and
protein levels, as well as the activation of the nuclear factor kappa light chain enhancer of activated B cells (NFκB) in
peripheral blood mononuclear cells (PBMCs). Moreover, plasmatic markers of oxidative stress, inflammation, and stress
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untrained young adult subjects. Even in the absence of an increased amount of protein or lipid oxidation, we confirmed a
PBMC upregulation of SOD1 (1.26 ± 0.07 fold change, p < 0.05), HSP70 (1.59 ± 0.28 fold change, p < 0.05), and HSP27 gene
expression (1.49 ± 0.09 fold change, p < 0.05) after 3 hours from the first bout of exercise, followed by an increase in proteins’
amount at 24 hours (SOD1, 1.80 ± 0.34 fold change; HSP70, 3.40 ± 0.58 fold change; and HSP27, 1.81 ± 0.20 fold change, p <
0.05) and return to basal levels after the 5 days of aerobic training. Indeed, the posttraining basal levels of oxidized molecules
in plasma and PBMCs were statistically lower than the pretraining levels (carbonyl content, 0.50 ± 0.05 fold change, p < 0.01),
paralleled by a lower expression of SOD2, GPx1, and TrxR1, at mRNA (SOD2, 0.63 ± 0.06; GPx1, 0.69 ± 0.07; and TrxR1, 0.69 ±
0.12 fold change, p < 0.05) and protein (TrxR1, 0.49 ± 0.11 fold change, p < 0.05) levels. These results verified the existence of
an early phase of redox adaptation to physical exercise already achievable after 5 days of moderate, regular aerobic training.
More interestingly, this phenomenon was paralleled by the degree of NFκB activation in PBMCs and the decrease of plasmatic
proinflammatory cytokines IL8, IL21, and IL22 in the postraining period, suggesting an interconnected, short-term efficacy of
aerobic exercise towards systemic oxidative stress and inflammation.

Research Article

Systemic Response of Antioxidants, Heat Shock Proteins, and Inflammatory Biomarkers to Short-Lasting Exercise Training in Healthy Male Subjects

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Received 27 May 2021; Accepted 29 October 2021; Published 22 November 2021

Academic Editor: Dimitrios Draganidis

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1. Introduction

There are substantial evidences that regular physical activity promotes health and healthy aging [1–3]. Indeed, regular exercise promotes multiple metabolic and immune benefits related to the decrease in the risk of various diseases, including, but not limited to, diabetes, cardiovascular disease, cancer, and Alzheimer’s disease [4–6].

Chronic inflammatory status and imbalance of the redox homeostasis are hallmarks of most of the biological and pathological conditions benefiting from an active lifestyle or exercise intervention [7–10]. Moreover, the activation of the anti-inflammatory pathways and/or the improvement of antioxidant and stress response with reduction of oxidative damage might represent key cellular processes ultimately leading to a reduction in mortality risk [11–13]. There is a growing body of evidence indicating the positive effects of regular moderate exercise (65–85% of maximum heart rate (HRmax)) on immune competency in healthy young and/or elderly subjects [14, 15], and it is recognized that the altered redox state in immune cells is connected to different metabolic- and cardiovascular-related conditions [16]. Nevertheless, few evidences support the interaction between oxidative stress and proinflammatory cytokine production, and the interconnection between the anti-inflammatory effect of exercise and the redox status (i.e., oxidants and antioxidants) of immune cells has not been elucidated after a short-term lasting exercise protocol, yet [17, 18].

It is widely known that peripheral blood mononuclear cells (PBMCs) represent the front line of the human immune system as well as the most essential mediators of stress and inflammation by producing cytokines, chemokines, and growth factors that may lead to beneficial or even pathological effects on tissues [19–21]. As other cell types (e.g., muscle cells) [22], PBMCs synthesize inflammatory proteins and reactive oxygen species (ROS) that can cause damage and dysfunction to arteries and other tissues, and it has been postulated that a proinflammatory/oxidant gene expression profile in PBMC may contribute to increased risk of cardiovascular and other diseases [23]. On the other hand, data from our and other laboratories on trained subjects show that the adaptive response of PBMCs in terms of the modulation of antioxidants and stress-induced markers is linked to rapid and substantial changes in the gene expression pattern, which correlates with an improvement of other parameters of healthy status [4, 24–30].

To date, studies concerning the effects of exercise on immune cells have focused mainly on athletes, with the overall goals of ascertaining the extent of immune decline due to excessive exercise training [31, 32], or on elderly, to identify the factors responsible for the improvement of immune response by regular exercise training [1]. Given the positive immunological effects that a regular exercise of moderate intensity can induce also in healthy young/adults, such as the decrease of cytokine levels, the enhancement of vaccine response, and the prompt response to viral infections [33–38], we aimed to analyze the effect of a moderate and short-lasting exercise period (5 days) on the modulation of plasma pro- and anti-inflammatory cytokines (i.e., IL6, IL8, IL10, IL17E, IL17F, IL21, IL22, and IL23) of healthy male subjects with a medium fitness level (38 < VO2max < 56 mL/kg/min), analyzing at the same time in the PBMCs the modulation of protein and lipid oxidation, antioxidant (i.e., SOD1, SOD2, GPx1, TrxR1, and CAT) and heat shock protein expression (i.e., HSP70 and HSP27), at both mRNA and protein levels, and the activation of the nuclear factor kappa light chain enhancer of activated B cells (NFκB). Moreover, several functions of lymphocytes are strongly regulated by redox status, including activation, proliferation, and apoptosis [39]. Therefore, the analysis of these parameters is a priority in defining the redox status of the lymphocytes.

Our results highlight the existence of an early phase for the exercise-induced adaptation of the redox components in immune cells, also suggesting an interconnected, short-term efficacy of aerobic exercise towards improved systemic oxidative stress and inflammation. In particular, after the 5-day training, the levels of both oxidized molecules and antioxidant enzymes verified a better redox buffering capacity paralleled by low levels of NFκB activation and a significant reduction of plasmatic proinflammatory cytokines IL8, IL21, and IL22.

2. Material and Methods

2.1. Study Design. A total of 10 healthy male subjects (26.6 ± 3.1 years) have been recruited for this study at the University of Rome “Foro Italico” (Table 1).

Specific eligibility criteria included male with an active lifestyle (<150 min/week recreational activity over the past 12 months) corresponding to a medium fitness level matched for age (36 < VO2max < 47 mL/kg/min), age 20–30 years, no illness or ongoing medication, and no signs of cardiovascular, metabolic, and pulmonary disease, orthopaedic injury or joint disease, and neurological or immunologic disease.

All participants underwent a detailed medical history and physical examination and provided informed written consent approved by the Ethics Committee of the University of Rome “La Sapienza” (RIF.CE: 4521). Moreover, they completed a detailed eating habit diary in which were recorded all food and drinks consumed during the 3 consecutive days before beginning the training protocol.

2.2. Evaluation of Physical Activity Level and Exercise Protocol. As previously reported [30], before starting the acute endurance exercise protocol, each participant performed a physical fitness assessment to estimate VO2max. Briefly, the aerobic capacity was assessed using the Balke treadmill test [40], a continuous incremental test on a treadmill (Skillrun Treadmill, Technogym, Italy). The test began with a warm-up of 5 min, the slope was 0% and the speed 5.3 km/h; then, the operator increased the slope of 1° after 1 min and then every minute. The score of the test is the time spent walking or running on the treadmill till exhaustion, in minutes. In most cases, time spent on the treadmill should be between 9 and 15 min. It is possible to estimate the VO2max score using the test time through the following formulas where the value “T” is the test time [VO2max =
Plasma and PBMC samples were isolated from whole blood by Ficoll gradient (Sigma-Aldrich, Milan, Italy), as previously described [46]. Human PBMCs were purified from whole blood by using the TRIzol (Invitrogen) reagent, according to the manufacturer’s procedure. As previously described [48], RNA was digested with RNase-free DNAsel (Ambion). Real-time quantitative RT-qPCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Life Technologies). Each reaction mixture contained Power SYBR Green RNA-to Ct 1stepMaster mix (2x) (Life Technologies), specific primer sets, RT Enzyme Mix (125x) (Life Technologies) of RNA samples. All samples were run in triplicate. Values obtained for the target gene were compared with values of an internal control gene, Cyclophilin A. A threshold cycle (Ct) was observed in the exponential phase of amplification, and quantification of relative expression levels was performed with standard curves for target genes (ΔΔCt) and the endogenous control (ΔΔCt). Geometric means were used to calculate the ΔΔCt (delta-delta Ct) values and expressed as 2^−ΔΔCt. The value of each control sample was set at 1 and was used to calculate the fold change of target genes. Primers were designed using Primer 3 Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and Primer-Blast using the reference and the alternative RefSeq accession numbers. The list of primers is reported in Supplementary Table 2.

2.7. Protein Carbonyl Content. The Protein Carbonyl Colorimetric Assay Kit (Cayman Chemical Company, USA) was performed in plasma samples using the manufacturer’s procedure. The protocol is based on the reaction between 2,4-dinitrophenylhydrazine (DNPH) and protein carboxyls forming Schiff base. The concentration of C=O is expressed per total protein content (nmol/mg protein).

2.8. Multiplex Cytokine Assay. Plasma levels of IL8, IL6, IL10, IL17E, IL17F, IL21, IL22, and IL23 were assayed in subjects experiencing the physical activity program, preexercise (before) and 24 h after the last training session (after 5 d), using a magnetic bead-based multiplex assay (Milliplex Human Cytokine, Chemokine Assay, Millipore-Sigma, non-reducing conditions and stained with Coomassie blue. The intensities of the bands were quantified by ImageJ 1.50 h software (National Institutes of Health, USA, http://imagej.nih.gov/ij). The list of antibodies utilized is reported in Supplementary Table 1.
Immediately after the training session, Figure 2(a) shows an increase of the blood lactate level immediately after (L1-L5, HR1-HR5) each training session. L: lactate; HR: heart rate.

Results in (data not shown).

Percentage of macro- and micronutrients consumed daily by the experimental group are shown in Table 1. All participants reveal a prompt response in terms of gene expression in PBMCs. This is a similar result is detected for lipid peroxidation, which demonstrates a decrease of HNE-protein-adducts at the end of the training period (Figure 3(a)).

As shown in Figure 2(b), the highest heart rate is recorded after the first exercise session (HR0 vs. HR1: 94.57 bpm ± 2.26 vs. 177.57 ± 9.78, p < 0.01), and compared with the baseline level, it remains higher immediately after the additional training sessions (HR0 vs. HR3, HR4, HR5: 94.57 bpm ± 2.26 vs. 154.00 ± 1.15, 151.43 ± 1.54, 149.14 ± 2.91, p < 0.01). Nevertheless, the values after the 2nd to the 5th training sessions (HR2-HR5) significantly decreased compared with the 1st session (HR1 vs. HR2: 177.57 bpm ± 9.78 vs. 154.14 ± 7.88, p < 0.01).

3.3. Systemic Oxidative Damage Measured at Plasma Level and in PBMCs. No changes in plasma protein carbonylation or PBMCs 4-HNE are found after 3 h and 24 h from the first exercise session (p > 0.05) (Figures 3(a) and 3(b)). The results show a significant reduction in plasma protein carbonylation content at the end of the training period (after 5 d) compared with the baseline levels (0.5 ± 0.05 fold change, p < 0.05) as well as its value measured after 24 h from the end of the first exercise session (p < 0.01) (Figure 3(a)).

A similar result is detected for lipid peroxidation, which demonstrates a decrease of HNE-protein-adducts at the end of the training period compared to the basal level (0.55 ± 0.10 fold change, p < 0.05) (Figure 3(b)).

3.4. Gene Expression and Protein Analysis of Antioxidants in PBMCs. As shown in Figure 4, the analysis of several antioxidants reveals a prompt response in terms of gene expression of SOD1, which is significantly increased already after 3 h following the first exercise session (after 3 h, 1.26 ± 0.07 fold change; after 24 h, 1.28 ± 0.11, p < 0.05), returning to the basal level at the end of the training period (before vs. after
The protein level of SOD1 is significantly increased only after 24 h of the first exercise section (after 24 h, 1.80 ± 0.34, \( p < 0.05 \)). No changes in mRNA and proteins are observed after 5 d of training with respect to the baseline level (\( p > 0.05 \)) (Figure 4(a)).

The analysis of SOD2 and GPx1 highlights a similar modulation with a significant decrease of their mRNA at the end of the training period (after 5 d, SOD2: 0.63 ± 0.06 fold change, \( p < 0.05 \); GPx1: 0.69 ± 0.07 fold change, \( p < 0.05 \)), while no differences are observed for the protein levels (\( p > 0.05 \)) (Figures 4(b) and 4(c)). No acute response is detected for both SOD2 and GPx1.

Interestingly, the mRNA level of TrxR1 is decreased both at 24 h following the first training session (after 24 h, 0.67 ± 0.09 fold change, \( p < 0.05 \)) and at the end of exercise training protocol (after 5 d, 0.69 ± 0.12 fold change, \( p < 0.05 \)) compared with the baseline level, whereas its protein level results are negatively modulated only at the last experimental point (after 5 d, 0.49 ± 0.11 fold change, \( p < 0.05 \)) (Figure 4(d)).

No significant changes are observed at mRNA and protein levels of CAT (\( p > 0.05 \)) at any experimental point (data not shown).

3.5. Modulation of Heat Shock Proteins at mRNA and Protein Levels in PBMCs. The analysis of HSP modulation highlights a significant increase of mRNA HSP70 already after 3 h from the first exercise session (after 3 h, 1.59 ±
Figure 4: Analysis of proteins and mRNA levels of antioxidants SOD1 (a), SOD2 (b), GPx1 (c), and TrxR1 (d) at baseline (——), after 3 hours (after 3 h) and 24 hours (after 24 h) following the first training session, and after 24 h following the last training session (after 5 d) in healthy subjects (n = 10). Bars in each histogram show the fold changes related to the baseline level measured before starting the training period. Data are presented as the means ± SEM. Statistical significance was determined using ANOVA with Bonferroni’s post hoc analysis. *p < 0.05 vs. baseline.
3.6. Cytokine Profile Analysis following the Exercise Training Period. The level of IL6, IL10, IL17E, IL17F, and IL23 is not changed significantly in response to exercise training ($p > 0.05$) (Figure 6). On the contrary, the mean levels of IL8, IL21, and IL22 are significantly decreased after the 5-day training protocol as compared to baseline values: IL8 decreased from $114.67 \pm 18.8$ to $68.9 \pm 10.89$ pg/mL ($p < 0.05$) (Figure 6(b)), IL21 is decreased from $101.68 \pm 23.56$ pg/mL to $61.47 \pm 18.79$ pg/mL ($p < 0.05$) (Figure 6(f)), and IL22 is decreased from $0.26 \pm 0.06$ pg/mL to $0.17 \pm 0.05$ pg/mL ($p < 0.05$) (Figure 6(g)).

3.7. NFkB Activation in PBMCs. As shown in Figure 7, the level of p-p65-NFkB is positively modulated by acute exercise, showing a significant increase after 24 h from the first exercise session (after 24 h, $1.81 \pm 0.17$ fold change, $p < 0.05$), whereas at the end of exercise training, it is found below the basal level (after 5 d, $0.88 \pm 0.09$ fold change, $p < 0.05$).

3.8. Matrix Correlation Analysis. To verify the impact of exercise training in the correlation among molecules belonging to stress proteins, antioxidant/oxidative stress, and cytokine response, we considered the fold change in the protein level at the end of training with respect to the baseline level. We identified a positive correlation of HSP70 with catalase (HSP70 vs. CAT: $r = 0.85 ; p = 0.033$) and SOD1 (HSP70 vs. SOD1: $r = 0.88 ; p = 0.021$), while only a negative correlation was found between IL21 and catalase (IL21 vs. CAT: $r = -0.95 ; p = 0.012$) (Supplementary Figure 1).
4. Discussion

To date, no studies conducted in humans have examined the effect of short-term exercise programs on circulating markers of oxidative stress, inflammation, and stress response proteins. For the first time, here, we show that five sessions of a regular and moderate exercise program, administered daily, are able to produce an improvement in immune health in untrained young adult subjects. In particular, we verified an acute response after the first exercise
session that activated the antioxidant response as evidenced by the increase in PBMC SOD1 expression, as well as the upregulation/activation of specific stress response proteins such as HSP70 and HSP27. Interestingly, at the end of the 5-day training protocol, we observed a significant decrease of the basal level of oxidized proteins and lipids, as well as the reduction in PBMC antioxidants and circulating proinflammatory cytokines, which may suggest a very early beneficial adaptation phase to physical exercise (Figure 8).

When comparing studies looking at the inflammatory and stress protein response to exercise, it is important to compare subjects at the same physical level and with homogeneous biological and anthropometric parameters, such as age and BMI. Moreover, results from the blood lactate (L) and heart rate (HR) analyses, considered reliable markers of physiological adaptation to exercise training, show a homogeneous response within subjects. As expected, L and HR values increased immediately after each bout of exercise, although the increment resulted significantly lower already after the second exercise session. Although the homogeneity and limited number of subjects could impact the actual consistency of these parameters, their reduction after training is considered a good indicator of adaptation [45, 52], suggesting the potential effect of our exercise protocol in improving aerobic training capacity in the subject group.

The reduction in protein carbonylation and lipid peroxidation observed at the end of the training period, paralleled to the downregulation of SOD2, Gpx1, and TrxR1, are important indicators of a better redox status, which can represent an efficient combination between oxidant and antioxidants [53, 54]. Indeed, the type and timing of modifications in the expression of antioxidant enzymes (SODs, TrxRs, and GPxs) may explain the reduction of oxidized molecules in both PBMCs and plasma determined in untrained subjects after the physical training program. In 2011, Jenkins et al. demonstrated that in untrained healthy males, whose age, BMI, and aerobic capacity were comparable to that of our experimental group, the baseline levels of reactive nitrogen and oxidative species in PBMCs were higher when compared to matched trained individuals because of an increased NADPH oxidase activity in the untrained group [55]. Moreover, we and other groups demonstrated that the baseline levels of SOD1 were lower in untrained than in trained matched subjects [30, 55]. Thus, even in healthy young individuals, the absence of regular exercise training correlates with a higher prooxidant environment in immune cells [55–58].

Our results also confirmed that unaccustomed moderate training did not increase oxidative damage [54]. On the contrary, it represents a clear example of oxidative eustress stimulus [59], able to activate a prompt cellular response, as demonstrated by the transient upregulation of SOD1, HSP70, and HSP27, at both mRNA and protein levels, possibly involving NFkB as an upstream or downstream mediator [60, 61]. We postulate that, in our protocol, an increase in cytoplasmic H$_2$O$_2$, from NADPH oxidase or mitochondrial activities, might represent the main driver for the acute response, largely involving the cytoplasmic superoxide dismutase and chaperone network [62].

It has been already demonstrated that regular participation in physical activity modulates in PBMCs the expression of molecules involved in the antioxidant response such as SOD2, TrxR1, OXR1, CAT, GPx, and UCP3 [8, 10, 12, 27, 63]. Similar to the aforementioned results, and matching both the subject’s characteristics recruited and the type of exercise protocol, we found a return to the basal level of SOD1 expression and a significant reduction at mRNA and/or protein levels for SOD2, GPx1, and TrxR1 at the end of the short-term aerobic training, correlated with the lower amount of lipid peroxidation in PBMCs and plasma protein carbonylation.

The results obtained so far confirm the crucial role of ROS signalling in the physical exercise response and as a moderate, short-term exercise training significantly ameliorating the redox homeostasis at the systemic levels, thereby causing a better-adapted antioxidant asset in active people [64]. In particular, it is known that an acute bout of exercise at sufficient intensity stimulates expression/activities of antioxidant enzymes in the first 3-24 hours postexercise [63, 65, 66]. This could be considered a defensive mechanism of the cell under oxidative stress. However, a repeated exercise may induce a transient reduction of specific antioxidants as

![Figure 7: Western blot analysis of p-p65-NFkB in PBMCs of subjects at baseline (---), after 3 hours (after 3 h) and 24 hours (after 24 h) following the first training session, and after 24 h following the last training session (after 5 d) in healthy subjects (n = 10). Bars in each histogram show the fold changes related to the baseline level measured before starting the training period. Data are presented as the means ± SEM. Statistical significance was determined using ANOVA with Bonferroni’s post hoc analysis. *p < 0.05 vs. baseline.](image-url)
adaptive response to exercise training and thereby index of a better redox balance [8, 10, 12, 27, 63, 67]. It has been demonstrated that after acute exercise, a significantly greater percentage of leukocytes express HSP70, HSP90, HSP60, and HSP27 depending upon exercise intensity [26, 68–70]. Differently, there are no studies investigating the modulation of HSPs in leukocytes of adult subjects after a period of endurance exercise training. However, it is known that trained subjects show at rest a downregulation of HSP positive cells, which may reflect adaptation mechanisms to regular endurance training [69, 71]. Similarly to the aforementioned results, we found that both HSP70 and HSP27 were upregulated in the PBMCs after acute exercise, whereas at the end of the training period their expression was returned to basal levels. These results confirm the prompt response of HSPs to exercise-induced stimuli and highlight the need for a longer training period for their adaptation. Protection and/or tolerance against exercise-induced oxidative, heat, cytokine, and inflammatory stress in leukocytes are provided by the modulation of other stress response proteins, including the HSPs [72, 73]. In different tissues, HSPs play a role in protein translocation, stabilization, assembly, and degradation processes, functions that could be important in leukocytes activated by physical exercise [30, 69, 74–77].

**Figure 8:** Proposed mechanism for the systemic response to moderate exercise in healthy adult subjects. Physiological increase of ROS concentration through exercise-induced NADPH oxidase is a potent intracellular stimulus for the transient expression/activation of molecules involved in antioxidant (GPX1, TrxR1, SOD1, and SOD2) and stress (HSP70 and HSP27) response, as well as in pro- and anti-inflammatory processes (IL8, IL10, IL17E, IL17F, IL21, IL22, and IL23). Following a single bout of moderate endurance exercise in untrained subjects, physiological levels of ROSs induce the transient upregulation of SOD1, HSP70, and HSP27, possibly involving NFκB as an upstream mediator. Differently, 5 days of exercise training appears to be more reflective of a longer-term training adaptation reducing the content of TrxR1, IL8, IL21, and IL22 and damaged macromolecules, as well as the activation of NFκB, indicative of positive effects of exercise training on the redox balance and the immunoresponse. ROS: reactive oxygen species; HSPs: heat shock proteins; PCC: protein carbonyl content; GPx1: glutathione peroxidase 1; NFκB: nuclear factor kappa B; SOD1: copper-zinc superoxide dismutase; SOD2: manganese superoxide dismutase; TrxR1: thioredoxin reductase 1; TF: transcription factors; NOXs: NADPH oxidases; 4HNE: 4-hydroxynonenal.
receptors 2 and 4 (TLR2 and TLR4) [78, 79]. TLRs are the critical sensors for the recognition of microorganisms whose expression patterns are closely related to the immunologic function of the cells [80].

Regular participation in physical exercise has a positive effect on the immune system [14]. Although it has been demonstrated that physiological response to acute and long-term adaptations of immunity to exercise is dependent on exercise characteristics (i.e., type, intensity, frequency, and duration), to date exercise training can be considered a kind of “immunotherapy” capable of promoting an anti-inflammatory environment or attenuating the acute response to exercise, possibly reducing the risk of developing inflammatory-related diseases [7, 81, 82]. Moreover, the available scholarly literature seems to suggest its positive effects on immune responses and outcomes to viral infections [33, 83].

To investigate in detail the relationships between short-lasting exercise protocol and systemic inflammatory markers, we analyzed the expression of numerous cytokines. Similar to previous researches [84–86], we found that a healthy amount of regular exercise reduces levels of inflammatory markers. Particularly, exercise training induced a tendency to decrease all cytokines, with someone reaching significance, including the proinflammatory IL8 and both IL21 and IL22 produced by Th17 cells, which play a critical role in the pathogenesis of autoimmune diseases [87].

Taken together, these data show that only 5 days of exercise training appear to be more reflective of a longer-term training adaptation and may be indicative of an effect of exercise training in reducing the risk of developing inflammatory-derived and autoimmune diseases.

With respect to the possible interconnection between the inflammatory, antioxidant, and HSP responses, nuclear factor (NF)κB is known to play a critical role in mediating immune and inflammatory/oxidative responses and apoptosis [88]. NFκB signalling pathways may be triggered by several stimuli such as ROS [54, 89], HSPs [79], HO-1 [56], and cytokines [90] induced by regular physical activity. As already demonstrated by Ji and colleagues [91], we found that p-p65-NFκB tends to increase after 3 h from the end of the first training session, reaching significance after 24 h. When this parameter was analyzed at the end of the training period, the activity of this protein was observed significantly reduced even compared with the basal level. Since the NFκB signalling pathway is induced in a redox-sensitive manner, the hypothesis that the activation of this protein is partly determined by the alteration of the redox state induced by exercise is convincing. Indeed, a reduction of systemic oxidative stress markers observed following the training period was parallel to the reduction of NFκB activity and antioxidant content [54]. Moreover, an increasing number of studies have shown that Hsp70 may be involved in the regulation of NFκB activity, protecting from the inflammatory response by preventing either NFκB activation directly [92] or via TRAF6, an essential activator of the NFκB pathway [93]. Another important protein regulating the fine balance of cellular redox status and responses to stress and inflammation in lymphocytes is the nuclear factor erythroid 2-related factor 2 (Nrf2) [94]. Although we did not measure the protein levels of Nrf2, as previously stated [95], it could speculate a further modulation of this transcription factor in response to physical exercise.

Finally, the correlation analysis among different categories of molecules (i.e., antioxidants, stress response, and inflammatory) performed at the end of the training period highlights a parallel increase of HSP70 expression, SOD1, and CAT, as well as an opposite trend for CAT and IL21. Although the causal link between these molecules remains purely speculative in this study, it is known that an acute prooxidant stimulus (acute exercise) can induce the expression of these biomolecules (i.e., HSP70, SOD1, and CAT) to maintain the cellular homeostasis, while in a condition of “exercise adaptation,” where the ROS production is better balanced by the various cellular systems, their expression can be reduced. The inverse correlation between IL21 and CAT could explain the further role of this interleukin in immune cells, where it could trigger an immunometabolic axis including improved mitochondrial fitness and cellular redox status [96]. Taken together, these results point out a conceivable relationship among antioxidants, HSP induction, and cytokines in immunocompetent cells. As already demonstrated by others [69, 97], these correlations help us to complete the complex puzzle where moderate exercise induces activation of immunocompetent cells, followed by an increase of ROS and cytokines, resulting in HSP induction (Supplementary Figure 1).

Some limitations of this study have to be considered. Our results were obtained on male subjects; this could make the results not generalizable to females; in the same instances, the small sample size may have reduced the number of statistical differences; lack of experimental point 48 h after the last exercise session and mechanistic measurements could better clarify the specific effect of exercise training on the analyzed parameters.

Moreover, although our results show trends in the direction of specific biomarker changes over time, we cannot attribute the absolute biomarker changes entirely to our intervention because the absence of a “nonexercised” control group does not allow the exclusion of additional factors.

5. Conclusion

To our knowledge, this is the first study to reveal an early adaptive response of immune cells after a successful 5 days of moderate exercise training in untrained healthy subjects with a medium fitness level.

The exact function of the differential regulation of all molecules analyzed in response to exercise training remains to be further investigated. However, it is intriguing to speculate that this type of physical activity conducted in frail people, such as the elderly, could lead to a reduction in chronic inflammation and oxidative stress, which result to be physiologically increased in PBMCs with age and linked to immunosenescence [98–100]. As demonstrated with other long-duration moderate aerobic exercises [33, 83], our results could also suggest in the long term an improvement of influenza risk and increased rates of vaccine efficacy.
To increase the reliability and applicability of the results, further studies need to investigate the effect of this exercise protocol on a greater number of subjects, possibly extending the analysis on female subjects and on a fragile population such as the elderly. Due to the dependence of changes in the activity of antioxidant enzymes after physical exercise, future research should also include this analysis in PBMCs and at the plasma/serum level, as well as a detailed analysis of blood lymphocyte phenotypic characteristics induced at the end of our exercise training. Moreover, they remain strongly recommended researches, preferably long-term standardized mechanistic studies, to understand the impact of the observed change in gene and protein expression in leukocytes and other organs, as well as to understand the complex interaction between white blood cells and muscle inflammatory response.

Data Availability
Data available on request.

Conflicts of Interest
The submitting authors are responsible for coauthors declaring their interests.

Acknowledgments
This work was supported by grants from the Foro Italico University of Rome (Research Grant CDR2.RIC182015) to DC.

Supplementary Materials
Supplementary Figure 1: heat map representation of the correlation matrix among fold changes (after 5 d/before) of molecules belonging to stress proteins, antioxidants/oxidative stress, and inflammatory response. The r value of the correlation is indicated in each cell of the matrix. Supplementary Table 1: list of primary antibodies utilized. Supplementary Table 2: sequences of the oligonucleotides used for RT-qPCR. (Supplementary Materials)

References
[1] J. E. Frankel, J. F. Bean, and W. R. Frontera, “Exercise in the elderly: research and clinical practice,” Clinics in Geriatric Medicine, vol. 22, no. 2, pp. 239–256, 2006.
[2] D. E. R. Warburton and S. S. D. Bredin, “Health benefits of physical activity: a systematic review of current systematic reviews,” Current Opinion in Cardiology, vol. 32, no. 5, pp. 541–556, 2017.
[3] G. D. Cartee, R. T. Hepple, M. M. Bamman, and J. R. Zierath, “Exercise promotes healthy aging of skeletal muscle,” Cell Metabolism, vol. 23, no. 6, pp. 1034–1047, 2016.
[4] H. M. Ahmed, M. J. Blaha, K. Nasir, J. J. Rivera, and R. S. Blumenthal, “Effects of physical activity on cardiovascular disease,” The American Journal of Cardiology, vol. 109, no. 2, pp. 288–295, 2012.
[5] I. M. Lee, E. J. Shiroma, F. Lobelo et al., “Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy,” The Lancet, vol. 380, no. 9838, pp. 219–229, 2012.
[6] E. Grazioni, I. Dimauro, N. Mercatelli et al., “Physical activity in the prevention of human diseases: role of epigenetic modifications,” BMC Genomics, vol. 18, no. 58, p. 802, 2017.
[7] M. Gleeson, N. C. Bishop, D. J. Stensel, M. R. Lindley, S. S. Mastana, and M. A. Nimmo, “The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease,” Nature Reviews Immunology, vol. 11, no. 9, pp. 607–615, 2011.
[8] M. R. Beltran Valls, I. Dimauro, A. Brunelli et al., “Explosive type of moderate-resistance training induces functional, cardiovascular, and molecular adaptations in the elderly,” Age, vol. 36, no. 2, pp. 759–772, 2014.
[9] M. Pittaluga, A. Sgadari, I. Dimauro, B. Tavazzi, P. Parisi, and D. Caporossi, “Physical exercise and redox balance in type 2 diabetics: effects of moderate training on biomarkers of oxidative stress and DNA damage evaluated through comet assay,” Oxidative Medicine and Cellular Longevity, vol. 2015, Article ID 981242, 7 pages, 2015.
[10] I. Dimauro, M. Scalabrin, C. Fantini et al., “Resistance training and redox homeostasis: correlation with age-associated genomic changes,” Redox Biology, vol. 10, pp. 34–44, 2016.
[11] B. K. Pedersen, “The anti-inflammatory effect of exercise,” Journal of Applied Physiology, vol. 98, no. 4, pp. 1154–1162, 2005.
[12] I. Dimauro, A. Sgura, M. Pittaluga et al., “Regular exercise participation improves genomic stability in diabetic patients: an exploratory study to analyse telomere length and DNA damage,” Scientific Reports, vol. 7, no. 1, article 4137, 2017.
[13] K. Suzuki, T. Tominaga, R. T. Ruhee, and S. Ma, “Characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress,” Antioxidants, vol. 9, no. 5, p. 401, 2020.
[14] B. K. Pedersen and L. Hoffman-Goetz, “Exercise and the immune system: regulation, integration, and adaptation,” Physiological Reviews, vol. 80, no. 3, pp. 1055–1081, 2000.
[15] N. Collao, I. Rada, M. Francaux, L. Deldicque, and H. Zbinden-Foncea, “Anti-inflammatory effect of exercise mediated by toll-like receptor regulation in innate immune cells—a review,” International Reviews of Immunology, vol. 39, no. 2, pp. 39–52, 2020.
[16] D. García-López, K. Håkkinen, M. J. Cuevas et al., “Effects of strength and endurance training on antioxidant enzyme gene expression and activity in middle-aged men,” Scandinavian Journal of Medicine & Science in Sports, vol. 17, no. 5, pp. 595–604, 2007.
[17] M. Sellami, M. Gasmi, J. Denham et al., “Effects of acute and chronic exercise on immunological parameters in the elderly aged: can physical activity counteract the effects of aging?,” Frontiers in Immunology, vol. 9, p. 2187, 2018.
[18] R. Tossige-Gomes, K. B. Costa, V. O. Ottone, F. C. Magalhães, F. T. Amorim, and E. Rocha-Vieira, “Lymphocyte redox imbalance and reduced proliferation after a single session of high intensity interval exercise,” PLoS One, vol. 11, no. 4, article e0153647, 2016.
[19] J. G. Tidball and M. Wehling-Henricks, “Macrophages promote muscle membrane repair and muscle fibre growth and regeneration during modified muscle loading in mice.
in vivo,” *The Journal of Physiology*, vol. 578, no. 1, pp. 327–336, 2007.

[20] J. N. Jarvis, H. R. Petty, Y. Tang et al., “Evidence for chronic, peripheral activation of neutrophils in polyarticular juvenile rheumatoid arthritis,” *Arthritis Research & Therapy*, vol. 8, no. 5, article R154, 2006.

[21] J. Hanna, D. Goldman-Wohl, Y. Hamani et al., “Decidual NK cells regulate key developmental processes at the human fetal- maternal interface,” *Nature Medicine*, vol. 12, no. 9, pp. 1065–1074, 2006.

[22] F. He, J. Li, Z. Liu, C. C. Chuang, W. Yang, and L. Zuo, “Redox mechanism of reactive oxygen species in exercise,” *Frontiers in Physiology*, vol. 7, p. 486, 2016.

[23] H. Chon, M. C. Verhaar, H. A. Koomans, J. A. Joles, and R. Ceci, M. R. Beltran Valls, G. Duranti et al., “Regulation of immune functions, *International Journal of Environmental Research and Public Health*, vol. 22, no. 17-18, pp. 2298–2306, 2004.

[24] J. A. Woods, K. T. Keylock, T. Lowder et al., “Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: the immune function intervention trial,” *Journal of the American Geriatrics Society*, vol. 57, no. 12, pp. 2183–2191, 2009.

[25] Y. Song, F. Ren, D. Sun et al., “Benefits of exercise on influenza or pneumonia in older adults: a systematic review,” *International Journal of Environmental Research and Public Health*, vol. 17, no. 8, p. 2655, 2020.

[26] M. D. Ferrer, X. Capó, M. Martorell et al., “Regular practice of moderate physical activity by older adults ameliorates their anti-inflammatory status,” *Nutrients*, vol. 10, no. 11, p. 1780, 2018.

[27] A. J. Wadley, Y. W. Chen, G. Y. Lip, J. P. Fisher, and S. Aldred, “Low volume-high intensity interval exercise elicits antioxidant and anti-inflammatory effects in humans,” *Journal of Sports Sciences*, vol. 34, no. 1, pp. 1–9, 2016.

[28] L. K. Stewart, M. G. Flynn, W. W. Campbell et al., “The influence of exercise training on inflammatory cytokines and C-reactive protein,” *Medicine and Science in Sports and Exercise*, vol. 39, no. 10, pp. 1714–1719, 2007.

[29] S. Cemerski, A. Cantagrel, J. P. M. van Meerwijk, and P. Romagnoli, “Reactive oxygen species differentially affect T cell receptor-signaling pathways,” *The Journal of Biological Chemistry*, vol. 277, no. 22, pp. 19585–19593, 2002.

[30] B. Balke and R. W. Ware, “An experimental study of physical fitness of Air Force personnel,” *United States Armed Forces Medical Journal*, vol. 10, no. 6, pp. 675–688, 1959.

[31] M. L. Pollock, R. L. Bohannon, K. H. Cooper et al., “A comparative analysis of four protocols for maximal treadmill stress testing,” *American Heart Journal*, vol. 92, no. 1, pp. 39–46, 1976.

[32] G. Borg, “Psychophysical scaling with applications in physical work and the perception of exertion,” *Scandinavian Journal of Work, Environment & Health*, vol. 16, Supplement 1, pp. 55–58, 1990.

[33] P. J. Donovan, R. J. Schoen, D. B. Braunstein, and P. M. Wolfson, “Cardiovascular response to exercise: physiology and clinical applications,” *The Journal of the American Osteopathic Association*, vol. 83, no. 3, pp. 243–253, 1983.

[34] J. A. Baecke, J. Burema, and J. E. R. Frijters, “A short questionnaire for the measurement of habitual physical activity in epidemiological studies,” *The American Journal of Clinical Nutrition*, vol. 36, no. 5, pp. 936–942, 1982.

[35] R. J. Favier, S. H. Constable, M. Chen, and J. O. Holloszy, “Endurance exercise training reduces lactate production,” *Journal of Applied Physiology*, vol. 61, no. 3, pp. 885–889, 1986.

[36] C. Fantini, P. Sgrò, M. Pittaluga et al., “Short-term, supra-physiological rhGH administration induces transient DNA damage in peripheral lymphocytes of healthy women,” *Journal of Endocrinological Investigation*, vol. 40, no. 6, pp. 645–652, 2017.

[37] F. Magi, I. Dimauro, F. Margheritini et al., “Telomere length is independently associated with age, oxidative biomarkers, and sport training in skeletal muscle of healthy adult males,” *Free Radical Research*, vol. 52, no. 6, pp. 639–647, 2018.

[38] A. L. Ilert, H. Kawaguchi, C. Antinozzi et al., “Targeted inactivation of nuclear interaction partner of AKL disrupts mitotic prophase,” *Development*, vol. 139, no. 14, pp. 2523–2534, 2012.

[39] L. Di Luigi, P. Sgrò, G. Duranti et al., “Sildenafil reduces expression and release of IL-6 and IL-8 induced by reactive oxygen species in systemic sclerosis fibroblasts,” *International Journal of Molecular Sciences*, vol. 21, no. 9, p. 3161, 2020.
Oxidative Medicine and Cellular Longevity

[50] G. F. Fletcher, P. A. Ades, P. Kligfield et al., “Exercise standards for testing and training: a scientific statement from the American Heart Association,” Circulation, vol. 128, no. 8, pp. 873–934, 2013.

[51] L. A. Kaminsky, M. T. Imboden, R. Arena, and J. Myers, “Reference standards for cardiorespiratory fitness measured with cardiopulmonary exercise testing using cycle ergometry: data from the Fitness Registry and the Importance of Exercise National Database (FRIEND) registry,” Mayo Clinic Proceedings, vol. 92, no. 2, pp. 228–233, 2017.

[52] A. Vallebona, G. Gigli, S. Orlandi, and G. Reggiardo, “Heart rate response to graded exercise correlates with aerobic and ventilatory capacity in patients with heart failure,” Clinical Cardiology, vol. 28, no. 1, pp. 25–29, 2005.

[53] K. H. Wagner, S. Reichhold, and O. Neubauer, “Impact of endurance and ultraendurance exercise on DNA damage,” Annals of the New York Academy of Sciences, vol. 1229, no. 1, pp. 115–123, 2011.

[54] M. C. Gomez-Cabrera, E. Domenech, and J. Viña, “Moderate exercise is an antioxidant: upregulation of antioxidant genes by training,” Free Radical Biology & Medicine, vol. 44, no. 2, pp. 126–131, 2008.

[55] N. T. Jenkins, R. O. Landers, S. R. Thakkar et al., “Prior endurance exercise prevents postprandial lipaemia-induced increases in reactive oxygen species in circulating CD31+ cells,” The Journal of Physiology, vol. 589, no. 22, pp. 5539–5553, 2011.

[56] A. Sureda, M. D. Ferrer, P. Tauler et al., “Effects of exercise intensity on lymphocyte H2O2 production and antioxidant defences in soccer players,” British Journal of Sports Medicine, vol. 43, no. 3, pp. 186–190, 2009.

[57] P. Tauler, A. Sureda, N. Cases et al., “Increased lymphocyte antioxidant defences in response to exhaustive exercise do not prevent oxidative damage,” The Journal of Nutrition, vol. 17, no. 10, pp. 665–671, 2006.

[58] N. V. Margaritelis, V. Paschalis, A. A. Theodorou, A. Kyparos, and M. G. Nikolaidis, "Redox basis of exercise physiology," Redox Biology, vol. 35, article 101499, 2020.

[59] H. Sies, "Oxidative eustress: on constant alert for redox homeostasis," Redox Biology, vol. 41, article 101867, 2021.

[60] M. C. Gomez-Cabrera, A. Martinez, G. Santangelo, F. V. Paliardo, J. Sastre, and J. Vina, "Oxidative stress in marathon runners: interest of antioxidant supplementation," The British Journal of Nutrition, vol. 96, Supplement 1, pp. S31–S33, 2006.

[61] L. Bhagat, V. P. Singh, R. K. Dawra, and A. K. Saluja, "Sodium arsenite induces heat shock protein 70 expression and protects against secretagogue-induced trypsinogen and NF-κB activation," Journal of Cellular Physiology, vol. 215, no. 1, pp. 37–46, 2008.

[62] Y. Wang, R. Branicky, A. Noë, and S. Hekimi, "Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling," The Journal of Cell Biology, vol. 217, no. 6, pp. 1915–1928, 2018.

[63] L. Hoffman-Goetz and P. A. Spagnuolo, "Effect of repeated exercise stress on caspase 3, Bcl-2, HSP 70 and Cu/Zn-SOD protein expression in mouse intestinal lymphocytes," Journal of Neuroimmunology, vol. 187, no. 1-2, pp. 94–101, 2007.

[64] Z. Radak, K. Ishihara, E. Tekus et al., "Exercise, oxidants, and antioxidants change the shape of the bell-shaped hormesis curve," Redox Biology, vol. 12, pp. 285–290, 2017.

[65] J. M. Antunes-Neto, M. H. Toyama, E. M. Carneiro, A. C. Boscherio, L. Pereira-da-Silva, and D. V. Macedo, "Circulating leukocyte heat shock protein 70 (HSP70) and oxidative stress markers in rats after a bout of exhaustive exercise," Stress, vol. 9, no. 2, pp. 107–115, 2006.

[66] A. K. Banerjee, A. Mandal, D. Chanda, and S. Chakraborti, "Oxidant, antioxidant and physical exercise," Molecular and Cellular Biochemistry, vol. 253, no. 1–2, pp. 307–312, 2003.

[67] A. Antonioni, C. Fantini, I. Dimauro, and D. Caporossi, "Redox homeostasis in sport: do athletes really need antioxidant support?" Research in Sports Medicine, vol. 27, no. 2, pp. 147–165, 2019.

[68] R. C. Walsh, I. Koukoulas, A. Garnham, P. L. Moseley, M. Hargreaves, and M. A. Febbraio, "Exercise increases serum Hsp72 in humans," Cell Stress & Chaperones, vol. 6, no. 4, pp. 386–393, 2001.

[69] E. Fehrenbach, A. M. Niess, E. Schlotz, F. Passek, H. H. Dickhuth, and H. Northoff, "Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners," Journal of Applied Physiology, vol. 89, no. 2, pp. 704–710, 2000.

[70] Y.-O. Shin, J. K. Oh, H.-S. Sohn et al., "Expression of exercise-induced HSP70 in long-distance runner’s leukocytes," Journal of Thermal Biology, vol. 29, no. 7-8, pp. 769–774, 2004.

[71] E. Fehrenbach, F. Passek, A. M. Niess et al., "HSP expression in human leukocytes is modulated by endurance exercise," Medicine and Science in Sports and Exercise, vol. 32, no. 3, pp. 592–600, 2000.

[72] E. Fehrenbach and A. M. Niess, "Role of heat shock proteins in the exercise response," Exercise Immunology Review, vol. 5, pp. 57–77, 1999.

[73] I. Dimauro, N. Mercatelli, and D. Caporossi, "Exercise-induced ROS in heat shock proteins response," Free Radical Biology & Medicine, vol. 98, pp. 46–55, 2016.

[74] S. H. Kaufmann, "Heat shock proteins and the immune response," Immunology Today, vol. 11, no. 4, pp. 129–136, 1990.

[75] A. Antonioni, I. Dimauro, C. Fantini et al., "αB-crystallin response to a pro-oxidant non-cytotoxic environment in murine cardiac cells an 'in vitro' and 'in vivo' study," Free Radical Biology & Medicine, vol. 152, pp. 301–312, 2020.

[76] I. Dimauro, A. Antonioni, N. Mercatelli et al., "The early response of αB-crystallin to a single bout of aerobic exercise in mouse skeletal muscles depends upon fiber oxidative features," Redox Biology, vol. 24, article 101183, 2019.

[77] E. Fehrenbach, A. M. Niess, R. Veith, H. H. Dickhuth, and H. Northoff, "Changes of HSP72-expression in leukocytes are associated with adaptation to exercise under conditions of high environmental temperature," Journal of Leukocyte Biology, vol. 69, no. 5, pp. 747–754, 2001.

[78] P. Rodriguez-Miguelez, R. Fernandez-Gonzalo, M. Almar et al., "Role of toll-like receptor 2 and 4 signaling pathways on the inflammatory response to resistance training in elderly subjects," Age, vol. 36, no. 6, p. 9734, 2014.

[79] A. Asea, S. K. Kraeft, E. A. Kurt-Jones et al., "HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine," Nature Medicine, vol. 6, no. 4, pp. 435–442, 2000.

[80] I. Rada, L. Deldicque, M. Francaux, and H. Zbinden-Foncea, "Toll like receptor expression induced by exercise in obesity
and metabolic syndrome: a systematic review,” *Exercise Immunology Review*, vol. 24, pp. 60–71, 2018.

[81] A. L. de Araújo, L. C. R. Silva, J. R. Fernandes, and G. Benard, “Preventing or reversing immunosenescence: can exercise be an immunotherapy?,” *Immunotherapy*, vol. 5, no. 8, pp. 879–893, 2013.

[82] P. Libby, “Inflammatory mechanisms: the molecular basis of inflammation and disease,” *Nutrition Reviews*, vol. 65, 12, Part 2, pp. S140–S146, 2007.

[83] T. Lowder, D. A. Padgett, and J. A. Woods, “Moderate exercise protects mice from death due to influenza virus,” *Brain, Behavior, and Immunity*, vol. 19, no. 5, pp. 377–380, 2005.

[84] M. P. Noz, Y. A. W. Hartman, M. T. E. Hopman et al., “Sixteen-week physical activity intervention in subjects with increased cardiometabolic risk shifts innate immune function towards a less proinflammatory state,” *Journal of the American Heart Association*, vol. 8, no. 21, article e013764, 2019.

[85] N. Sallam and I. Laher, “Exercise modulates oxidative stress and inflammation in aging and cardiovascular diseases,” *Oxidative Medicine and Cellular Longevity*, vol. 2016, Article ID 7239639, 32 pages, 2016.

[86] J. B. Farinha, F. M. Steckling, S. T. Stefanello et al., “Response of oxidative stress and inflammatory biomarkers to a 12-week aerobic exercise training in women with metabolic syndrome,” *Sports Medicine-Open*, vol. 1, no. 1, p. 19, 2015.

[87] C. Wu, J. C. Goodall, R. Busch, and J. S. H. Gaston, “Relationship of CD146 expression to secretion of interleukin (IL)-17, IL-22 and interferon-γ by CD4+ T cells in patients with inflammatory arthritis,” *Clinical and Experimental Immunology*, vol. 179, no. 3, pp. 378–391, 2015.

[88] N. D. Perkins and T. D. Gilmore, “Good cop, bad cop: the different faces of NF-κB,” *Cell Death and Differentiation*, vol. 13, no. 5, pp. 759–772, 2006.

[89] N. Mercatelli, I. Dimauro, S. A. Ciafré, M. G. Farace, and D. Caporossi, “αB-crystallin is involved in oxidative stress protection determined by VEGF in skeletal myoblasts,” *Free Radical Biology & Medicine*, vol. 49, no. 3, pp. 374–382, 2010.

[90] S. Uwe, “Anti-inflammatory interventions of NF-κB signaling: potential applications and risks,” *Biochemical Pharmacology*, vol. 75, no. 8, pp. 1567–1579, 2008.

[91] L. L. Ji, M. C. Gomezcabra, N. Steinhaefel, and J. Vina, “Acute exercise activates nuclear factor (NF)-κB signaling pathway in rat skeletal muscle,” *The FASEB Journal*, vol. 18, no. 13, pp. 1499–1506, 2004.

[92] P. W. Sheppard, X. Sun, M. Khammash, and R. G. Giffard, “Overexpression of heat shock protein 72 attenuates NF-κB activation using a combination of regulatory mechanisms in microglia,” *PLoS Computational Biology*, vol. 10, no. 2, article e1003471, 2014.

[93] L. C. Wang, L. X. Liao, H. N. Lv et al., “Highly selective activation of heat shock protein 70 by allosteric regulation provides an insight into efficient neuroinflammation inhibition,” *eBioMedicine*, vol. 23, pp. 160–172, 2017.

[94] C. Morzadec, M. Macoch, L. Sparfel, S. Ker dine-Römer, O. Fardel, and L. Vernhet, “Nrf2 expression and activity in human T lymphocytes: stimulation by T cell receptor activation and priming by inorganic arsenic and tert-butylhydroquinone,” *Free Radical Biology & Medicine*, vol. 71, pp. 133–145, 2014.

[95] N. Vargas-Mendoza, Á. Morales-González, E. O. Madrigal-Santillán et al., “Antioxidant and adaptive response mediated by Nrf2 during physical exercise,” *Antioxidants*, vol. 8, no. 6, p. 196, 2019.

[96] R. Loschinski, M. Böttcher, A. Stoll, H. Bruns, A. Mackensen, and D. Mougiakakos, “IL-21 modulates memory and exhaustion phenotype of T-cells in a fatty acid oxidation-dependent manner,” *Oncotarget*, vol. 9, no. 17, pp. 13125–13138, 2018.

[97] A. M. Niess, F. Passek, I. Lorenz et al., “Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes: acute and adaptational responses to endurance exercise,” *Free Radical Biology and Medicine*, vol. 26, no. 1-2, pp. 184–192, 1999.

[98] T. M. Hagen, “Oxidative stress, redox imbalance, and the aging process,” *Antioxidants & Redox Signaling*, vol. 5, no. 5, pp. 503–506, 2003.

[99] R. Njemini, M. Lambert, C. Demanet, R. Kooijman, and T. Mets, “Basil and infection-induced levels of heat shock proteins in human aging,” *Biogerontology*, vol. 8, no. 3, pp. 353–364, 2007.

[100] D. Zhou, M. Borsa, and A. K. Simon, “Hallmarks and detection techniques of cellular senescence and cellular ageing in immune cells,” *Aging Cell*, vol. 20, no. 2, article e13316, 2021.